STRUCTURE ELUCIDATION OF AND SYNTHETIC APPROACHES TO MONATIN, A METABOLITE FROM SCHLEROCHITON ILICIFOLIUS

by

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VERKLARING

Ek die ondergetekende verklaar hiermee dat die werk in hierdie tesis vervat, my eie oorspronklike werk is wat nog nie vantevore in die geheel of gedeeltelik by enige ander Universiteit ter verkryging van 'n graad voorgelê is nie.

L G J Ackerman **Datum**

OPSOMMING

Monatien, of 4-hidroksi-4-(3-indolielmetiel) glutamiensuur is 'n aminosuur met 'n hoë-intensiteit soet smaak, wat in die wortelbas van die inheemse plant *Schlerochiton ilicifolius* voorkom. In die proefskrif word die isolasie, struktuuropklaring en 'n aantal sintetiese benaderings tot die totaalsintese daarvan beskryf.

Omvattende fraksionering van 'n waterige ekstrak van die wortelbas van *S. ilicifolius* met behulp van AG50WX8 sterksuur-katioonuitruilhars gevolg deur opeenvolgende gelfiltrasies op Biogel P2 en Sephadex G10 filtrasiegel met die intense soet smaak as indikator, het gelei tot die isolasie van monatien as 'n mengsel van soute waarin die natriumsout oorheers het. Kalium en kalsium is die ander twee katione wat ook voorgekom het.

Die struktuuropklaring is hoofsaaklik gebaseer op ^{1}H en ^{13}C k.m.r.-data wat vir monatien en die laktoonester van die *N*-2,4-dinitrofeniel derivaat verkry is. Laasgenoemde verbinding is berei deur reaksie van monatien met Sanger se reagens en diasometaan. Die *X*-straalkristallografiese studie van beide monatien en die derivaat het teleurstellende resultate gelewer aangesien swak refleksie-intensiteite die daaruitspruitende verfyning beperk het. Die verkreë strukture toon egter wel die raamwerkatome van beide verbindings waaruit die relatiewe stereochemie van die twee chirale sentra afgelei kon word. Vergelyking van die spesifieke rotasie van monatien met dié van die verwante 4-hidroksi-4-metielglutamiensure dui daarop dat monatien die (2*S*,4*S*) konfigurasie mag besit.

Retrosintetiese analises van monatien het 'n aantal sintetiese roetes aangedui wat gevolg kon word vir die sintese van monatien en/of monatienanaloë waarin die indoolbrokstuk deur 'n feniel of arielgroep vervang is. Sewe benaderings waarin modelverbindings gebruik is om die gunstigste reaksietoestande te bepaal, is ondersoek. Slegs die laaste benadering, gebaseer op 'n 1,3-dipolêre siklo-addisiereaksie was gedeeltelik suksesvol, naamlik reaksie van die 1,3-dipolêre verbinding gevorm uit *N*-t-Boc-indool-3-aldehied en metiel *N*-bensielglisinaat met 'n dipolarofiel, metiel-2-asetoksi-akrilaat, wat 'n pirrolidien gelewer het met die vereiste substituente benodig vir die monatienstruktuur. Die laaste stap, naamlik splyting van die C-2--N binding van die gesubstitueerde pirrolidienring was onsuksesvol.

Die ondersoek van die 1,3-dipolêre sikio-additiereaksie het verskeie gesubstitueerde pirrolidiene as rasemiese stereoisomere gelewer. Hiermee saam het 'n aantal oksasoliene en pirrolisidiene as byprodukte gevorm en hulle strukture is met behulp van proton/proton kern-Overhauser-effekstudies en *X*-straalkristallografie afgelei.

SUMMARY

Monatin, or 4-hydroxy-4-(3-indolylmethyl) glutamic acid is a high-intensity sweet tasting amino acid found in the root bark of the indigenous plant *Schlerochiton ilicifolius*. In the thesis the isolation, structure elucidation and a number of synthetic approaches toward the total synthesis of monatin are described.

Extensive fractionation of an aqueous extract of the root bark of *S. ilicifolius* using AG50WX8 strong acid cation exchange resin followed by successive gel filtration procedures using Biogel P2- and Sephadex G10 gels and guided by the intense sweet taste resulted in the isolation of monatin as a mixture of salts in which the sodium salt predominates. Potassium and calcium are the two other cations present.

The structure elucidation is based mainly on the analysis of data obtained for monatin and the lactone ester of the *N*-2,4-dinitrophenyl derivative, prepared by reaction of monatin with Sangers reagent and diazomethane, by ^{1}H and ^{13}C n.m.r. techniques. The *X*-ray crystallographic study of both monatin and the derivative proved disappointing in that the reflections measured were weak and as a consequence refinement of the data was severely curtailed. However the resultant structures do show the skeletal atoms of the two compounds and in each case the relative stereochemistry of the two chiral centres could be deduced. A comparison of the specific rotation of monatin with those of related 4-hydroxy-4-methylglutamic acids indicates that monatin could have the (2*S*,4*S*) configuration.

Retrosynthetic analysis of the monatin molecule identified a number of routes which could be utilized for the synthesis of monatin and analogues in which the indole moiety is replaced by a phenyl or aryl group. Seven approaches toward the synthesis of monatin were investigated using in most instances model compounds to establish optimum reaction conditions. Only the last approach, based on a 1,3-dipolar cyclo-addition reaction met with a measure of success: reaction of the 1,3-dipolar compound formed by reaction of *N*-t-Boc-indole-3-aldehyde with methyl *N*-benzylglycinate, with the dipolarophile methyl 2-acetoxyacrylate, generated a pyrrolidine with the requisite substituents needed for the monatin structure. In the event the final step, the cleavage of the C-2--N bond of the substituted pyrrolidine ring to give monatin, failed.

During the investigation of the 1,3-dipolar cycloaddition reaction several substituted pyrrolidines were prepared. In each case several racemic stereoisomers were formed. In addition a number of substituted oxazolines and pyrrolizidines were obtained as minor by-products during these reactions. The stereochemistry of these compounds was deduced from the proton-proton nuclear Overhauser effect studies and *X*-ray crystallographic data.

Peter Molopyane for recording many n.m.r spectra;

Drr R M Horak, L M du Plessis en P J van Wyk vir hulle volgehoue belangstelling in die vordering van die werk;

Mnr H Vahrmeyer wat oorspronlik die plant waarop gewerk is onder my aandag gebring het;

Owen Frisby, Karl Bischofberger, Robert Robinson, Heinrich Taljaard, Piet Masemula, Phineas Masoga en Edward Kekana wat behulpsaam was met die versameling van die plantmateriaal;

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Laastens, my vrou en kinders vir die geduld wat hulle moes beoefen en die begrip wat hulle getoon het veral gedurende die periode waarin die proefskrif geskryf is.

Opgedra aan my vrou Bettie - baie dankie vir al die jare en baie aande van geduld.

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CHAPTER 1

AN OVERVIEW ON SWEETENERS

1.1 INTRODUCTION

Humans have the ability to distinguish between four primary taste sensations namely sweet, sour, bitter and salty and of those it would seem that the greatest regard is for sweetness. In Biblical days honey was the only available sweetener and its use is inferred from Egyptian tomb drawings of about 2000 B.C. in which apiculture is pictured. The modern day sweetener sucrose, was first described in the Hindu literature of about 1000 B.C.. However, it was only during the 14th century that sugar was imported into England via Venice as a rare commodity and sold at exorbitant prices. It took almost two more centuries before Columbus brought the sugar cane from the Canary Islands to the Western hemisphere.[1] As a result of modern agricultural techniques sugar is nowadays freely available and this has led to overconsumption with the concomitant detrimental effects such as obesity and tooth decay. This excessive use is apparent also from the annual consumption in South Africa which was greater than 50 kg/capita during 1984.[2] In a First World country such as the United States the consumption of sugar in 1985 was as high as 62 kg/capita.[1]

Since people seem to be incapable of controlling their craving for sweetness, a great amount of work has been and still is being devoted to the search for alternative sweeteners which lack undesirable side-effects.

Most of the research is aimed at non-carbohydrate sweeteners and these compounds can be divided into three groups: (i) naturally occurring compounds, (ii) synthetic compounds and (iii) a group which is best described as semi-synthetic compounds. The reason for this classification is simply to have a convenient grouping of the sweeteners.

This also allows one to single out the synthetic compounds as these are regarded with greater suspicion by health authorities than the natural compounds. This prejudice would seem to be unfounded considering that the main cause of overconsumption problems still lies with the natural sweeteners, sucrose and glucose.

Several symposia have been organized[3-5] and a wealth of books[1,6,7] as well as reviews have been published on the subject of sweeteners. It would seem that the review of Crosby *et al.*[8] best covers the complete field of sweeteners. This review is very comprehensive and includes data such as sensory evaluation and synthetic routes of the more important compounds aspartame and neohesperidin-dihydrochalcone. The reviews of Kinghorn *et al.*[9] and Morris[10] emphasize the natural compounds and include data such as plant sources, taste quality and chemical structures. The synthetic compounds are summarized in the review of Unterhalt[11] which also gives data on the respective taste intensities, the mineral analyses of the different saccharin salts and even metabolic data on cyclamate.

1.2 NATURALLY OCCURING COMPOUNDS

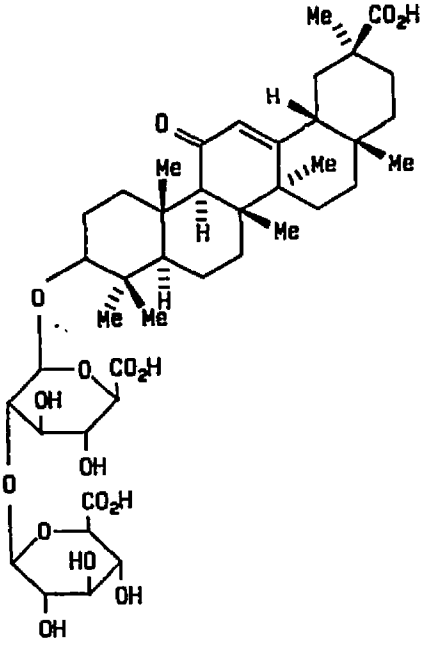
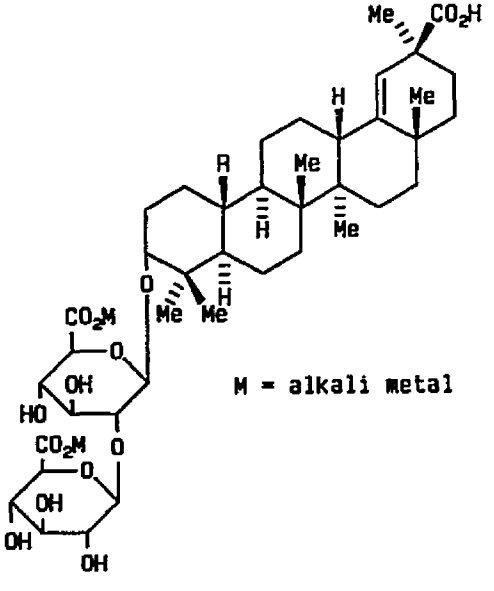
Sweet compounds found in nature cover a broad spectrum of structural types. Some have been consumed by a group or different groups of people for extended periods of time, mostly as a crude extract.

The FDA as the health regulating body in the USA has to approve the use of any new compound as a food additive for humans. As a rule the programme for proving the safety of a compound can be very time-consuming and expensive. Therefore, if a plant or product from which such a compound is extracted is known to have been consumed by humans for extended periods of time without ill-effects, the release of the compound for general consumption may be sanctioned more easily.

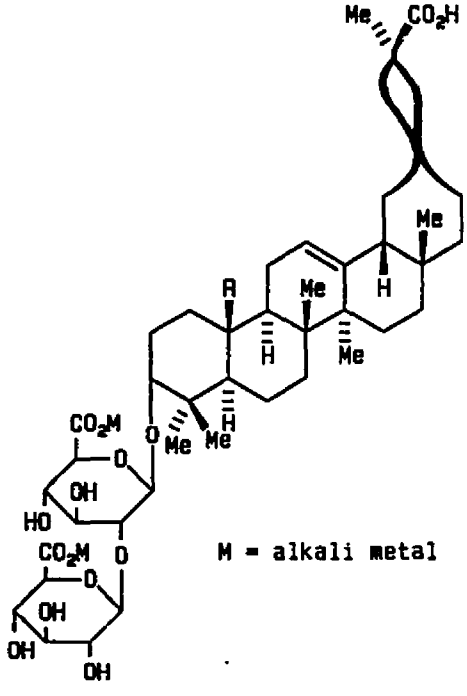
The known natural sweet compounds together with their relative sweetness level as well as certain properties such as stability and taste quality are summarized in Table 1.

Table 1 Naturally Occuring Compounds

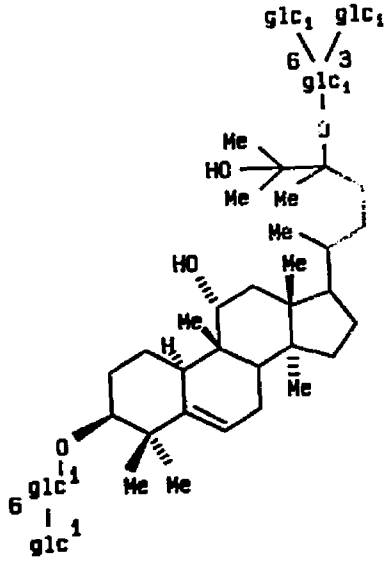
Compound	Sweetness*	Properties	Ref
(i) **Monellin** A protein consisting of 94 amino acid residues.	2000x	Lingering taste, heat and acid labile.	8 12 13
(ii) **Thaumatin** (Talin) A protein consisting of 207 amino acid residues.	2000x	Lingering taste, heat labile, fair-ly pH stable-range 2-10, expensive.	12 14 15 16
(iii) **Miraculin** A glycoprotein - amino acid sequence not known.	not sweet	After con-sumption changes sour-tasting foods to a sweet/-sour taste perception.	12 17

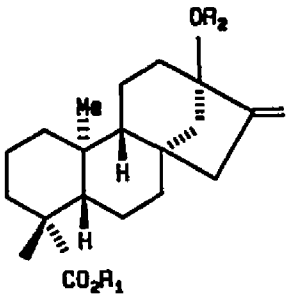
(iv) Glycyrrhizin 	50x	Strong liquorice after-taste. Widely used in confectionary and pharmaceutical industries	18 19 20
(v) Periandrine I R = CHO **(iv) Periandrine II** R = CH_2OH  M = alkali metal	not indicated	Taste the same as liquorice, not widely used.	21 22

(vii) Periandrine III R = CHO **(viii) Periandrine IV** R = CH_2OH	not indicated	Taste the same as liquorice, not widely used.	22



(ix) Mogroside	Unkown	Unknown	23





(x) Stevioside: $R_1 = glc_1$, $R_2 = glc_2\text{-}glc_1$	300x	Stevioside is a suspected mutagen with an unpleasant aftertaste. Allowed for use in Japan.	24

(xi) Steviol:

$R_1 = H$, $R_2 = H$

(xii) Rebaudioside A:

$R_1 = glc_1$, $R_2 = {}^{3}glc_2\text{-}glc_1$ (glc$_1$)

(xiii) Rebaudioside C = **Dulcoside**

$R_1 = glc_1$, $R_2 = {}^{2}glc_3\text{-}glc_1$ (rham$_1$)

(xiv) Rebaudioside D:

$R_1 = glc_2\text{-}glc_1$

$R_2 = {}^{3}glc_2\text{-}glc_1$ (glc$_1$)

(xv) Rebaudioside E:

$R_1 = glc_2\text{-}glc_1$

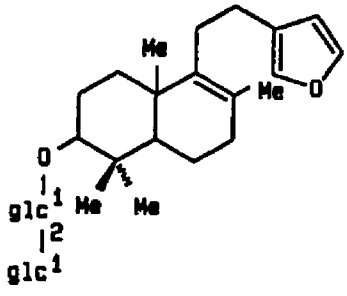
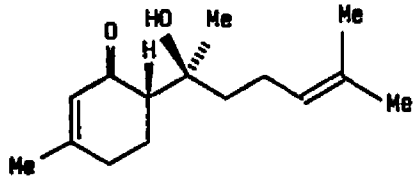
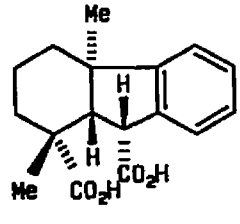
$R_2 = glc_2\text{-}glc_1$

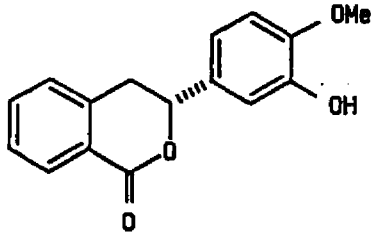
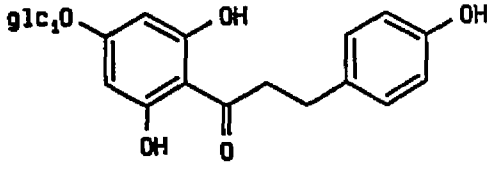
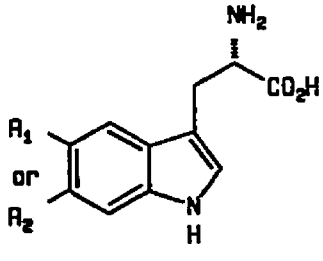
(xvi) Dulcoside A:

$R_1 = glc_1$, $R_2 = glc_2\text{-}rham_1$

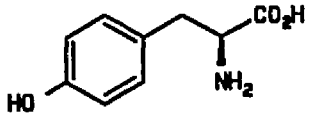
(xvii) Rubososide:

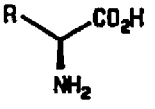
$R_1 = glc_1$, $R_2 = glc_1$

(xx) Bajunoside 	500x	Unknown	25
(xx) Hernandulcin 	1000x	Less pleasant than sucrose with an off- and after-taste	26
(xxii) Pine tree resin sweetener 	12-18	Bitter aftertaste.	27

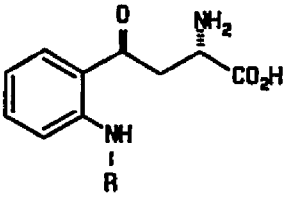
(xxiii) **Phyllodulcin** 	400x	Unknown	28 29
(xxiv) **Glucophloritin dihydrochalcone** 	Not determined due to possible toxicity.	Causes glucosuria	30
(xxv) **Tryptophan** and 5,6-substituted tryptophan  R_1, R_2 = halogens, Me, Et, Pr, Bu or alkoxy groups	35x		31 32

(xxvi) Tyrosine	5.5x	31

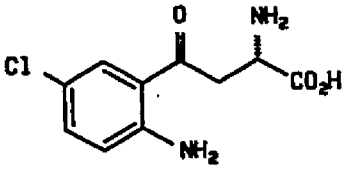
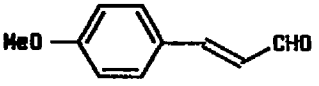
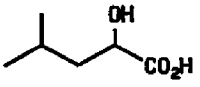


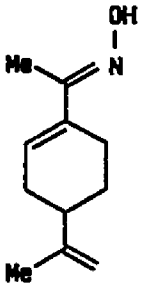


(xxvii) Glycine: R = H	1,5x	31
(xxviii) Phenylalanine:	7x	
R = CH_3		
(xxvix) Leucine:	4.3x	
R = $CH_2CH(CH_3)_2$		



(xxx) *N*-Formyl		
and *N*-Acetyl	35x	33
Kynurenine		

(xxxi) 3-(4-chloro-anthranyl) alanine 	80x	Taste closely resembles that of sucrose.	34
(xxxii) *p*-Methoxy-cinnamaldehyde 	unknown	unknown	35
(xxxiii) Leucic acid (2-hydroxy-4-methyl-pentanoic acid) 	30x	Used as a flavouring agent for tobacco. Promotes root-growth.Active stereoisomer not known.	36

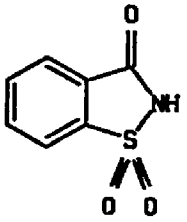
(xxxiv) **Perrilartine** 	370x	Unpleasant aftertaste. Unstable in acid.	37

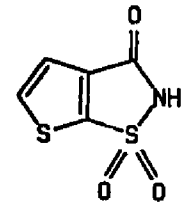
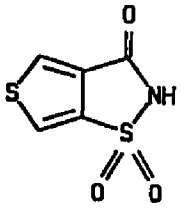
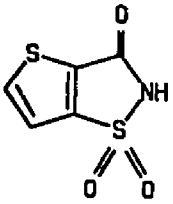
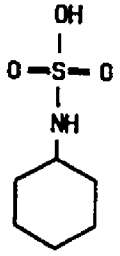
* relative to sucrose = 1

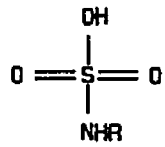
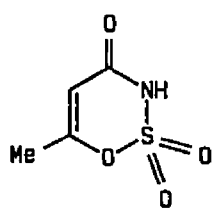
1.3 SYNTHETIC COMPOUNDS

In many scientific discoveries serendipity plays an important role and this holds true especially for synthetic sweeteners. These compounds can be divided into three main groups namely (i) the commercially important sulphonamides, (ii) the substituted aromatic compounds and (iii) the heterocyclic compounds, as indicated in Table 2.

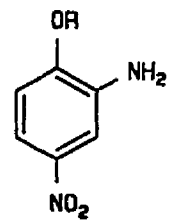
Table 2. Synthetic Compounds

Compound	Sweetness*	Properties	Ref
(i) **Saccharin** 	250x	Safety questioned. Bitter aftertaste which can be masked. Commercially very important.	38

(ii) Thiophene saccharins   	400x	Claimed to lack the unpleasant aftertaste of saccharin. Not commercially available.	39
(iii) Cyclamate 	30x	Safety questioned. Legislation forced its withdrawal from the market in the United States.	40

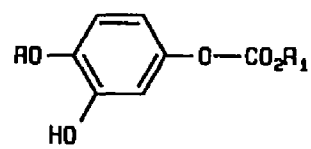
(iv) Cyclamate analogues	Less sweet than cyclamate.	No data	41 42 43 44
			
R = alkylated cyclohexyl = cyclopentyl = macrocycles C6-C12 = heterocyclohexyl = alkyl group	Same as cyclamate when hetero atom is S		45
(v) Acesulfam (Sunett)	130x	Non-toxic, Stable	46 47
			

(vi) Alkoxynitroanilines		Not in use-	48
(n-Pr = P-4000)	4000x	Causes kidney damage.	49



R = H, Me, Et, n-Pr

(vii) Alkoxy-hydroxyphenyl carboxylates	No data	No data	50



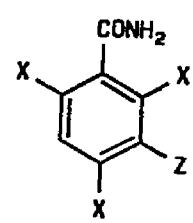
R = Me, Et, n-Pr

R_1 = aryl

= alkyl

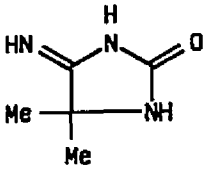
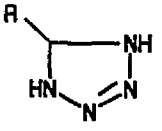
= heterocyclic alkyl

(viii) Benzamides	No data	No data	51



X = halogen

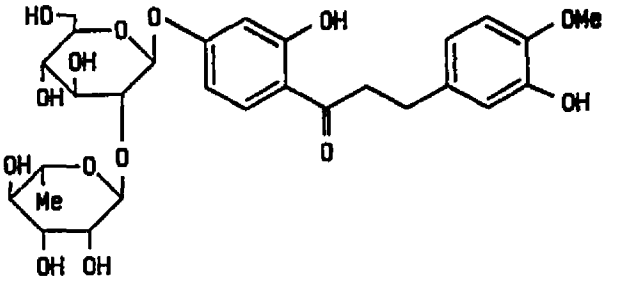
Z = carboxyl group

(ix) **Imidazolidinone** 	No data	Small changes in structure renders compound non-sweet	52
(x) **Tetrazoles**  R = 3-hydroxyphenoxy = 2,3-, 2,4-, 3,4-dihydroxyphenoxy = carbocyclic amines	No data	No data	53 54

1.4 SEMI-NATURAL COMPOUNDS

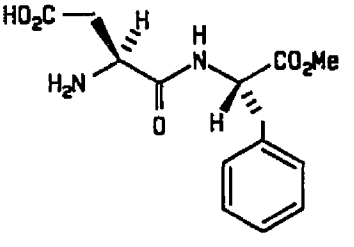
At present there are only two compounds namely neohesperidin dihydrochalcone (1) and the methyl ester of the dipeptide aspartylphenylalanine, better known as aspartame (2), which are part of this catagory.

Neohesperidin dihydrochalcone (1) was unexpectedly discovered during a study to determine the relationship between bitterness and certain structural features.[55] The compound (1) is about 1000 times sweeter than sucrose but has the disadvantage of a menthol-like after-taste and the sweetness tends to linger in the mouth after tasting. The use of (1) as a sweetener has not gained acceptance.



(1)

The second compound, aspartame (2), must rate as the greatest success of the non-carbohydrate sweeteners. Aspartame was also discovered by accident: Schlatter, during a peptide synthesis program, while crystallizing the peptide spilt some of it on his hand, brought it in contact with his mouth and hence made the taste observation.[56]



(2)

Aspartame (2) is about 150-200 times sweeter than sucrose but still has two drawbacks in that it is heat and acid labile. Furthermore people who suffer from phenylketonuria should be careful as aspartame may be hydrolysed to its constituent amino acids, aspartic acid and phenylalanine.

1.5 SYNTHETIC STUDIES ON SWEETENERS

Whenever a chemist attempts the synthesis of a compound he has to bear two aspects in mind. The first concerns especially the natural products where one has to consider the stereochemistry involved. This in turn leads to the second aspect namely the cost to achieve this goal. If a compound is aimed at the commercial market the cost-effectiveness of the synthetic process is a major factor towards the viability of a project and this poses a great challenge to the chemist. One will therefore find that commercial syntheses often do not involve elaborate techniques or routes. There was a time when enantiomeric mixtures were tolerated but present-day chemical methodology and commercial specifications demand the synthesis of single enantiomers.

1.5.1 THE NATURAL COMPOUNDS

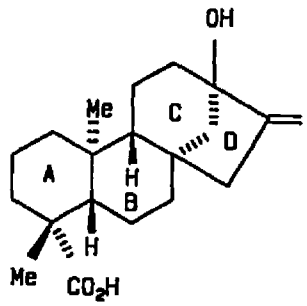
The important sweeteners glycyrrhizin, stevioside and talin are extracted from their natural sources on a commercial basis.

The synthetic work on stevioside was aimed firstly at the total synthesis of the compound and secondly at modifying the molecule in order to study taste-structure relationships. Other natural compounds which serve as synthetic targets in research projects are the sweeteners phyllodulcin, hernandulcin, perrilartine and a number of tryptophan derivatives.

The structural complexity of both glycyrrhizin, with its nine chiral centers, and talin, the protein with 207 amino acid residues have to date discouraged studies aimed at their total synthesis.

1.5.1.1 STEVIOSIDE

The dried leaves of the shrub *Stevia rebaudiana* when extracted yields 7% of a crude sweet extract which was originally named stevioside.[1] When this extract was subsequently shown to consist of seven sweet compounds the name stevioside (3) was retained for the major component.[24] These compounds share a common aglycone, steviol (4) and are differentiated by the structures of the glycosidic sugars. It is therefore logical that more emphasis has been placed on the synthesis of the aglycone since the attachment of the sugars to the aglycone is a straight-forward exercise in carbohydrate chemistry.

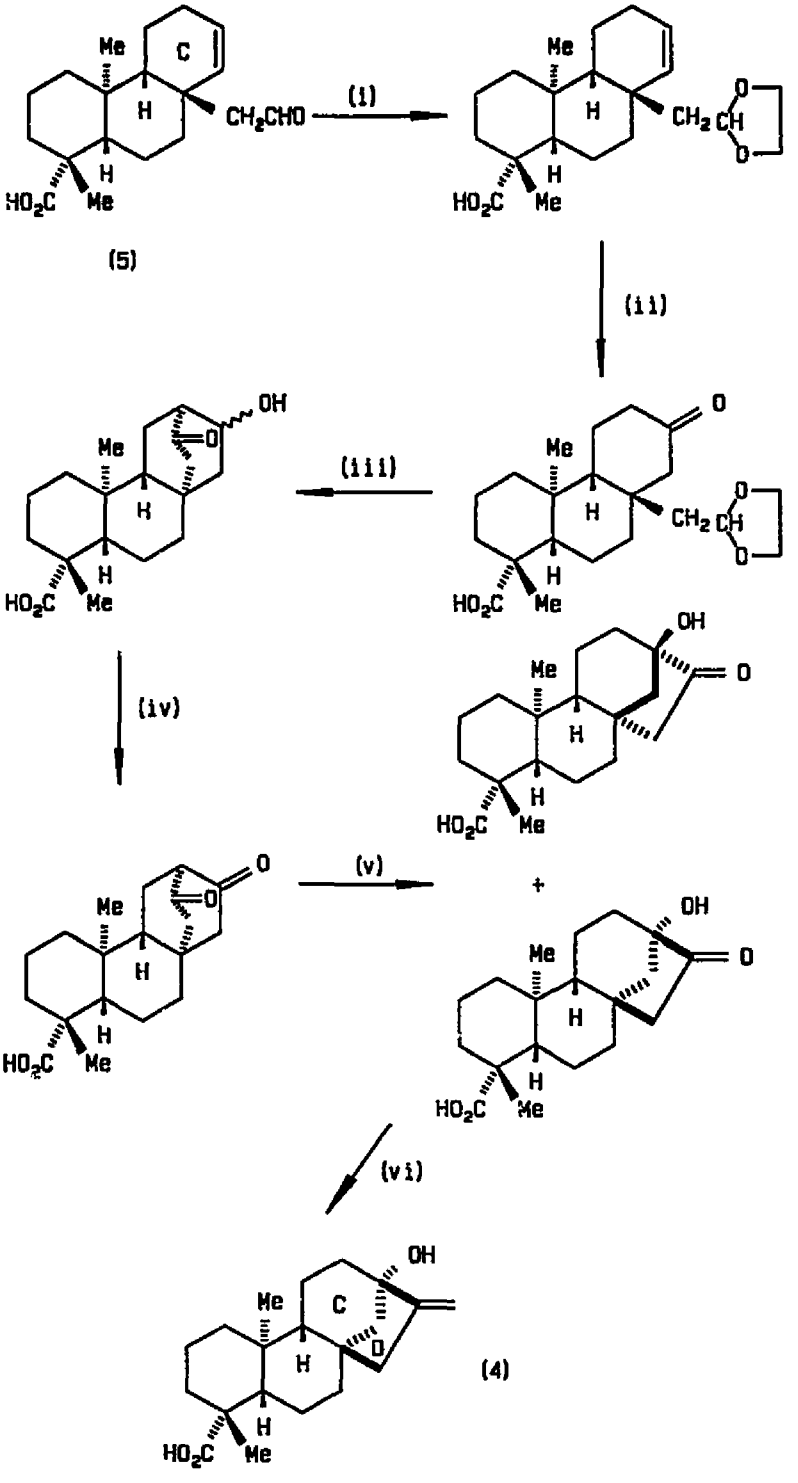


(4)

The part of the molecule which received the most attention was the bicyclo[3.2.1]octane system constituting the C/D-rings. Three papers[57-58] describe the work on the total synthesis of steviol and in all three cases the final product was the racemate.

The first procedure utilised a hydrolysis product (5) of stevioside as starting material and the synthetic strategy involved the elaboration of the C-ring of compound (5) to form the bicyclic ring system of compound (4) as shown in Scheme 1.[57] No unusual chemistry was involved in the procedure and although a purification of the isomers was effected after step (iv), the last two steps still rendered the final product as a racemic mixture.

Scheme 1. Total Synthesis of Steviol - Procedure 1

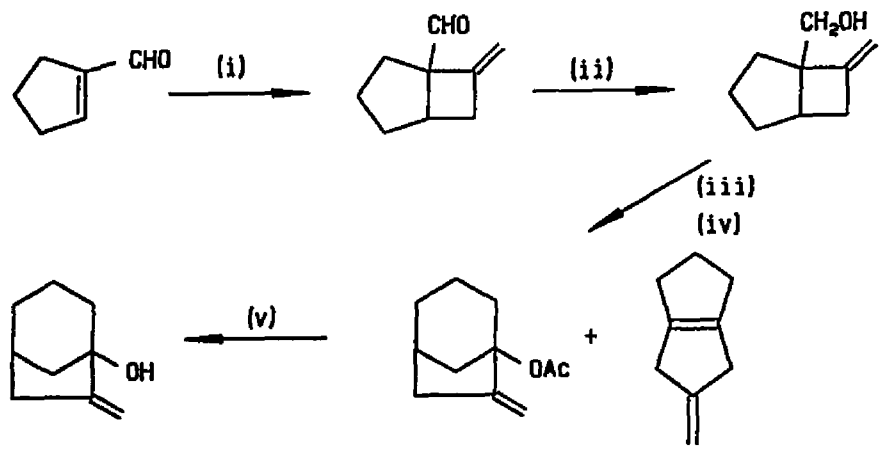


Reagents: (i) $HOCH_2CH_2OH$, TsOH (ii) a. B_2H_6/H_2O_2, b. Jones oxidation (iii) H^+, H_2O, acetone (iv) Jones (v) Zn/Hg, HCl (Clemmensen), (vi) methylenetriphenyl-phosphorane (Wittig).

In contrast the method of Nakahara *et al.*[58] (Procedure 2) consisted of a lengthy 17 step route which involved the synthesis of the tricyclic system (5) which was subsequently converted into racemic steviol (4) as depicted in Scheme 1.

The concept for the third procedure for the synthesis of the C/D ring system, which is found in both steviol (4) and in gibberelic acid, was tested on a model compound[59] and is outlined in Scheme 2.

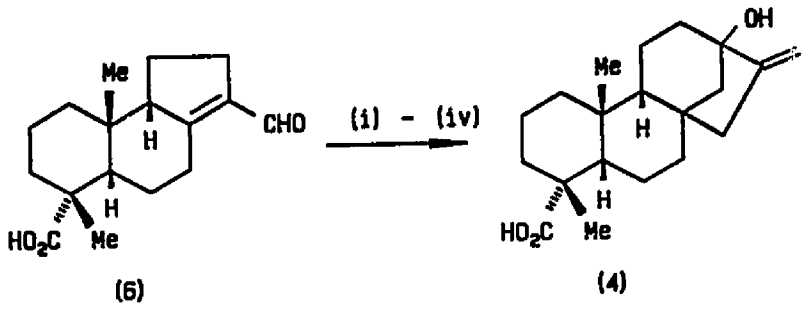
Scheme 2. Synthesis of a Model Compound for Steviol



Reagents: (i) allene, hν, ether (ii) lithium aluminium hydride (iii) tosyl chloride, pyridine (iv) NaOAc, HOAc (v) lithium aluminium hydride.

The aldehyde (6) required for this transformation was synthesized via a 16 step sequence. At that stage it was found that both the tosylation step and the subsequent treatment with sodium acetate failed and the synthetic strategy had to be modified as shown in Scheme 3 to give racemic steviol in a low yielding (3%) final step.

Scheme 3. Total synthesis of racemic steviol



Reagents: (i) allene, hν (ii) lithium aluminium hydride (iii) mesyl chloride (iv) 2,6-lutidine.

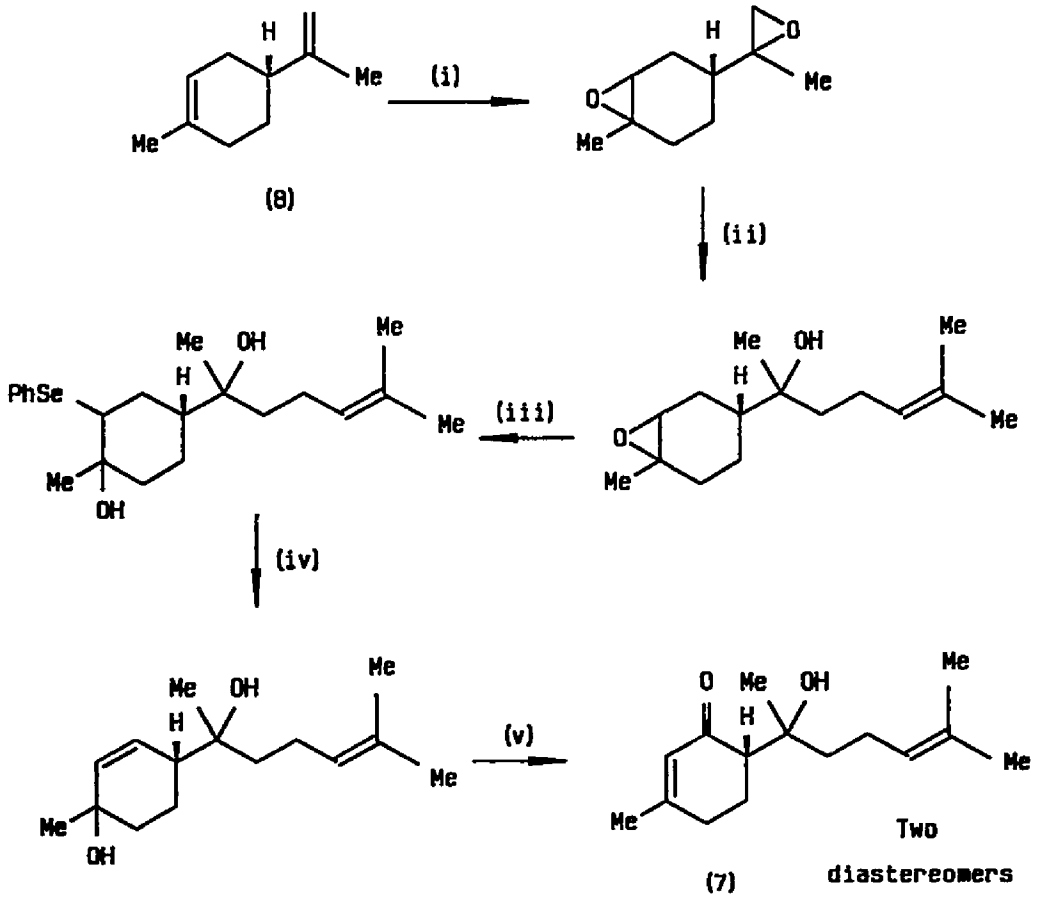
A formal synthesis of stevioside can be achieved using the aglycone steviol (4), which can be produced from stevioside by two routes. The first way involves a two-step procedure namely (i) a periodate oxidation of the sugar moieties to a hexa-aldehyde followed by (ii) base hydrolysis of the aldehyde containing moieties.[60] The second procedure utilises the enzymatic hydrolysis of the sugar moieties.[61]

With the aglycone steviol (4) in hand three different objectives were investigated. The first was to reinstal the sugar moieties into steviol (4) to give stevioside (3). This was achieved by linking the disaccharide to the C-13 oxygen function of steviol and subsequent introduction of the the monosaccharide to position 4.[60] Since rebaudioside A (see Table 1, compound xii) is said to be more sweet and pleasant tasting than stevioside the second objective was to convert stevioside to rebaudioside A. This route was initiated by hydrolysis of the terminal glucose moiety of the disaccharide sophorose. Stepwise addition of two glucose units then leads to rebaudioside A.[61] The third goal was to vary the sugar moieties linked to the aglycone to establish whether these changes would improve the taste of stevioside. However these attempts proved to be unsuccessful.[62,63]

1.5.1.2 HERNANDULCIN

Hernandulcin (7), a sweet sesquiterpene is at present not one of the commercially important sweeteners. Thus much of the synthetic work was aimed at establishing the absolute configuration of this compound.[64] The synthesis is shown in Scheme 4.

Scheme 4. Synthesis of Hernandulin



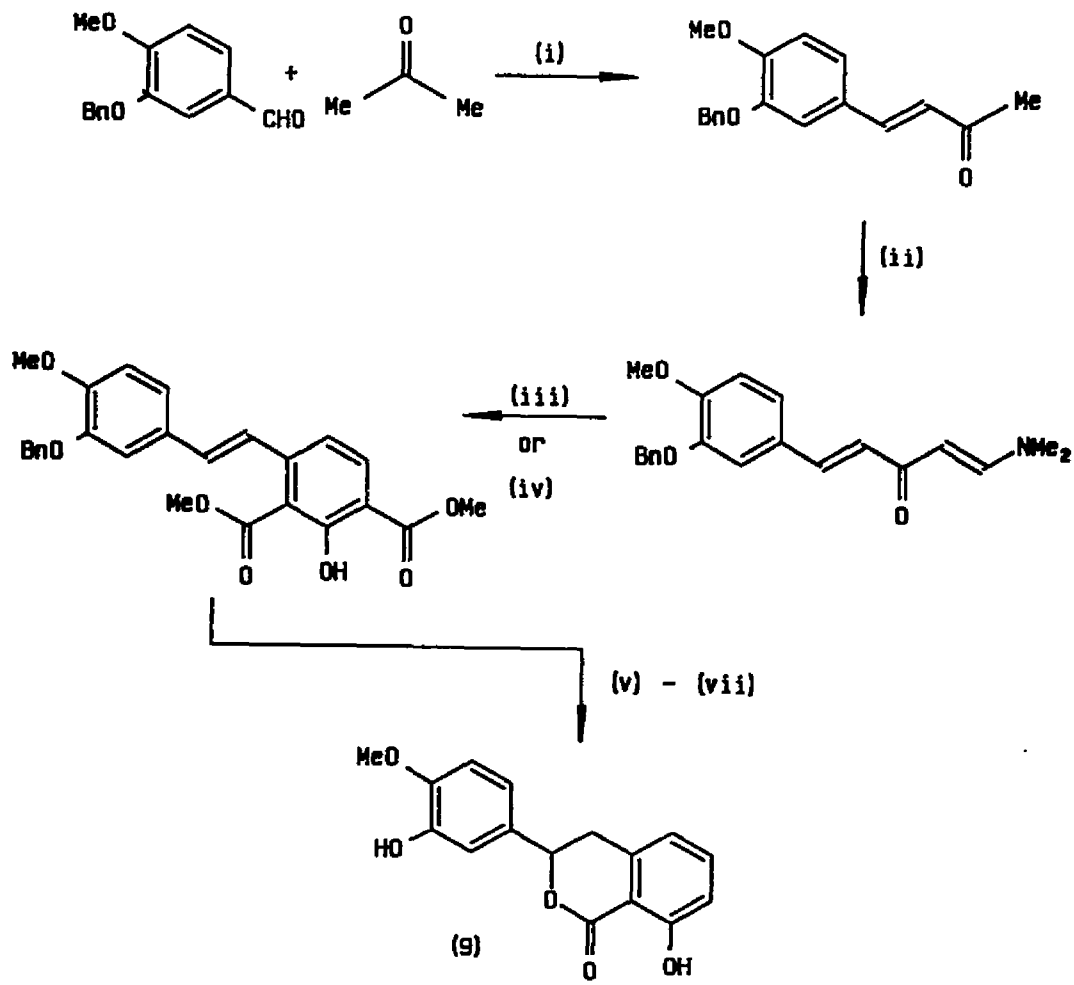
Reagents: (i) *m*-chloroperbenzoic acid (ii) $Me_2C{=}CHCH_2MgCl$, CuCl (iii) PhSeNa (iv) H_2O_2 (v) CrO_3, pyridine, HCl.

Although the chirality of (+)-limonene (8) is retained throughout the synthesis the final step proceeds in low yield (5,6%) to produce two diastereomers which renders the synthesis of little practical value.

1.5.1.3 PHYLLODULCIN

Phyllodulcin (**9**) a sweet dihydro-isocoumarin has been synthesised in racemic form by Takeuchi *et al.*[65,66] using two approaches to the problem. The first synthesis is based on a "biogenetic type" approach and makes use of a polyketide intermediate. The second and simpler of the two syntheses, is shown in Scheme 5.

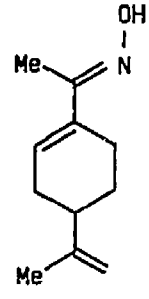
Scheme 5. Synthesis of Phyllodulcin



Reagents: (i) NaOH (ii) DMF (iii) KF, HOAc (iv) NaOAc, HOAc, 18-crown-6, dimethyl oxoglutarate (v) 10% KOH (vi) H^+ (e.g. H_2SO_4, CF_3COOH) (vii) 180°C, H_2O.

1.5.1.4 PERRILARTINE

Perrilartine (10) is a sweet ketdoxime found in the plant *Perylla nankinensis*.[37] This sweetener unfortunately also causes an unpleasant aftertaste.



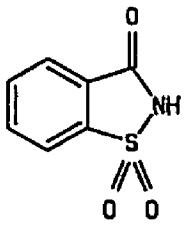
(10)

The synthesis of this compound and a series of aldoximes is based on the addition of hydroxylamine to an aldehyde or ketone. More than seventy aldoximes have been prepared and evaluated.[67,68] The properties of only a few of these compounds were an improvement on that of perrilartine. Although this work formed the basis for some patents[69] not one of these compounds has yet been exploited commercially.

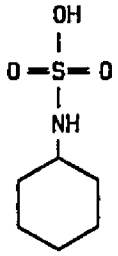
1.5.2 THE SYNTHETIC COMPOUNDS

At present more than thirty sweet tasting synthetic compounds have been described in the literature. Some of these compounds are known to be detrimental to health, e.g. P4000, but for most of the others there is little or no data available. At present only saccharin (11) is, with certain reservations, still allowed for human consumption. Cyclamate (12) used to be a very important sweetener. For example in 1970 it had an annual production in the United States of about 10,000 tons.[6] Although it has been withdrawn from the United States market because of FDA legislation it is still allowed in South Africa where it is used in admixture with saccharin in certain beverages. A third

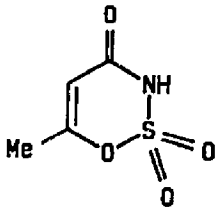
compound, acesulfam-K, marketed as Sunett (13), which is gaining importance is not yet exploited commercially. It is of interest to note that all three compounds (11- 13) are sulphonamides.



(11)



(12)



(13)

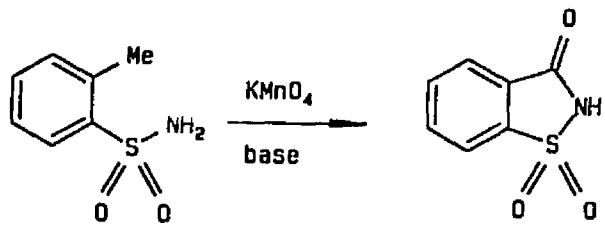
1.5.2.1 CYCLAMATE AND ANALOGUES

The syntheses of compounds in this series meet the requirements of a commercial viable procedure with regard to ease of execution and low cost. They are formed by the reaction of chlorosulphonic acid with an amine to give the sulphonamide. Although there is quite a variation in the amines used, namely cyclohexylamine[40], cyclopentyl-amines[70,71], various ring size cycloamines[72] and heterocyclic amines, no real improvement as compared to cyclamate was achieved with respect to taste quality and intensity.[40] None of the analogues have reached the market place.

1.5.2.2 SACCHARIN AND ANALOGUES

The original synthesis of saccharin (11) was unintentional. Fahlberg and Remsen[38] were in fact studying the oxidation of *o*-toluenesulphonamide to *o*-sulphonamidebenzoic acid, but the basic conditions employed in the reaction favoured the cyclization to the sulfam (Scheme 6).

Scheme 6. Synthesis of Saccharin



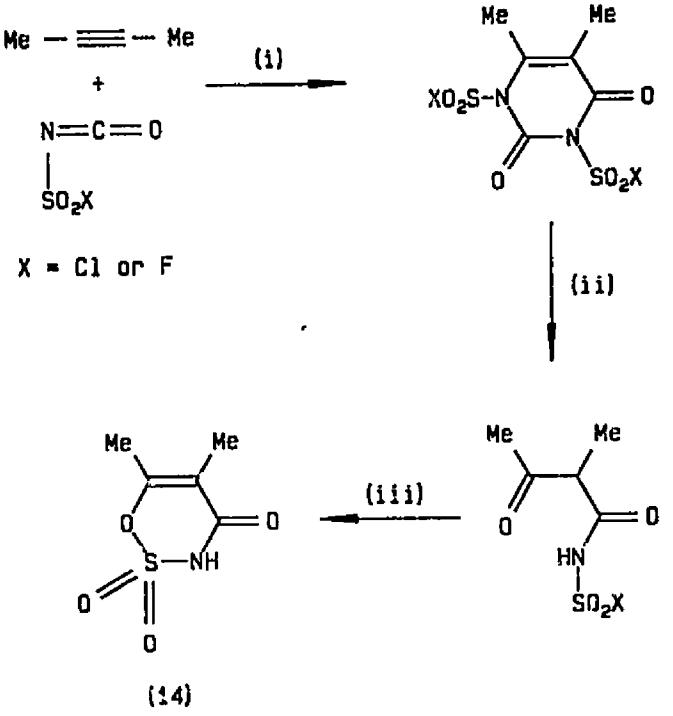
(11)

This sulfam (11) was for many years the major non-carbohydrate sweetener on the market and its importance can be seen from the annual production which during 1983 was about 3000 tons in the United States. However the unpleasant aftertaste associated with this compound prompted the search for saccharin analogues which lack this unwanted characteristic. Compounds claimed to satisfy this requirement are the so-called thiosaccharins (see Table 2) which contain various substituted thiophene rings and which have been patented[74] but up to now none have found any commercial use.

1.5.2.3 ACESULFAM

The formation of 5,6-dimethyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide (14) when either fluoro- or chloro-sulphonylisocyanate (FSI or CSI) is reacted with 2-butyne (Scheme 7) was reported in 1970.[46] When the sweetness of compound (14) was discovered whole series of compounds were synthesised by reaction of FSI or CSI with a variety of substrates.[75]

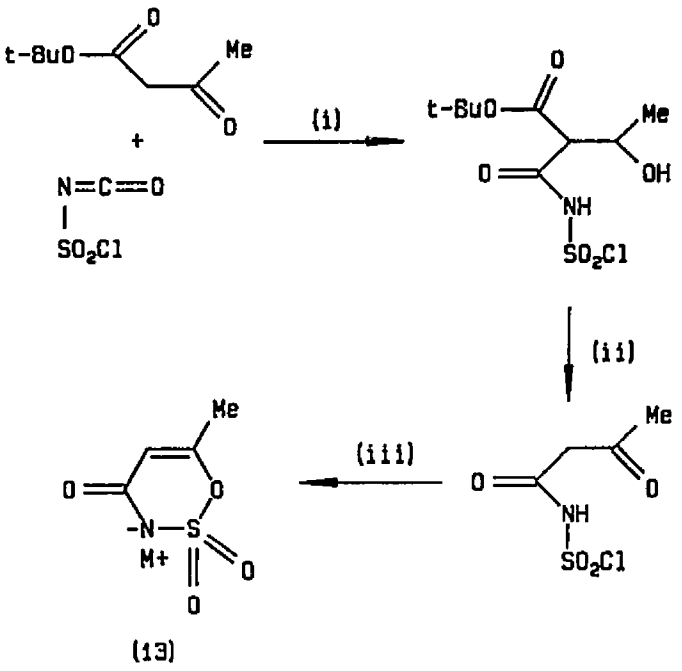
Scheme 7. Synthesis of Sulfam (14)



Reagents: (i) reflux (ii) H_2O (iii) H_2O, OH^-.

The compound which eventually gained in importance was 3,4-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide (13) and it was prepared according to the procedure outlined in Scheme 8.[76] Chlorosulphonylisocyanate is the preferred reagent since the use of fluorosulphonylisocyanate results in the formation of metal fluorides during the base induced cyclisation step. The fluorides proved difficult to remove from the sweetener and could have caused health problems.

Scheme 8. Synthesis of Acesulfam (13)



(13)

Reagents: (i) reflux (ii) heat (iii) aqueous base.

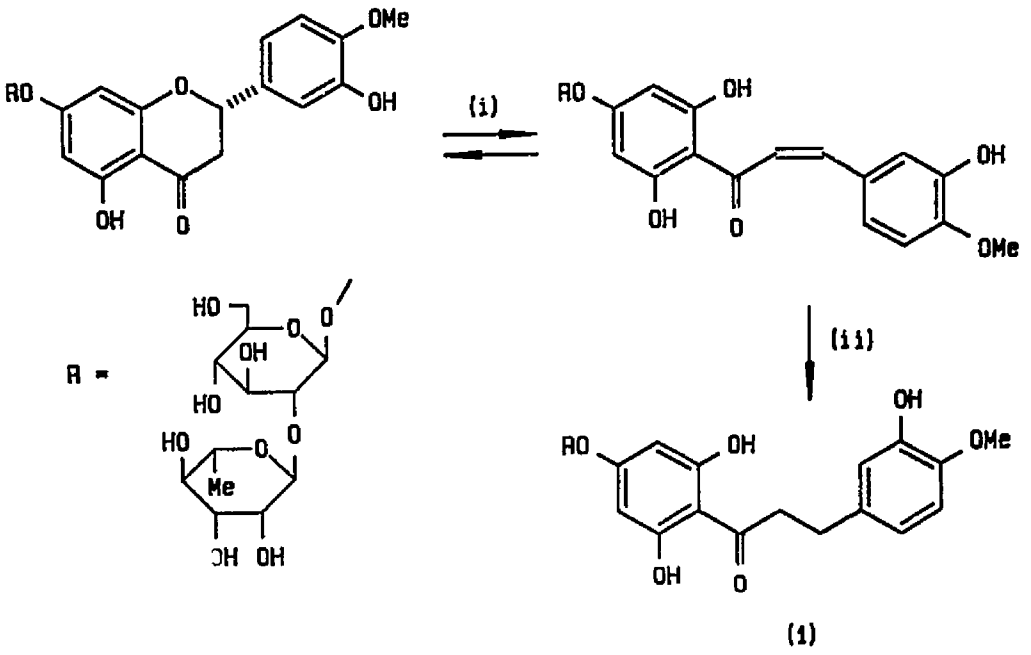
1.5.3 THE SEMINATURAL SWEETENERS

1.5.3.1 DIHYDROCHALCONES

The first of the seminatural compounds to be discussed is neohesperidin dihydrochalcone (**1**). It must be noted that several dihydrochalcones are known to possess a sweet taste but up to date neohesperidin dihydrochalcone is the compound which exhibits the highest level of sweetness in this series.

The syntheses of the dihydrochalcones revel in their simplicity. The conversion of e.g. (2*S*)-hesperitin to neohesperidin dihydrochalcone is a two-step process[55] as shown in Scheme 9.

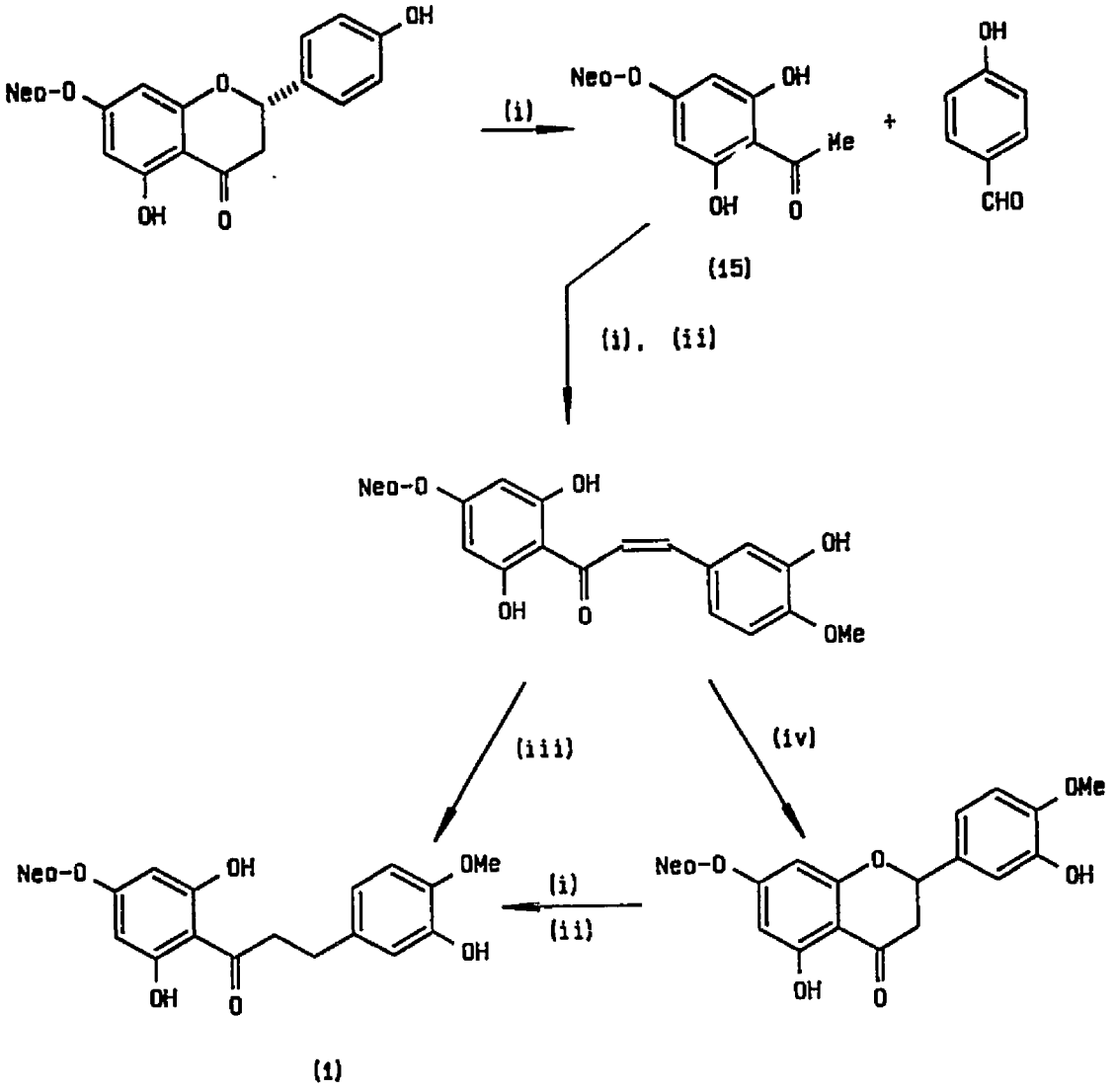
Scheme 9. The Conversion of Hesperitin to Neohesperidin Dihydrochalcone



Reagents: (i) NaOH (ii) H_2, Pd/C.

However, as another flavanone namely naringin, the main flavanoid constituent of grapefruit, is commercially available, a process was developed to convert naringin to neohesperidin dihydrochalcone (1).[77] The motivation for this synthesis is the fact that the dihydrochalcone derived from naringin which is about 100 times sweeter than sucrose can be converted to the dihydrochalcone derived from (2*S*)-hesperitin with a concomitant tenfold increase in sweetness. The first step in the conversion is the base-catalysed retro-aldol degradation of naringin. The resultant compound (15) is then coupled with isovanillin by means of an aldol-condensation. The complete procedure is shown in Scheme 10.

Scheme 10. Conversion of Naringin to Neohesperidin Dihydrochalcone



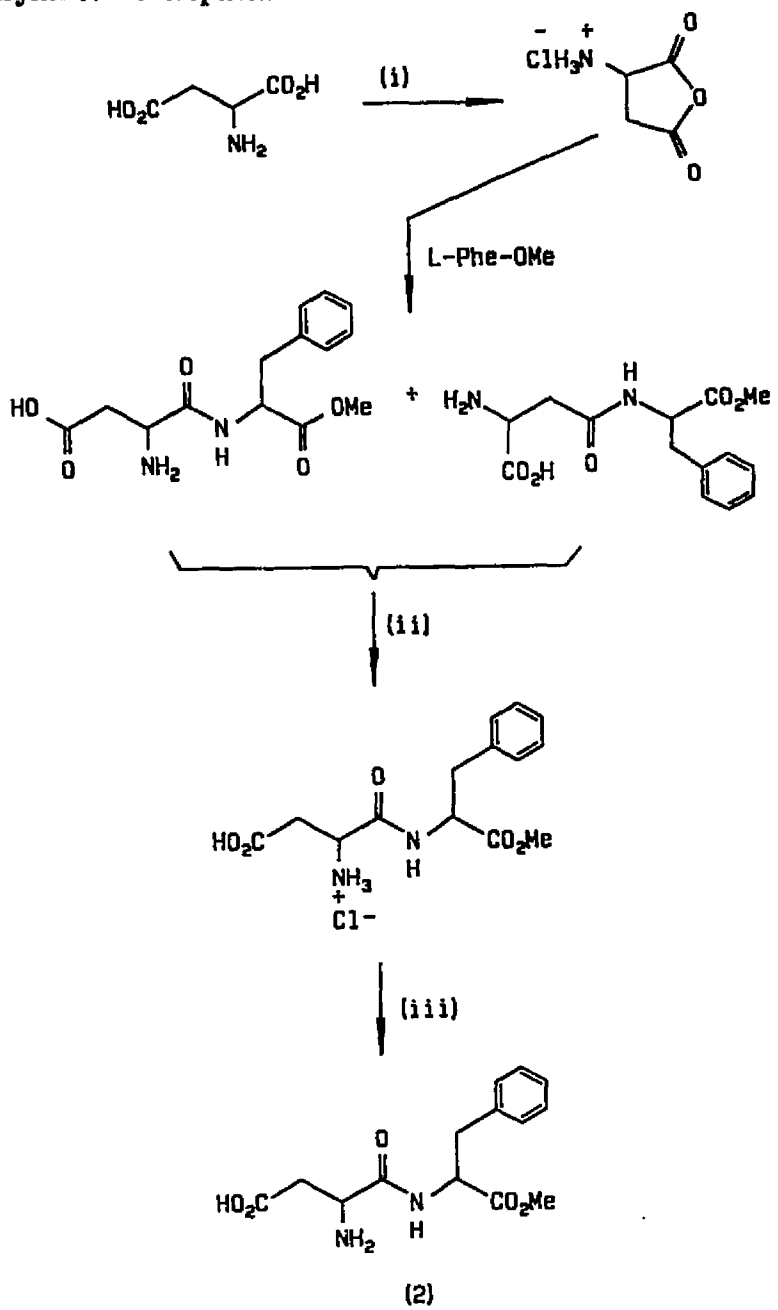
Reagents: (i) KOH (ii) isovanillin (iii) H_2, Pd - C (iv) heat 100°C

1.5.3.2 ASPARTYLPHENYLALANINE METHYL ESTER · ASPARTAME

The rise of L-aspartyl-L-phenylalanine methyl ester, aspartame (**2**), to its position of eminence as low calorie sweetener has resulted in the investigation and design of an ever increasing number of commercially viable syntheses for this compound. At present there are two basic chemical procedures for the synthesis of aspartame. The first makes use of either N-protected or unprotected L-aspartic acid anhydride which is reacted with

L-phenylalanine methyl ester to give after deprotection L,L-aspartame.[8] The procedure involving the unprotected aspartic acid is shown in Scheme 11. A drawback of this route is the fact that the phenylalanine moiety can react with either of the carbonyls of the anhydride. Fortunately the desired reaction predominates and furthermore during the acidification step it is the desired regeoisomer which precipitates.

Scheme 11. Synthesis of Aspartame

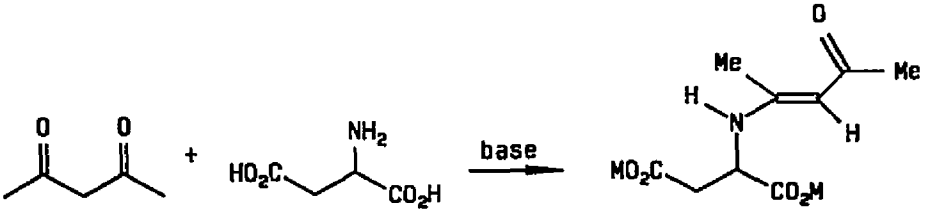


(2)

Reagents : (i) PCl_3 (ii) 1N HCl (iii) Na_2CO_3.

A patented procedure for the synthesis of aspartame[78] makes use of the 1-methyl-2-acylvinyl group as a novel nitrogen-protecting moiety for aspartic acid as illustrated in Scheme 12. The subsequent steps then follow the procedure as outlined in Scheme 11. However it is claimed that by the correct choice of solvent(s) the *in situ* formed anhydride reacts exclusively at the α-amino carbonyl group with the phenylalanine moiety to give, after deprotection, aspartame.

Scheme 12. N-protection of Aspartic Acid

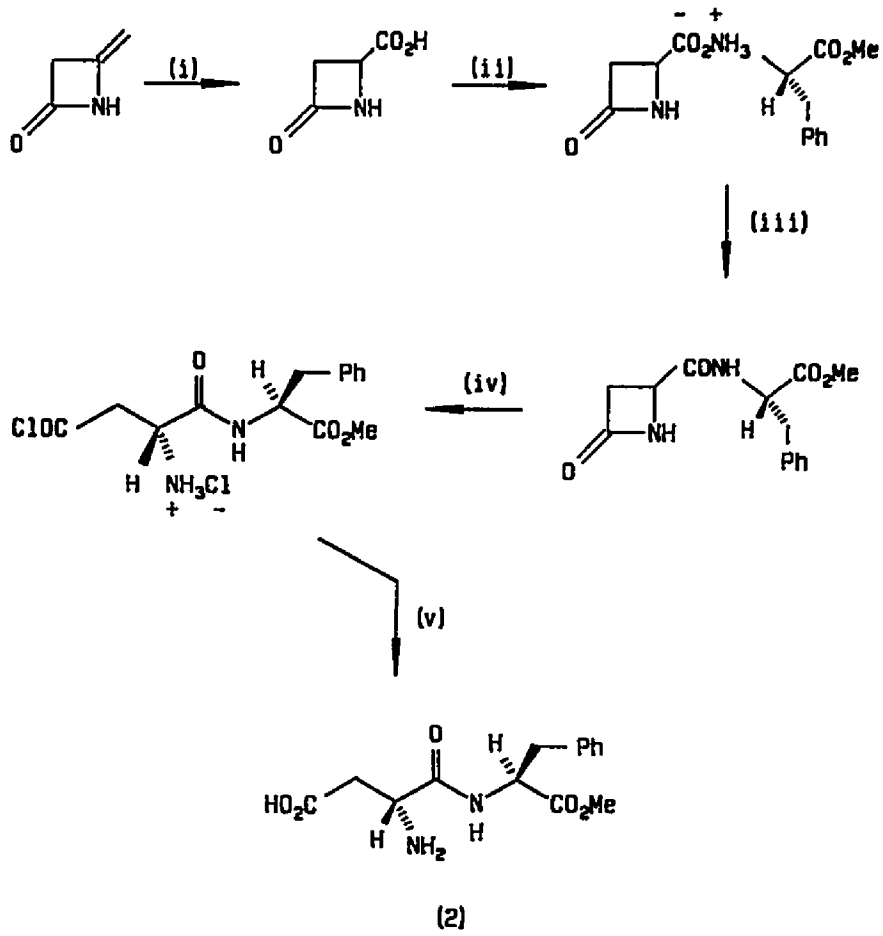


The second chemical procedure[79] (Scheme 13) entails the use of a ß-lactam as starting material. The chirality of the starting lactam is retained throughout the synthesis. Certain steps such as the permanganate degradation/oxidation of the exocyclic methylene group of the lactam to an acid proceeds in typical moderate yield. Subsequent steps also proceed in moderate yield and render this procedure non-competitive in comparison with the previously mentioned procedures.

1.6 STRUCTURAL REQUIREMENTS FOR SWEET TASTE

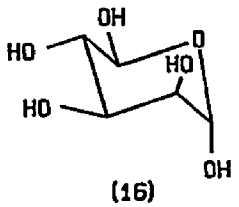
The biological properties of organic compounds can in most cases be traced back to certain structural features such as functional groups and/or stereochemistry. Taste can vary immensely with slight changes in the stereochemical arrangement of the atoms in a molecule. Thus, for example, α-D-mannopyranose (**16**) is sweet whilst ß-D-mannopyranose (**17**) is bitter.[6]

Scheme 13. Synthesis of Aspartame

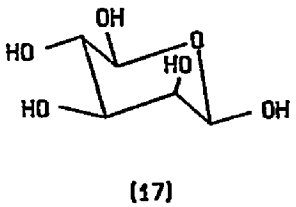


(2)

Reagents: (i) $KMnO_4$, H^+(ii) L-phenylalanine methyl ester, (iii) DCC (iv) HCl gas (v) $NaHCO_3$.



(16)

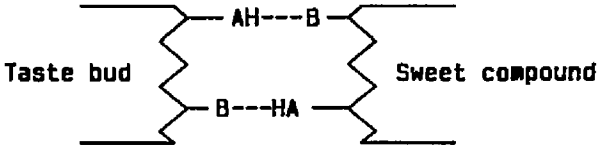


(17)

1.6.1 THE AH-B THEORY

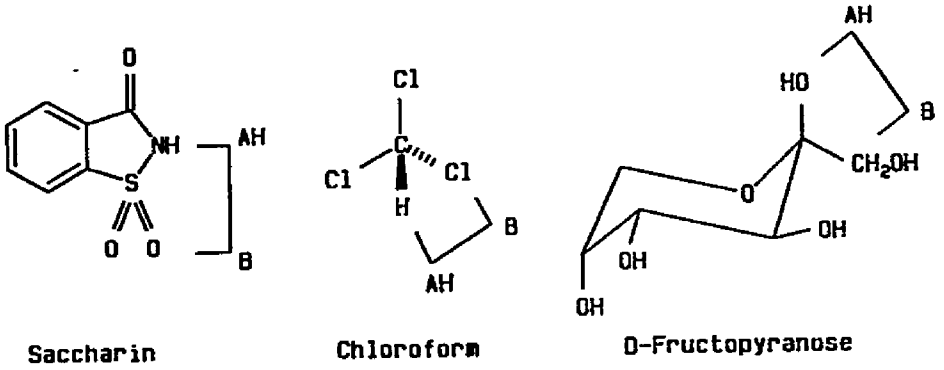
The first attempt to correlate sweet taste with molecular structure was described by Schallenberger and Acree in 1967.[80] This theory proposes that a bipartite chemical unit is common to the various substances that taste sweet. This common unit was described as an AH-B couple and is usually used to describe either an intra- or an intermolecular hydrogen bond. It was also established that the AH proton to B distance needed to be about 3 Å for optimum sweetness.[81] The points AH and B will then correspond with complementary AH-B sites on the taste buds of the tongue as shown in Figure 1.

Figure 1. Matching of Taste Bud Sites with AH-B Sites on Sweet Compounds



The positioning of the AH-B sites on some known sweet compounds is demonstrated in Figure 2.

Figure 2. AH-B Sites on some Sweet Compounds

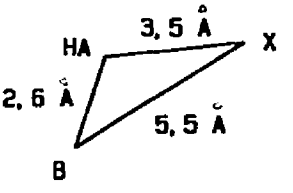


Saccharin Chloroform D-Fructopyranose

1.6.2 THE AH-B-X THEORY

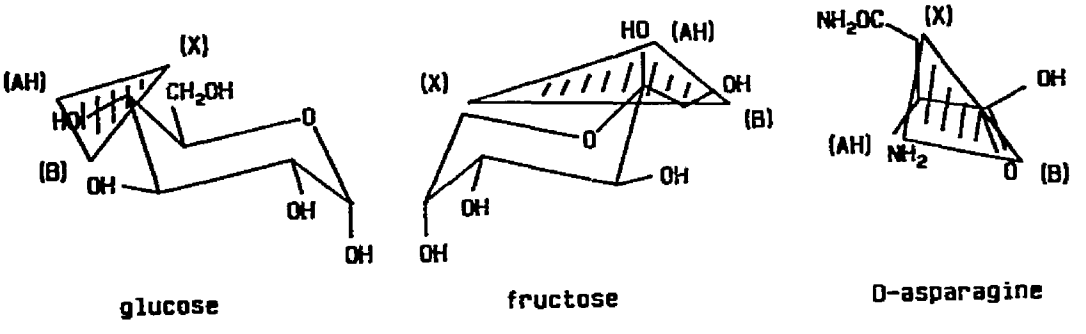
The AH-B theory seems to satisfy the requirement for sweetness encountered in carbohydrates. Since this requirement satisfies the non-sweet L-tryptophan as well as the sweet D-tryptophan it was clear that the theory had to be expanded or revised. Kier[83] by studying sweet and non-sweet amino acids expanded the previous theory to include a third electron-rich site in a molecule. He also stated that the AH point is better defined as a polarized bond rather than a labile or acidic hydrogen atom. As a result of these two additions he formulated the so-called triangle of sweetness, the dimensions of which are shown in Figure 3.

Figure 3. Triangle of Sweetness



This concept was subsequently accepted by other workers.[84] The way this triangle fits the structure of some sweet compounds is depicted in Figure 4.

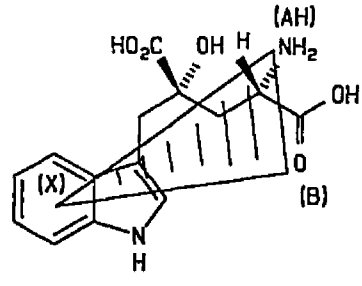
Figure 4. Sweetness Triangle Fitted onto some Sweet Compounds



1.6.3. FITTING TRIANGLE OF SWEETNESS ONTO MONATIN

The triangle of sweetness fits monatin (18), a novel sweetener which is the subject of this thesis (see Figure 5). A triangle of the dimensions given in Figure 3 was fitted onto a Dreiding model of monatin and the three touch points were found to be as follows: (i) the AH point corresponds to the amino group, (ii) the B point of the triangle fits the oxygen of the carbonyl group of the amino acid moiety and (iii) the X point is placed at the phenyl ring of the indole nucleus.

Figure 5 **Triangle of Sweetness Fitted onto Monatin**



1.6.4. EXPERIMENTALLY DEDUCED REQUIREMENTS FOR SWEETNESS

Neither of the theories mentioned above is able to explain the properties of the different sweet compounds. It was therefore decided by researchers in this field to synthesise a series of compounds in each class of sweeteners and from the data thus obtained deduce the structural requirements for that class of sweeteners. In this manner data were obtained for the coumarins, oximes, cyclamate analogues, dihydrochalcones and the dipeptide sweeteners. Due to the importance of the dipeptide aspartame it is not unexpected that most data was obtained for the dipeptide sweeteners.

1.6.4.1 THE OXIMES

A vast number of aldoximes were synthesized[67,68] and the requirements deduced:

(i) the oxime moiety must be conjugated with a double bond and

(ii) have a free hydroxy group.

1.6.4.2 THE "CYCLAMATES"

As the simple structure of cyclamate does not allow any great variation, the following requirements serve only as a guideline:

(i) The substituent on the sulphonamide nitrogen can vary from cycloalkyl[41-43] to heterocycloalkyl[44] to alkyl[45] moieties. The cyclohexyl derivative, i.e. cyclamate, proved to be the sweetest.

(ii) The second substituent on the amide nitrogen must be a proton.

(iii) The metal ion involved in the salt formation, which incidently improves solubility, is not critical.

1.6.4.3 THE COUMARINS

The following structural requirements for sweetness were deduced from the compounds synthesized[85-87] in the coumarin series. (For ring indication see the parent compound **(19)** in Figure 6)

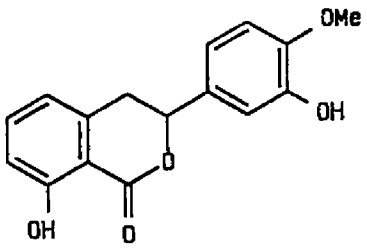
(i) The C-8 hydroxy group (ring A) is not necessary for sweetness but it does influence the taste intensity.

(ii) The substitution pattern on the C ring must be 2'-H, 3'-OH and 4'- OCH_3 as any alteration results in loss of sweetness.

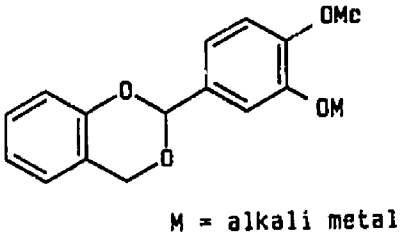
(iii) When the lactone (ring B) is changed to a lactam sweetness is lost or at least greatly reduced.

On the basis of these requirements the compound 2-(3-hydroxy-4-methoxyphenyl)-1,3-benzodioxane (**20**) was proposed to have sweet properties, synthesized[88] and in the event turned out to be 3000 times sweeter than sucrose!

Figure 6. Proposed Sweetener



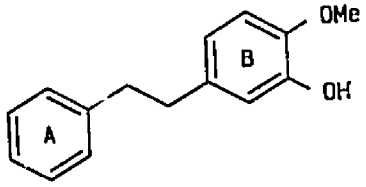
Parent compound (19)



Proposed compound (20)

1.6.4.4 THE DIHYDROCHALCONES

A sweet compound, 1-(3-hydroxy-4-methoxyphenyl)-2-phenylethane (**21**), was the result of work done in the coumarin series.[85] Note that this compound's structure is closely related to the aglycone of the dihydrochalcones.



(21)

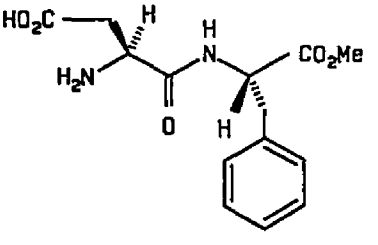
This compound was also used to study the substitution pattern of the B-ring needed for sweet taste. Once again it was found that the 2-H, 3-OH, 4-OCH_3 substitution pattern for the C-ring of the coumarins also applied to the dihydrochalcone sweeteners.[89] It was shown that the terminal sugar of the disaccharide must be rhamnose whilst the other

sugar could be either glucose or galactose. However the linkage of rhamnose via its anomeric carbon must involve position 3 of the glucose or galactose moiety as otherwise the sweetness intensity is greatly reduced.[90]

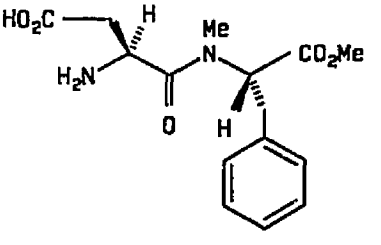
1.6.4.5 ASPARTAME

A wealth of synthetic procedures has been developed for the synthesis of aspartame and its analogues. The following points illustrate the structural requirements for sweetness:

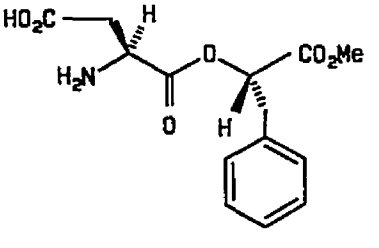
(i) The basic skeleton and stereochemistry of aspartame as determined by X-ray crystallography[91] is important. Various analogues of aspartame *viz.* the N-methylated derivative (22), the ester analogue (23) and the ß-amino acid analogue (24) were synthesised. Of these compounds only aspartame is sweet.[92]



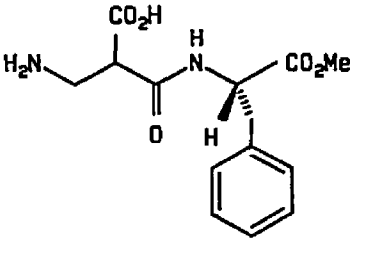
Aspartame (2)



(22)



(23)



(24)

(ii) The aspartic acid moiety must have the L-configuration.[93]

(iii) The aspartic acid amides are also sweet provided the substituent on the nitrogen atom is chiral.

(iv) Replacement of phenylalanine with either tyrosine or the aromatic ring of phenylalanine with a cyclohexyl ring created an analogue which was sweet. In fact the latter compound is even sweeter than aspartame.[94]

(v) On substituting the phenylalanine moiety with different amino acids it was found that it is not the stereochemistry but the shape and size of the different side-chains which determine sweetness i.e. the amino acid only acts as a carrier of alkyl or aryl groups.[96]

(vi) The distance between the ionic part of aspartic acid and the chiral centre on the amide nitrogen is critical for sweetness (triangle of sweetness!).[94]

(vii) A change in chirality for the second amino acid changes the taste of the dipeptide. There is, however, no fixed rule for the phenomenon since it was found that L-Asp-D-Ala-OMe is sweet while L-Asp-L-Ala-OMe is bitter. On the other hand L-Asp-L-Phe-OMe is sweet whereas in complete contrast L-Asp-D-Phe-OMe is tasteless.[97]

(viii) Several combinations of the side chain and ester group on the second amino acid have been synthesised.[98,99]

From the data obtained for these compounds it was deduced that sweetness reached a maximum for the n-propyl esters.[99] Grosch and Belitz[100] postulated that for the maximum sweetness in the dipeptide series the volume of the alkyl group of the ester moiety should exceed 30 Å^3 and should have a length that lies between 4,8 and 8,8 Å. The calculated volume[101] of the n-propyl group is about 60 Å^3 and its length is about 7,5 Å which fits the requirements of the Grosch and Belitz's postulate. It must be noted that the methyl, ethyl and n-butyl esters which fall outside the required limits are less sweet. The nature of the amino acid side-chain showed no pattern which could be correlated with sweetness.

1.7 CONCLUSION

Despite the present extensive body of experimental evidence there is not yet a satisfactory way to predict on the basis of stereochemical requirements whether a compound will be sweet or not. If such a theory could be developed it would greatly aid in the formulation and production of the perfect sugar substitute.

In conclusion it should be noted that at present only saccharin and aspartame have been granted general approval for human use. Another sweetener, acesulfam K is at an advanced stage of commercialisation and may be added to the list in the near future. In Japan stevioside is allowed for human use but its use has not yet gained approval in the USA. Since all these compounds as well as those which have not yet been accepted as sweeteners have shortcomings such as aftertaste and instability, it is clear that more research to find better sugar substitutes is quite justified.

CHAPTER 2

ISOLATION AND STRUCTURE ELUCIDATION OF MONATIN

2.1 INTRODUCTION

The rocky hills in the north-western Transvaal are the habitat of a spiny leaved hardwood shrub that grows to a height of about two metres. The plant, *Schlerochiton ilicifolius* is known, as a result of its spiny leaves, by the Afrikaans name of satansbos. The sweet-tasting bark of the roots prompted the black tribes to aptly name it "molomo monate". A direct translation of the Sepedi name means "mouth nice" and based on the Sepedi word "monate" it was decided to name the sweet-tasting amino acid which was isolated from the root bark "monatin".

Except for people who chew the root bark for its sweet taste, witch doctors have two medicinal uses for the roots. The first is as an emetic. The intense sweetness of a crude concentrated aqueous extract drunk by a patient induces vomiting.[102] The second use is of a psychological nature. Men believe that by eating the root they become more attractive to women. In the areas where this second belief is accepted the plant is also known as "pelua ea ketwana" which is best translated as "gladden the heart".

As there exists a need for a non-carbohydrate high intensity sweetener it was decided to investigate this plant as a possible source of such compounds.

2.2 ISOLATION OF MONATIN (18)

Roots of *Schlerochiton ilicifolius* were collected in the vicinity of the Waterberge in Northern Transvaal. The roots were freeze-dried to facilitate the easy removal of the bark. The latter operation was achieved by rolling-rubbing the roots on a large sheet of

coarse sandpaper with a hard brick. The bark was then ground in a hammermill and extracted with water. The aqueous extract was freeze-dried and the sweet compound isolated from this crude extract using two procedures.

The first procedure made use of paper chromatography. The crude redissolved extract was applied in a band on Whatman 3MM chromatography paper sheets. The chromatogram was developed with 10% water in ethanol. The sweet tasting area between two fluorescent bands (visible under illumination at 254 nm) was cut out, extracted with water and freeze-dried. This extract was then chromatographed on two gel filtration columns, first on Biogel P2 and then Sephadex G10 to give the pure compound. However, this procedure is tedious as 1200 sheets of chromatography paper were needed to process 9 kg of roots!

The second procedure which is described in detail in the experimental section can be summarized as follows:
After collection the roots were air-dried in a cool place, ground in a farmtype hammermill and extracted with water. The aqueous extract was stirred with AG50W-X8 cation resin in the H^+-form. The basic components bound to the resin were removed by treatment with an aqueous ammonia solution. The ammonia solution was freeze-dried and the crude basic extract was chromatographed first on Biogel P2 and then Sephadex G10 gel filtration resin to give pure monatin.

As in the previous procedure pure monatin was obtained as a mixture of salts in which the sodium salt predominated. This salt mixture was used in the structure elucidation and to determine the relative sweetness intensity.

2.3 STRUCTURE ELUCIDATION OF MONATIN

2.3.1 BACKGROUND

The strategy for the structure elucidation of monatin involved the collection of data about the constitution of this natural product using spectroscopic techniques and chemical reactions.

The molecular formulae, where applicable, were obtained from accurate mass determinations of the molecular ion or other significant ions observed in the mass spectra as well as elemental analyses. A detailed study of the ^{1}H and ^{13}C n.m.r. spectra led to the assignment of specific structures.

N.m.r. spectroscopy is one of the most powerful tools for structural analysis available to the organic chemist. The detailed analysis of both the ^{1}H and ^{13}C n.m.r. spectra of monatin and its derivatives was facilitated by the results of a number of n.m.r. techniques.

Assignments based on first order analysis of the spin systems in the 500,13 MHz ^{1}H n.m.r. spectra were confirmed by homonuclear ^{1}H-{^{1}H} decoupling experiments. The 125,76 MHz ^{13}C n.m.r. data, *viz.* chemical shifts , most multiplicities and one-bond (C,H) coupling constants were obtained from proton-decoupled and single frequency nuclear Overhauser enhanced (n.O.e.) spectra.

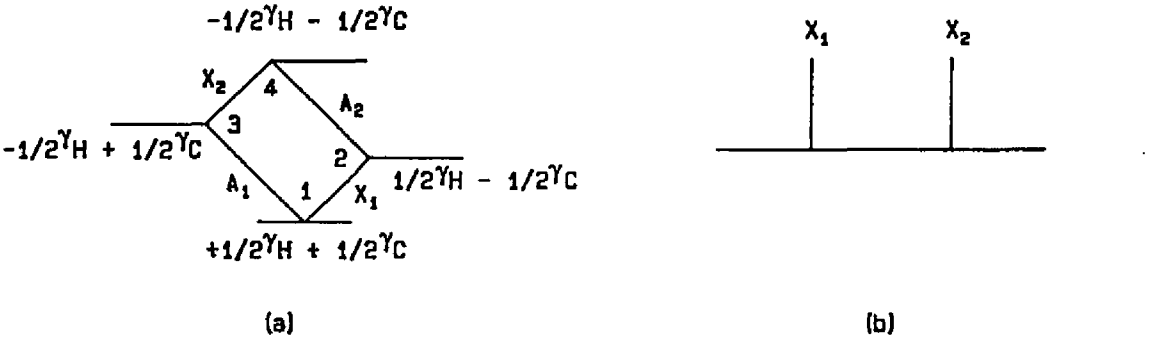
The signals of the proton bearing carbon atoms were correlated with specific proton resonances in two-dimensional (2-D) ^{13}C-{^{1}H} heteronuclear shift correlation experiments utilizing the one-bond (^{13}C,^{1}H) spin-spin couplings.

2.3.2 THE SPI TECHNIQUE

Heteronuclear ^{13}C-{^{1}H} selective population inversion (SPI), a pulsed double resonance technique, was applied in the structure elucidation to determine the long-range (more than one bond) (^{13}C,^{1}H) connectivity patterns, by using long-range (^{13}C,^{1}H) spin-spin couplings.[103-6]

The basic principle of this technique is demonstrated for a simple AX system of spin -½ nuclei consisting of a ^{1}H (A part) and a ^{13}C nucleus (X part). Figure 7 shows the AX energy level diagram with the relative populations at thermal equilibrium and the allowed single quantum transitions, as well as the corresponding spectrum of the X part.

Figure 7. Energy Level Diagram and ^{13}C Spectrum



(a) Energy level diagram for an AX spin system at thermal equilibrium and (b) corresponding spectrum of the X part (^{13}C spectrum).

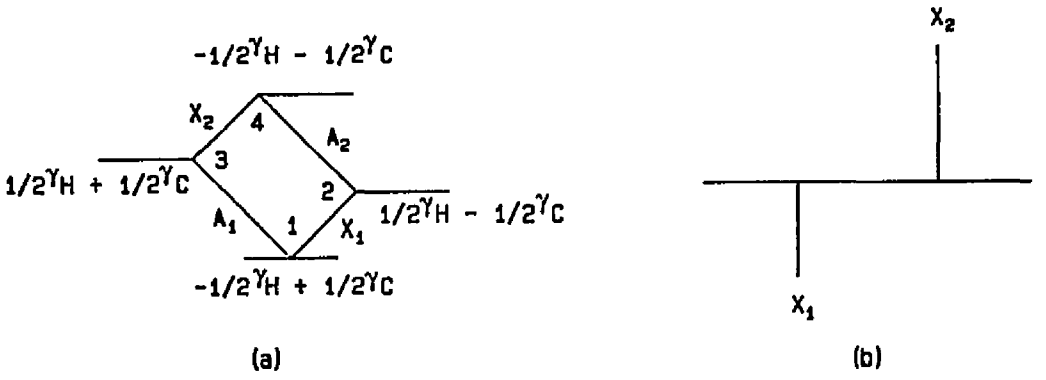
The population difference between the two energy levels, and the intensity of a transition is proportional to the gyromagnetic ratio, γ , of the nucleus that changes its spin state during the transition. The relative populations and transition intensities of an AX system before and after inversion of the A_1 transition are collated in Table 3.[106]

Table 3

	Populations			Intensities	
Energy Level	At Equi-librium	After Inversion of A_1 Transition	Transi-tion	At Equi-librium	After In-version of A_1 Transi-tion
1	$\frac{1}{2}\gamma_A + \frac{1}{2}\gamma_X$	$-\frac{1}{2}\gamma_A + \frac{1}{2}\gamma_X$	$A_1(13)$	γ_A	$-\gamma_A$
2	$\frac{1}{2}\gamma_A - \frac{1}{2}\gamma_X$	$\frac{1}{2}\gamma_A - \frac{1}{2}\gamma_X$	$A_2(24)$	γ_A	γ_A
3	$-\frac{1}{2}\gamma_A + \frac{1}{2}\gamma_X$	$+\frac{1}{2}\gamma_A + \frac{1}{2}\gamma_X$	$X_1(12)$	γ_X	$-\gamma_A + \gamma_X$
4	$-\frac{1}{2}\gamma_A - \frac{1}{2}\gamma_X$	$-\frac{1}{2}\gamma_A - \frac{1}{2}\gamma_X$	$X_2(34)$	γ_X	$\gamma_A + \gamma_X$

The energy level diagram and ^{13}C n.m.r. spectrum shown in Figure 8 result when the respective spin population are interchanged through selective spin population inversion involving the A_1 transition. The result of this inversion is that the population of the two energy levels of the A_1 transition is interchanged. The effect on the population differences however, is much more pronounced. For the two A transitions the population differences are the same, γ_A, except that the one is now negative. For the X transitions which previously had a population difference of γ_X, we now find $-\gamma_A + \gamma_X$ for the X_1 transition and $\gamma_A + \gamma_X$ for the X_2 transition. This result implies that the population differences for the A transitions have been transfered to the X transitions and added to the existing differences. This phenomenon is known as polarization transfer.

Figure 8. Energy Level Diagram and ^{13}C Spectrum After Inversion



(a) Energy level diagram for an AX spin system after inversion of the A_1 transition and (b) corresponding spectrum of the X-part (^{13}C spectrum)

Experimentally, selective population inversion is achieved through a radio-frequency pulse applied at the frequency of the A_1 transition such that $\gamma H_2.\tau = \pi$, where γH_2 is the power level and τ the duration of the π-pulse. As a result, the intensities of the lines in the X part of the spectrum are changed by an amount proportional to $\pm\gamma_A$ (*cf.* Table 3). In a normal X-{A} SPI experiment maximum signal enhancement factors are $(\gamma_X \pm \gamma_A)/\gamma_X$.

In the case under consideration, where X = ^{13}C and A = ^{1}H, $\gamma_A/\gamma_X = 4$ and the application of a π pulse at the low-field ^{1}H transition (A_1) will result in enhancements of -3 and +5 for the low-field and high-field ^{13}C transitions, respectively.

Application of a π-pulse with irradiating power $\gamma H_2 = 5$ Hz at a position 5 Hz to high-field (or to low-field) of a proton transition generally affects only the carbon atoms two- and three-bonds removed, as the corresponding (^{13}C,^{1}H) couplings are in the range 0-10 Hz.[107] The magnitude of four-bond (^{13}C,^{1}H) couplings (ca 1-2 Hz) precludes their detection under these experimental conditions.[108,109]

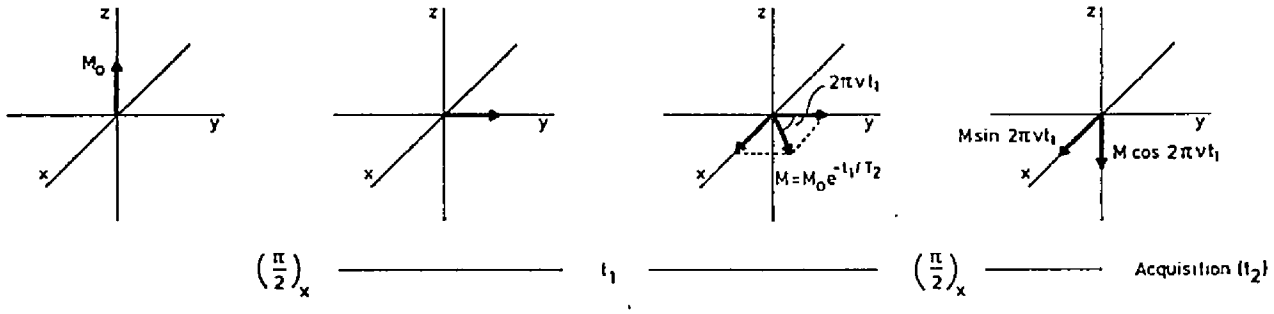
Interpretation of the SPI experiments was facilitated by using difference SPI spectroscopy in which a control spectrum is subtracted from the perturbed spectrum so that only changes between the two spectra are obtained.[110]

2.3.3 TWO-DIMENSIONAL (2-D) N.M.R. SPECTROSCOPY

The concept of two-dimensional (2-D) n.m.r. spectroscopy is best explained by considering a sample which gives rise to only one signal with chemical shift value ν : for instance a solution of chloroform in a deuterated solvent with proton observation.

At the start of the experiment the magnetization vector M is aligned along the z axis as indicated in Figure 9. Application of a $(\pi/2)_X$ pulse generates transverse magnetization (coherence) in the xy plane where it begins to precess with frequency ν for a time t_1. At the end of the interval t_1, a second $(\pi/2)_X$ pulse is applied and the magnetization is measured as a normal free induction decay (FID).

Figure 9.



If a series of experiments with different values of t_1 is performed, a separate FID is detected in t_2 for each t_1 value.[111] Fourier transformation of each FID with respect to t_2 generates a set of spectra in which the amplitude of the signal oscillates (with

frequency ν) as a function of t_1 *viz.* $M \sin 2\pi\nu t_1$. A second Fourier transformation over t_1 of this sine function generates a signal that is centered at ν_1 Hz. The two-dimensional Fourier transform converts the original dataset into a two-dimensional frequency spectrum $f(\nu_1,\nu_2)$ with ν_1 and ν_2 representing the chemical shift, ν of the signal in the two dimensions.

In general experiments are arranged such that the magnetization which evolves with some frequency during t_1, evolves with a different frequency during t_2; the latter frequency will invariably be a normal chemical shift value and could include spin-spin couplings.

In a coupled spectrum of two nuclei e.g. ^{1}H and ^{13}C the first ($\pi/2$) pulse creates ^{1}H magnetization (coherence) which is transfered to the ^{13}C nucleus through (^{13}C,^{1}H) coupling by the simultaneous application of a second ^{1}H ($\pi/2$) pulse as well as a ^{13}C ($\pi/2$) pulse. The two-dimensional spectrum which is obtained after Fourier transformation contains a signal at the coordinates (ν_1,ν_2) where ν_1 represents the ^{1}H chemical shift and ν_2 the ^{13}C chemical shift. This is the fundamental scheme for heteronuclear chemical shift correlation. The underlying phenomenon of the technique, the transfer of coherence amongst coupled spins, is most simply understood in the context of the previously described SPI technique. The technique can be utilized to correlate ^{1}H and ^{13}C chemical shifts through either the one-, two- or three-bond (^{13}C,^{1}H) coupling constants by changing the delay times which are a function of $J(^{13}\text{C},^1\text{H})$ in the pulse sequence.[106]

In contrast to the one-dimensional SPI experiment, excitation is nonselective *i.e.* all resonances of the ^{1}H n.m.r. spectrum are excited at the same time and the polarization transfer is t_1-dependent. The elegance of this type of two-dimensional correlation experiment must be seen in the fact that, using only one experiment, connections

between two types of nuclei can be established. The correlation character of the method allows an assignment made for one type of nucleus to be transfered immediately to another type.

2.3.4 THE NUCLEAR OVERHAUSER EFFECT (n.O.e.)

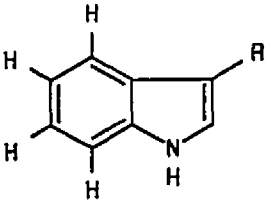
The nuclear Overhauser effect (n.O.e.) is a very useful n.m.r. parameter for chemical structure elucidation and conformational analysis.[112] Irradiation at the resonance frequency of a ^1H nucleus produces a perturbation in the observed signal of another ^1H nucleus if the irradiated nucleus contributes to the dipolar relaxation of the observed nucleus. Unlike chemical shifts and coupling constants that depend in part on through-bond effects, n.O.e.s are through-space effects. In an environment providing multiple dipole-dipole relaxation pathways, the n.O.e. between two protons is essentially inversely proportional to the sixth power of the distance between them. However the ability to observe small n.O.e.s using difference techniques allows one to determine long-range through-space connections between protons.

2.4 STRUCTURE ELUCIDATION

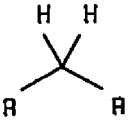
As stated earlier monatin was isolated from *Schlerochiton ilicifolius* as a mixture of salts in which the sodium salt predominated (>95%). The remainder of the material was made up of the potassium and calcium salts. The free acid form of monatin was obtained by treating an aqueous solution of monatin with acetic acid followed by addition of ethanol. Upon standing monatin (**18**) crystallized in the form of fine rosettes which analysed for $C_{14}H_{16}N_2O_5.½H_2O$. The compound gave positive Ehrlich and ninhydrin colour reactions which pointed to the presence of a 2-unsubstituted indole and an amino acid moiety in the molecule, respectively. The u.v. spectrum was reminiscent of the

spectrum of indole and had λ_{max} 279 nm (ϵ 5455). In spite of several attempts no mass spectrum could be obtained for either the salt or the free acid form of monatin using either the electron impact or fast atom bombardment technique.

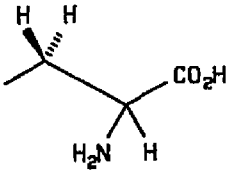
The ^{1}H and ^{13}C n.m.r. data for the monatin salt (in D_2O) are collated in Table 4. The signal at δ_H 7,192 in the ^{1}H n.m.r. spectrum was assigned to the C-2 proton of the putative indole moiety. The remainder of the signals in the ^{1}H n.m.r. spectrum of the monatin salt exhibited fine structure. First order analysis of these multiplets yielded the value of the proton chemical shifts and proton-proton coupling constants. On the basis of these results three fragments (I), (II) and (III) could be constituted.



(I)



(II)



(III)

Fragment (I).- The proton chemical shift and coupling constants of this four-proton spin system are typical of the protons of an indole moiety.[113]

Fragment (II).- The chemical shifts at δ_{II} 3,243 (10-H_a) and δ_{II} 3,051 (10-H_b) and coupling constant (J 14,3 Hz) of this isolated two proton spin system suggest that these protons are bonded to an sp^2 hybridized carbon atom.

Fragment (III).- The one proton doublet at δ_{II} 3,168 (J 11,6 1,8 Hz) serves as the terminus of this three-proton ABX system and is indicative of the methine proton of an amino acid. The chemical shifts of the AB part of the spin system (δ_{II} 2,651 and 2,006) are in agreement with the values reported for the ß-methylene group of amino acids.[114]

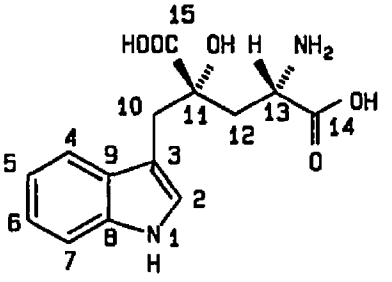
Table 4 **N.m.r. Data for Monatin Salt**

Atom	δ_C[a]	J (CH)/Hz	δ_H[b]	J (HH)/Hz
2	126,03D	182,2	7,192 s	
3	110,31S	-	-	-
4	120,46D	159,0	7,686 d	7,9
5	120,25D[c]	159,4	7,102 dd[d]	8,0 8,0
6	122,74D[c]	159,6	7,176 dd[d]	8,0 8,0
7	112,79D	160,3	7,439 d	8,1
8	137,06S	-	-	-
9	129,23S	-	-	-
10	36,53T	128,2	3,243 d	14,3
			3,051 d	14,3
11	81,41S	-	-	-
12	39,31T	130,2	2,651 dd	15,3 1,7
			2,006 dd	15,3 11,7
13	54,89D	144,2	3,168 dd	11,6 1,8
14	175,30S	-	-	-
15	181,18S	-	-	

[a] Relative to internal dioxane at 67,80 p.p.m.

[b] Relative to internal dioxane at 3,70 p.p.m.

[c,d] May be interchanged.



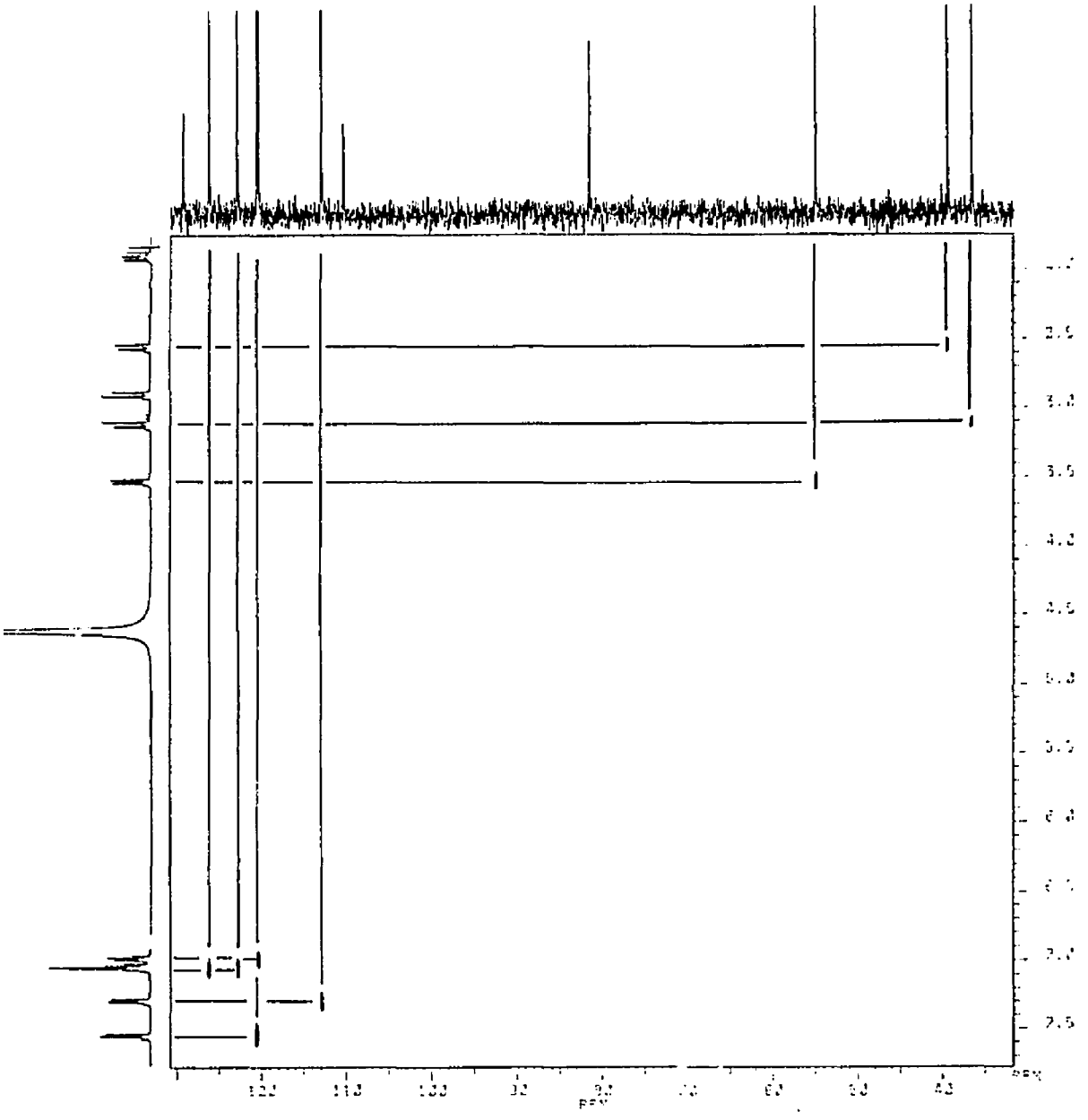
(18)

The (^{1}H,^{1}H) connectivity pattern of monatin followed from an analysis of the coupling constants and was confirmed by (^{1}H,^{1}H) homonuclear decoupling experiments.

The ^{13}C n.m.r. spectrum of the monatin salt confirmed the presence of 14 carbon atoms as indicated by the elemental analysis of monatin (**18**). The single frequency n.O.e. ^{13}C n.m.r. spectrum revealed that the fourteen resonances observed in the proton-decoupled ^{13}C n.m.r. spectrum are due to two methylene, six methine and six quaternary carbon atoms. These resonances represent on the basis of their chemical shift values two carbonyl groups, five methine and three quaternary sp^2 hybridized carbon atoms as well as one quaternary, one methine and two methylene sp^3 hybridized carbon atoms. The signals of the proton-bearing carbon atoms were correlated with specific proton resonances in a 2-D heteronuclear ^{13}C-{^{1}H} chemical shift correlation experiment (see Figure 10).

The connections between the different structural units and the assignment of the resonances in the proton-decoupled ^{13}C n.m.r. spectrum of the monatin salt were determined by heteronuclear ^{13}C-{^{1}H} SPI experiments utilizing the two- and three-bond coupling constants. The results obtained from these experiments are collated in Table 5 and Figure 12.

Figure 10. Two-dimensional (2D)(^{13}C,^{1}H) Correlation of Monatin

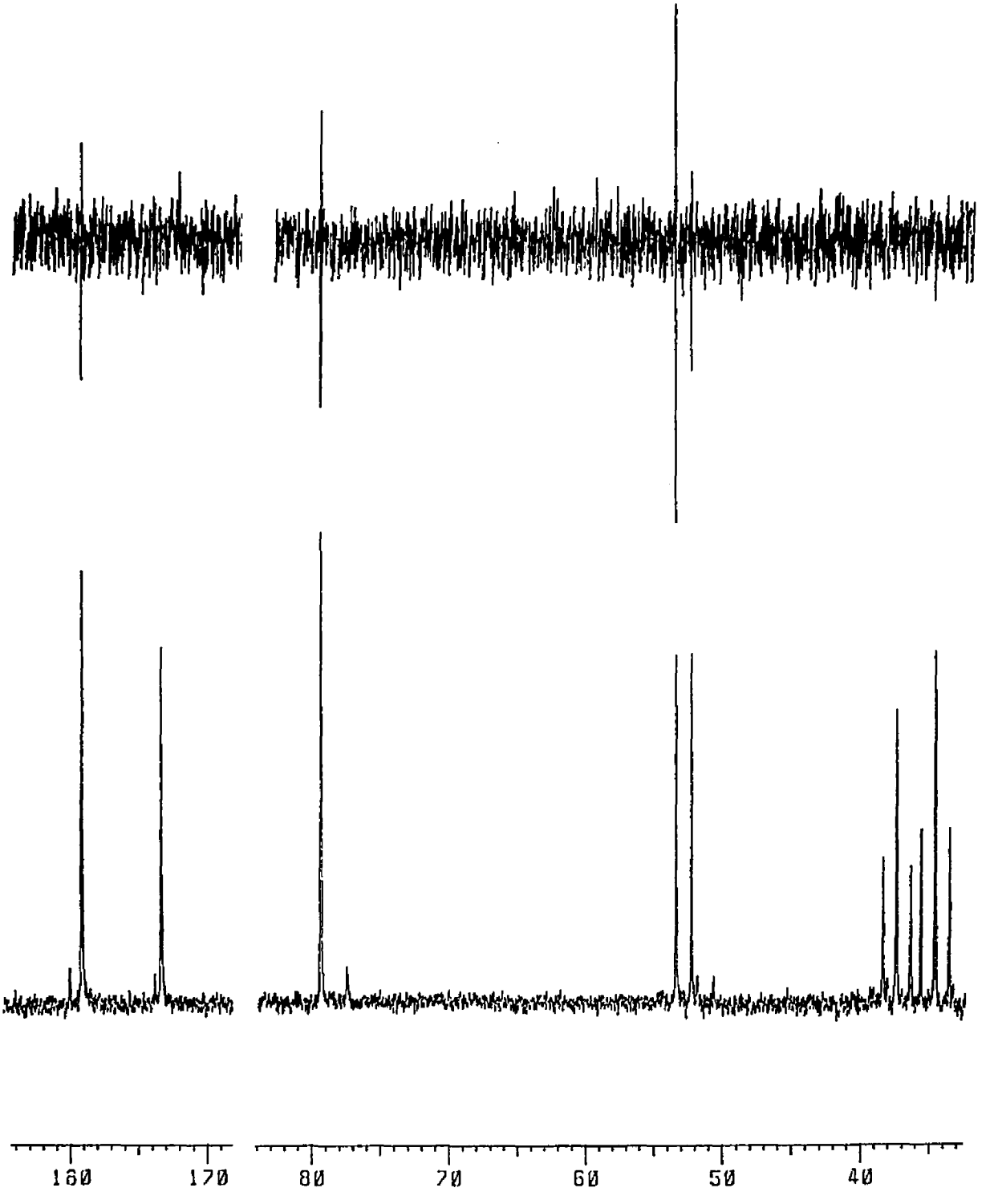


The singlet signal at δ_H 7,192 which correlates with the resonance at δ_C 126,03D is assigned to the C-2 proton of the indole ring on the basis of the chemical shift value and the one-bond (C,H) coupling constant [^{1}J(CH) 182,2 Hz]. Application of a π-pulse at a position 5,0 Hz to low-field of the 2-H resonance in a heteronuclear ^{13}C-{^{1}H} SPI experiment affected the resonances at δ_C 110,31S (C-3), 137,06S (C-8) and 129,23 (C-9). However no effect was observed for any of the non-indole carbon resonances.

Table 5. Results From SPI Experiments on Monatin Salt

Proton Transition Irradiated	δ_H/p.p.m.	^{13}C resonance Affected	Assignment
2-H	7,192	110,31S	C-3
		137,06S	C-8
		129,23S	C-9
10-H_a	3,243	126,03D	C-2
		110,31S	C-3
		129,23S	C-9
		81,41S	C-11
10-H_b	3,051	126,03D	C-2
		110,31S	C-3
		129,23S	C-9
		81,41S	C-11
		181,18S	C-15
12-H_a	2,651	81,41S	C-11
		54,89D	C-13
		175,30S	C-14
12-H_b	2,006	81,41S	C-11
		54,89D	C-13
		181,18S	C-15
13-H	3,168	81,41S	C-11
		39,31T	C-12
		175,30S	C-14

Figure 11. SPI Effects on Irradiation of the 12-H_b Proton Transition



The resonance at δ_C 110,31S was assigned to C-3 and those at δ_C 137,06S and 129,23S to C-8 and C-9 by comparison with the chemical shift values of other indole compounds e.g. tryptophan.[115] The link between fragment (II) and the indole ring was established by the

(C,H) connectivity pattern determined for the C-10 methylene proton in a SPI experiment. Irradiation in separate experiments of each of the C-10 protons affected the resonances at δ_C 126,03D (C-2), 110,31S (C-3) and 129,23S (C-9). As a result the last resonance is assigned to C-9 and as a consequence the resonance at δ_C 137,06S must be assigned to C-8. The two- and three-bond (C,H) connectivity pattern locates the C-10 methylene group at C-3 of the indole ring. However 10-H must furthermore be linked to quaternary carbon atoms two- and three-bonds removed as the resonance at δ_C 81,41S and 181,18S are also affected in these SPI experiments. The chemical shift value of the δ_C 81,41S resonance points to the presence of an sp^3 hybridized carbon atom substituted by an oxygen-substituent whereas the resonance at δ_C 181,18S is typical of an sp^2 hybridized carbon atom of a carbonyl group. The resonance at δ_C 81,41S was also affected when the 12-H_b resonance at δ_H 2,006 was irradiated in a SPI experiment (see Figure 10). In this instance it is the resonance at δ_C 175,30S, assigned to another carbonyl atom three-bonds removed, which was affected. In contrast it is the resonance at δ_C 181,18S which is affected when 12-H_a is irradiated. In both cases the C-13 resonance at δ_C 54,89D is also affected. Irradiation of 13-H in a SPI experiment confirmed the above two- and three-bond (C,H) connectivity pattern (see Figure 12)

On the basis of the two- and three-bond (C,H) connectivity pattern the structure as shown in (**18**) is assigned to the acid form of monatin. A less likely alternative in which the hydroxy and amino groups have been interchanged is shown in (**25**). Although the chemical shift values of the C-11 and C-13 resonances (δ_C 81,41S and 54,89D) militate against structure (**25**), the value of J(CH) of 144,2 Hz for C-13 favours either structure.

Figure 12. **Monatin (^{13}C,^{1}H) Connectivities as Observed in a Series of SPI Experiments**

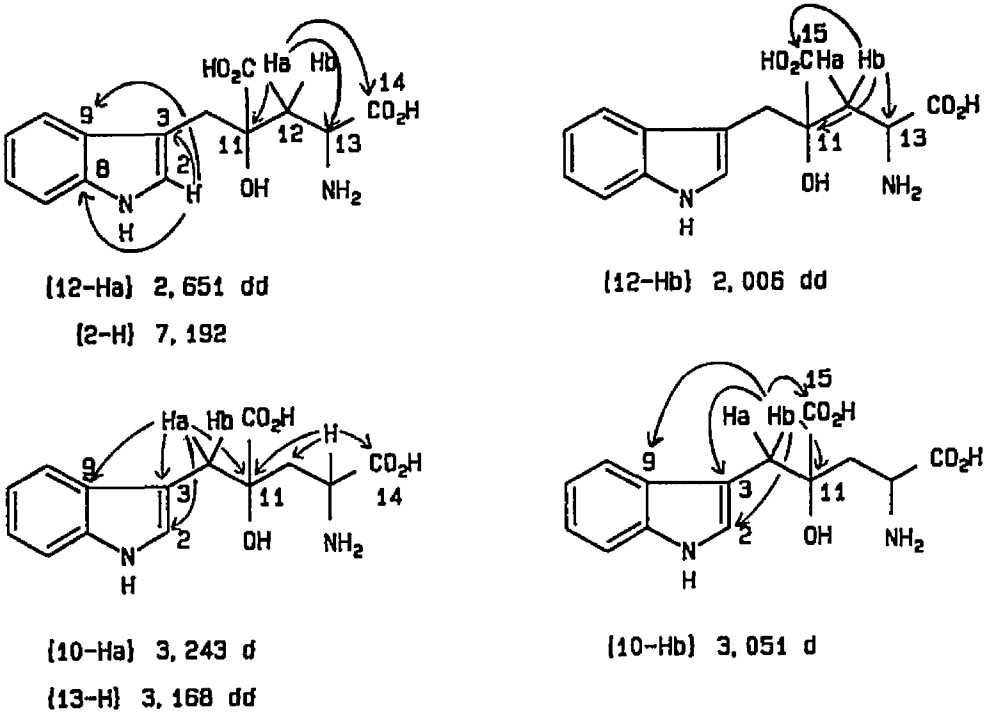
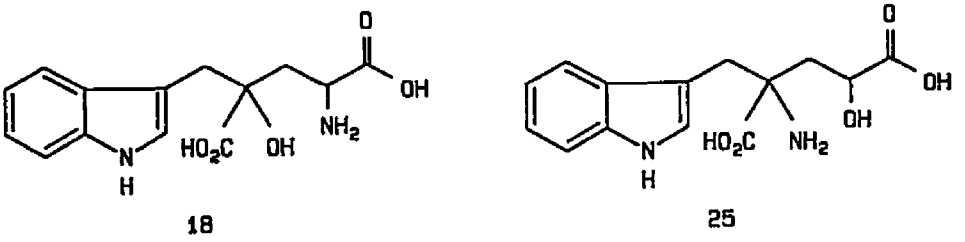


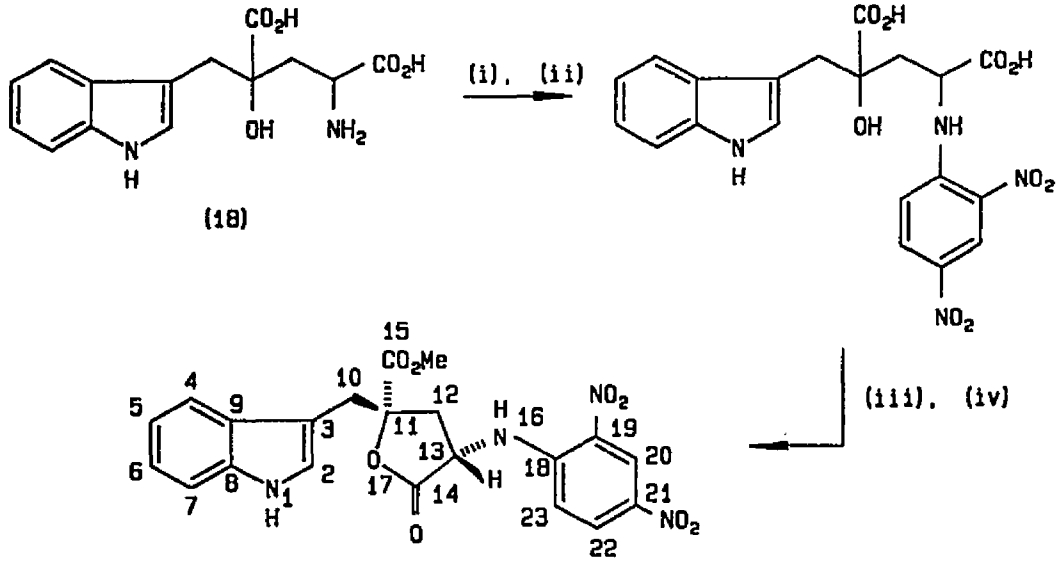
Figure 13. **Possible Monatin Structures Deduced from N.M.R. Data**



Additional evidence in favour of structure (**18**) for monatin was obtained in a number of ways. The ^{1}H chemical shift of the α-proton of α-amino acids is pH dependent.[116] Under basic conditions this proton resonance shifts upfield and under acidic conditions it moves downfield compared to its position under neutral conditions. Although the other protons

behave in a similar fashion the effect is less pronounced. The data for selected protons are collated in Table 6. From Table 6 it can be seen that the X part of the AB-X system *i.e.* 13-H of structure (**18**) appears at δ_H 3,529 (dd, *J* 11,6 and 1,8 Hz) but moves upfield by 0,781 p.p.m. at pH 13 and downfield by 0,345 at pH 1. These pH dependent shifts can not be accommodated by the alternative structure (**25**) and indicated the structure (**18**) for monatin. Although 12-H_a and 12-H_b also experience substantial upfield shifts at pH 13 this is due to the cumulative effect of the two carboxylic acid moieties.

Scheme 14



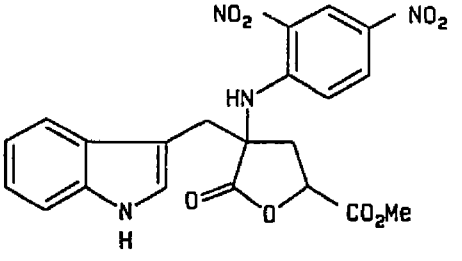
Reagents: (i) $NaHCO_3$ (ii) 2,4-dinitrofluorobenzene (iii) HOAc (iv) Diazomethane.

A derivative of monatin was prepared by reaction of the amino group of monatin with Sanger's reagent (2,4-dinitrofluorobenzene) to give the *N*-2,4-dinitrophenyl (DNP) derivative. The product was converted to the dimethyl ester by treatment with an ethereal solution of diazomethane and purified by column chromatography. Elemental analysis as well as accurate mass determination of the molecular ion at *m/e* 454 gave the molecular formula as $C_{21}H_{18}N_4O_8$. This molecular formula as well as the ^1H and ^{13}C

n.m.r. spectra (see later) militate against the presence of two methyl ester groups in the product. These results can be rationalized by the involvement of the hydroxy group in the formation of a lactone ring by reaction with either the C-11 or the C-14 carbomethoxy group, respectively, depending on the proposed structures (**18**) and (**25**) for monatin.

Table 6 pH Dependence of Chemical Shifts of Monatin in D_2O

Proton Condition	10-H_a	10-H_b	12-H_a	12-H_b	13-H
Neutral	3.243	3,051	2,651	2,006	3,618
pH 1	3,245	3,084	2,618	2,083	3,967
δ	0,002	0,033	0,030	0,077	0,345
pH 13	3.077	2.907	2,237	1,597	2,837
δ	0,166	0,144	0,414	0,409	0,781



(27)

The ^1H and ^{13}C n.m.r. data of the derivative are collated in Table 7. The resonances of a single methyl ester group appear at δ_H 3,798 s and δ_C 53,21Q in the n.m.r. spectra. The 1,2,4-oriented protons of the 2,4-dinitrophenyl moiety appear at δ_H 8,981d (*J* 2,5 Hz), 8,088dd (*J* 2,6 and 9,4 Hz) and 8,743d (*J* 9,4 Hz).* The C-12 methylene protons appear at 2,775dd (*J* 10,3 and 13,2 Hz) and 3,195dd (*J* 9,2 and 13,1 Hz). It is of interest to note that the 16-NH proton appears as a doublet (*J* 9,5 Hz) at δ_H 6,371 as a result of vicinal

coupling with the C-13 proton. This result confirms the structure of monatin as **(18)** as no such coupling is possible in the derivative **(27)** derived from the alternative structure **(25)** for monatin.

*As the assignments have not been correlated with specific carbon resonances the assignment of the ^{13}C resonances of this moiety must be regarded as tentative.

2.5 CONFIGURATION OF MONATIN

The X-ray crystallographic study of both the free acid form of monatin **(18)** (see earlier) and the methyl ester of the 2,4-DNP derivative **(26)** proved disappointing in that the reflections measured were weak, due to the small size and quality of the crystals, and the refinement of the data was thus only possible to an *R*-factor of 30-35%. The resultant structures generated do show the skeletal atoms of the two compounds and in each case, the relative stereochemistry at the two chiral centres.(see Figure 14).

Figure 14. Skeletal Structure of Monatin (18) and its Ester 2,4-DNP Derivative (26)

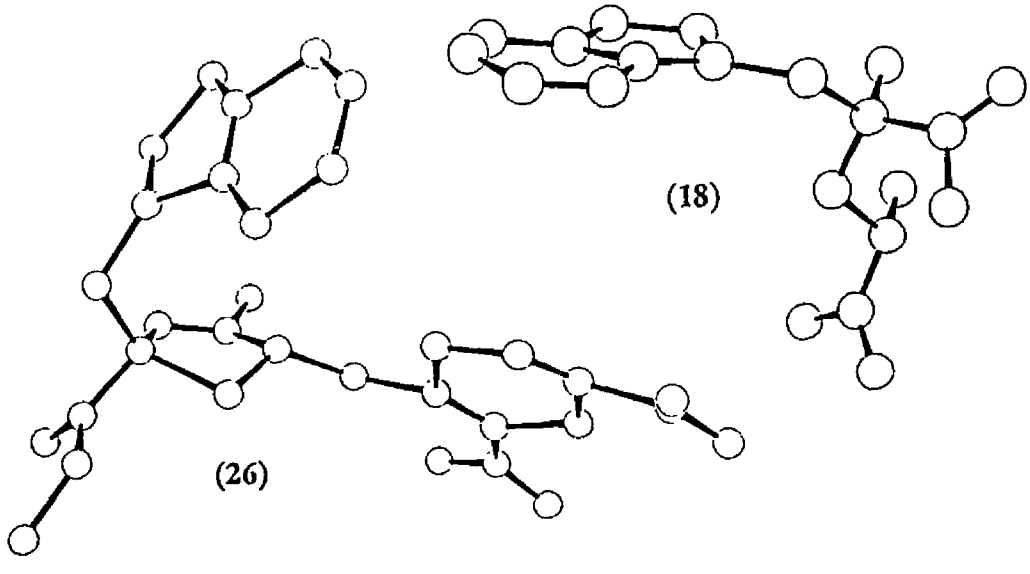


Table 7 N.m.r. Data for the Methyl Ester of the Monatin 2,4-DNP Derivative[a] (26)

Atom	δ_C	J (CH)/Hz	δ_H	J (HH)/Hz
2	126,42D	168,8	7,371s	-
3	108,12S	-	-	-
4	120,43D	151,2	7,804d	7,9
5	119,49D	158,0	7,180dd	7,9; 8,0
6	122,84D	159,1	7,235dd	8,0; 8,1
7	112,72D	160,1	7,502d	8,1
8	137,59S	-	-	-
9	132,08S	-	-	-
10	33,58T	129,8	3,513d	14,9
			3,599d	14,9
11	86,01S	-	-	-
12	36,79T	138,1	2,775dd	10,3; 13,2
			3,195dd	9,2; 13,1
13	53,22D	148,3	3,916m	not resolved
14	171,47S[b]	-	-	-
15	172,94S[b]	-	-	-
16	-	-	6,371d	9,5
18	126,26S[c]	-	-	-
19	128,95S[c]	-	-	-
20	124,17D	172,2	8,918d	2,6
21	128,28S[c]	-	-	-
22	130,64D	169,5	8,088dd	2,5; 9,4
23	115,27D	167,0	8,743d	9,4
OMe	53,21Q	115.7	3,798s	-

[a] Recorded in acetone-d_6

[b,c] May be interchanged

The available data on monatin do not allow us to unambiguously determine the absolute configuration of the two chiral centres. Circumstantial evidence, however indicates that monatin has the (2*S*,4*S*)-4-hydroxy-4-(indolylmethyl) glutamic acid structure as depicted in (**18**).

The two diastereomers of 4-hydroxy-4-methylglutamic acid (**28** and **29**), isolated from natural sources[117] have the following specific rotations:

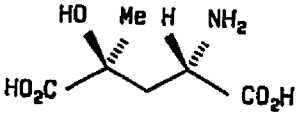
(**28**) (2*S*,4*S*): $[\alpha]_D$ - 30,3° (H_2O); - 8,3° (0,2M HCl)

(**29**) (2*S*,4*R*): $[\alpha]_D$ + 0,5° (H_2O); + 23,2° (0,2M HCl),

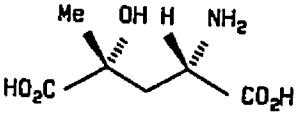
whereas for monatin salt the following specific rotations were determined:

$[\alpha]_D$ - 49,6° (*c* 1,00 in H_2O)

$[\alpha]_D$ - 7,6° (*c* 1,00 in 1M HCl)



(28)



(29)

This finding is in line with the so-called Clough-Lutz-Jirgenson's rule[118] which states that acidification of an aqueous solution of an L-amino acid [e.g. (**28**) and (**29**)] results in a more possitive value for the molecular rotation whereas this value for a D-amino acid becomes more negative upon acidification. These observations can be formulated as follows:

L-amino acid: $[M]_{acid} - [M]_{water} = + [M]$

D-amino acid: $[M]_{acid} - [M]_{water} = - [M]$

This rule was validated by the results obtained for more than 60 amino acids.

2.6 SWEETNESS OF MONATIN

The sweetness of monatin salt was determined[119] by a taste panel trained according to recognised procedures.[120] The recognition threshold value was used and the sweetness determined relative to sucrose. Monatin salt was used since the free acid is very insoluble in almost every solvent.

Monatin's relative sweetness value was determined as 1100-1200 times greater than that of sucrose and it exhibits only a very slight liquorice aftertaste. This finding makes monatin a very attractive proposition as a high intensity sweetener.

CHAPTER 3

SYNTHETIC ENDEAVOURS TOWARDS THE TOTAL SYNTHESIS OF MONATIN

3.1 INTRODUCTION

The synthetic organic chemist has marvelled at and envied Nature's tools in producing the awesome array of molecular structures with such efficacy and stereochemical precision, in a timespan that defies human reference terms. In attempting to emulate Nature, the practice of organic synthesis, particularly as it relates to natural products, continues to flourish and expand as it accepts new challenges of conquering increasingly complex synthetic targets.

The ability of chemists to synthesize organic compounds has evolved through a number of discernible stages over the past 160 years in a progression which is marked historically by ascendence to a new and qualitative higher level of sophistication of roughly 20 years. One clear sign of this advance is the achievement in a particular period of syntheses which are conceptually more complex and technically well beyond those realized in the preceeding stage.

In conceptualizing the synthesis of organic molecules the modern organic chemist is guided by the innovative strategies introduced by Corey which constitute the basis of retrosynthetic analyses.[121]

Retrosynthetic or antithetic analysis is a problem-solving technique for transforming the structure of a synthetic target molecule into a sequence of progressively simpler structures along a pathway which ultimately leads to simple or commercially available starting materials for a chemical synthesis. The transformation of a molecule into a synthetic precursor is accomplished by the application of a 'transform', the exact reverse

of a synthetic reaction, to a target structure. In order for a transform to operate on a target structure to generate a synthetic predecessor the requisite structural subunit or retron for that transform must be present in the target. The retron for the Diels-Alder reaction for instance, is a six-membered ring containing a π-bond.

The general strategies which are available for devising retrosynthetic pathways fall into several classes.

3.1.1 TRANSFORM-BASED STRATEGIES

This strategy involves the application of a powerful simplifying transform (or a tactical combination of transforms) to a synthetic target molecule containing certain appropriate keying features. Once again the Diels-Alder transform serves as an example: The retron for the Diels-Alder reaction is a six-membered ring containing a π-bond and it is this structural subunit which represents the minimal keying element for the transform function.

Usually the retron required for the application of a transform is not present in a complex synthetic target molecule and a number of antithetic steps are needed to establish it.

3.1.2 STRUCTURE-GOAL STRATEGIES

This is the oldest and most traditional of all synthetic strategies and has long been the dominant strategy in organic synthesis. In this instance the basic structure of the target molecule is already present and only a limited substructural region is modified, for example to accommodate the retron for a simplifying transform.

3.1.3 TOPOLOGICAL STRATEGIES

This procedure involves the identification of one or more individual bond disconnections as strategic. Topological strategies may also lead to the recognition of a key substructure for disassembly or the use of rearrangement transforms.

3.1.4 STEREOCHEMICAL STRATEGIES

Stereochemical strategies are general strategies which remove chiral centres and spatial relationships under stereocontrol. Such stereocontrol can arise from transform-mechanism control or substrate control. In the former case the retron for a particular transform contains certain critical stereochemical information (absolute or relative) at one or more stereocentres. Stereochemical strategies may also dictate the retention of certain stereocentre(s) during the retrosynthetic processing.

3.1.5 FUNCTIONAL GROUP BASED STRATEGIES

The retrosynthetic reduction of molecular complexity involving functional groups encompasses many important general problem solving tactics. Single or pairs of groups (and the interconnecting atom path) can key directly the disconnection of a target skeleton to form simpler molecules.

Functional group interchange is a commonly used tactic for generating from a target molecule retrons which allow the application of simplifying transforms. Functional groups frequently key transforms which stereoselectively remove stereocentres or break topologically strategic bond(s) so that in effect they play a role in the other types of retrosynthetic strategies.

3.1.6 OTHER STRATEGIES

Certain other strategies may result from the requirements of a particular problem, for example economic requirements or a requirement that several related target structures be synthesized from a common intermediate. A target molecule or retron which resists retrosynthetic simplification may require that new chemical methodology be developed for a synthesis and thus suggest a new line of research.

The basic ideas of retrosynthetic analysis become much more tangible when illustrated by specific applications.

The problem of the synthesis of a deceptively simple compound such as monatin (**18**) provides a useful testing ground. Retrosynthetic analyses of the monatin structure was carried out by the concurrent use of several different strategies to guide the antithetic search for appropriate starting molecules for molecular construction.

3.2 THE SYNTHETIC ENDEAVOURS TOWARDS MONATIN

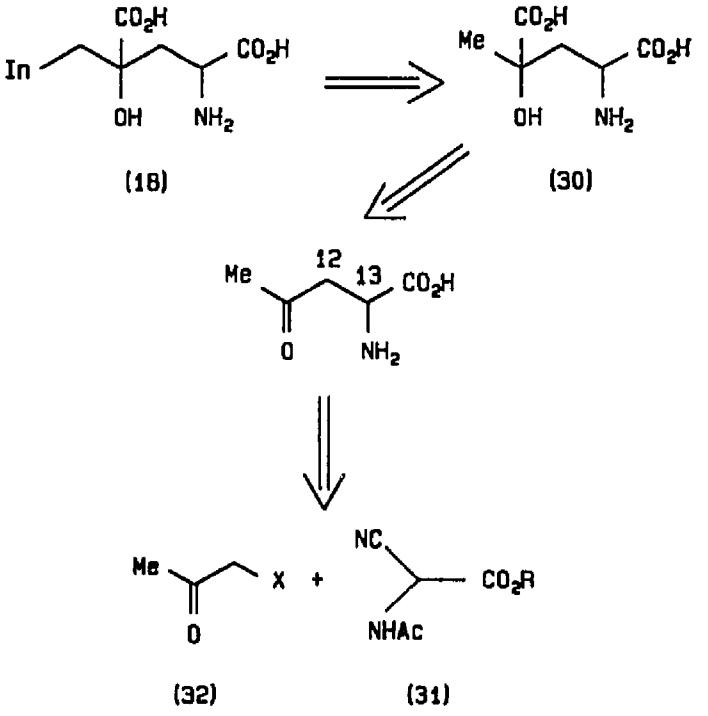
Several different retrosynthetic analyses of the monatin structure were carried out in order to arrive at a synthetic procedure which would allow for the synthesis of both monatin and a number of analogues where the indolyl moiety is replaced for example by a phenyl or a *p*-hydroxyphenyl group. A number of synthetic approaches were subsequently investigated and these are outlined in the following pages.

3.2.1 APPROACH 1

The retrosynthetic interchange of the indolyl group in the monatin structure with a hydrogen atom as shown in Scheme 15 provides an intermediate (**30**) which in a synthetic direction, would lead to an analogue of monatin. The subsequent retrosynthetic strategy

involves a functional group interchange which replaces the hydroxy-acid moiety at C-11 with a carbonyl group. The strategic disconnection of the C-12--C-13 bond generates two synthons which must meet certain requirements if they are to be of use in a proposed synthesis.

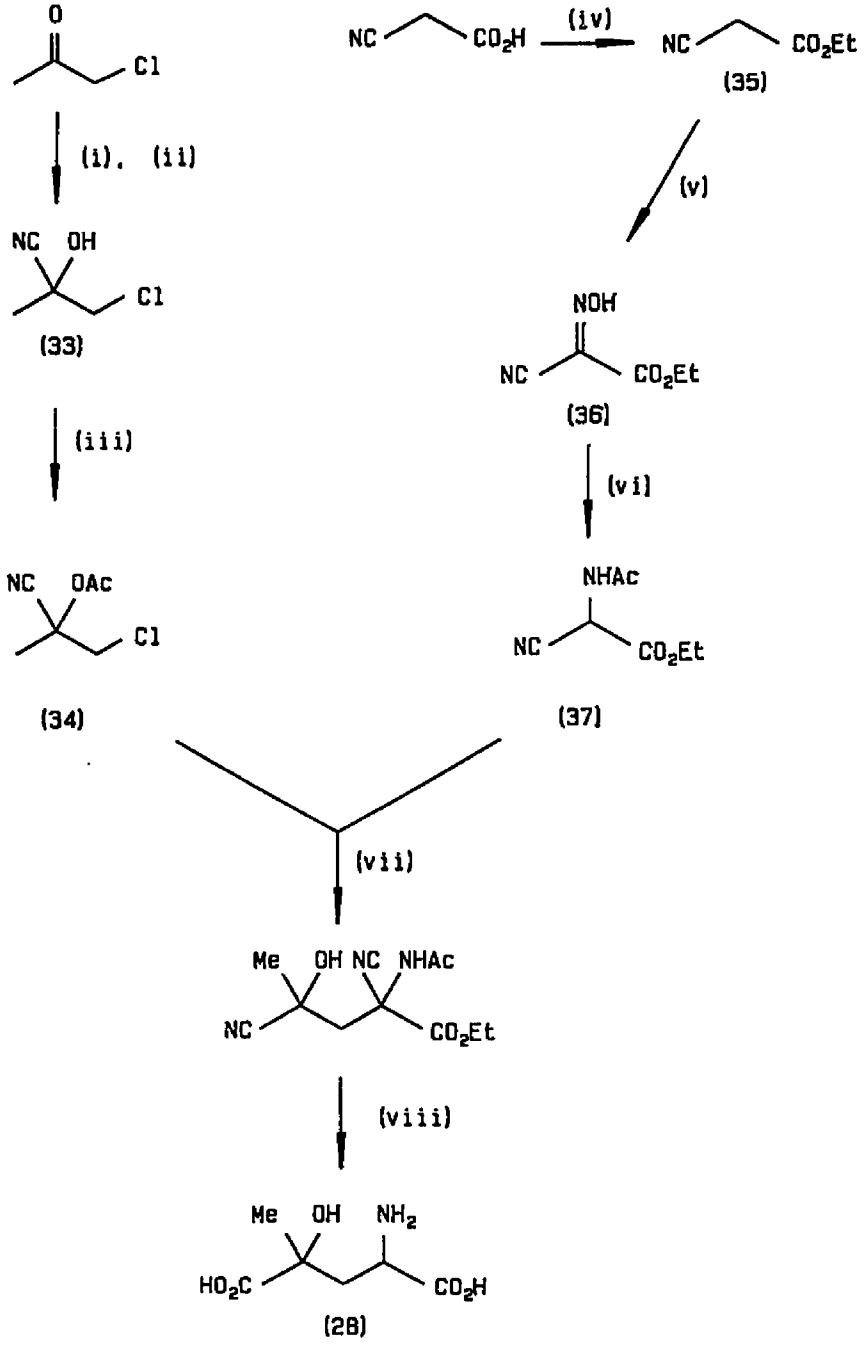
Scheme 15



Thus synthon **(31)** is an activated masked amino acid compound in which the functional groups are suitably protected. Carbon-carbon bond formation by nucleophilic attack of the anion generated by proton abstraction from synthon **(31)** necessitates the masking of the electrophilic character of the carbon atom of the carbonyl group as well as the presence of an appropriate leaving group in synthon **(32)**.

The synthesis of 4-hydroxy-4-methylglutamic acid **(28)** as shown in Scheme 16, is based on the approach by Kristensen.[122]

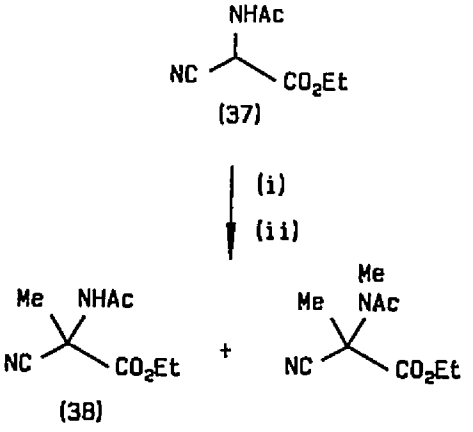
Scheme 16



Reagents: (i) $NaHSO_3$ (ii) NaCN (iii) Ac_2O (iv) $EtOH,H_2SO_4$ (v) $NaNO_2$,HOAc (vi) H_2,Pt on C,Ac_2O (vii) NaOEt (viii) 5M HCl reflux.

The synthesis of the protected chlorocyanohydrin (**34**), a masked hydroxy-acid equivalent and the activated protected amino acid synthon (**37**) was achieved by established literature procedures.[123-126] The subsequent carbon-carbon bond formation by nucleophilic attack on the cyanohydrin by the anion generated by treatment of (**37**) with sodium ethoxide, inexplicably failed and the protected amino acid synthon was recovered unchanged. Evidence for the formation of a carbanion on treatment of the protected amino acid synthon with sodium ethoxide was obtained by quenching the reaction with methyl iodide to give 2-acetamido-2-cyano-propionate (**38**) (33%) as well as a small amount of the *N*-methyl derivative (Scheme 17), the product of dimethylation.

Scheme 17

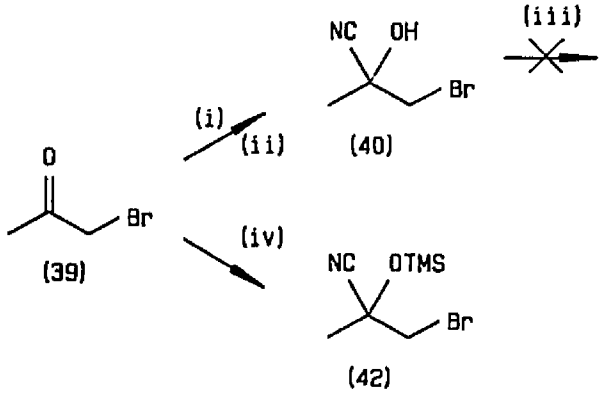


Reagents: (i) NaOEt (ii) MeI

It was felt that the use of a better leaving group, such as bromine, in the halocyanohydrin moiety would facilitate the carbon-carbon bond formation process. Initial attempts to synthesize the *O*-acetate derivative of bromoacetone cyanohydrin (**40**) were unsuccessful probably due to the compound's instabiblity under the reaction conditions. This problem was solved by combining the cyanohydrin formation and the subsequent protection of the hydroxy group into one step using cyanotrimethylsilane (**41**), a reagent readily prepared from chlorotrimethylsilane and potassium cyanide.[129] Thus, treatment of bromoacetone

with cyanotrimethylsilane resulted in the formation of the *O*-trimethylsilyl derivative **(42)**.

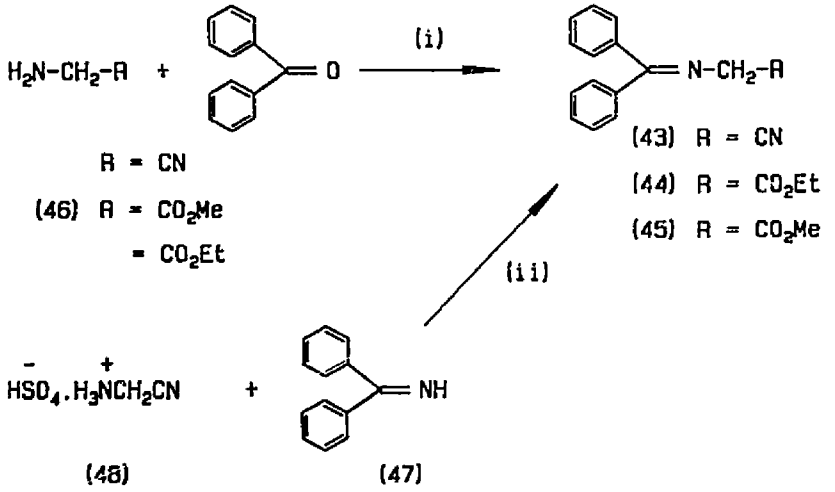
Scheme 18



Reagents: (i) $NaHSO_3$ (ii) NaCN (iii) Ac_2O (iv) Me_3SiCN, (41)

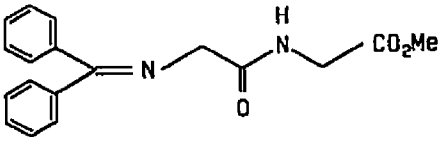
As a further elaboration of the work of Kristensen[122] it was decided to use protected amino acid synthons which would require less vigorous conditions for deprotection in the final steps of the synthesis. These compounds, *N*-diphenylketimine glycinonitrile **(43)**, ethyl *N*-diphenylketimine glycinate **(44)** and methyl *N*-diphenylketimine glycinate **(45)** are readily available by borontrifluoride catalysed reaction of benzophenone and glycinonitrile, ethyl glycinate or methyl glycinate **(46)** (Scheme 19).[130,131]

Scheme 19



Reagents: (i) BF_3/etherate, reflux (ii) Acetonitrile reflux.

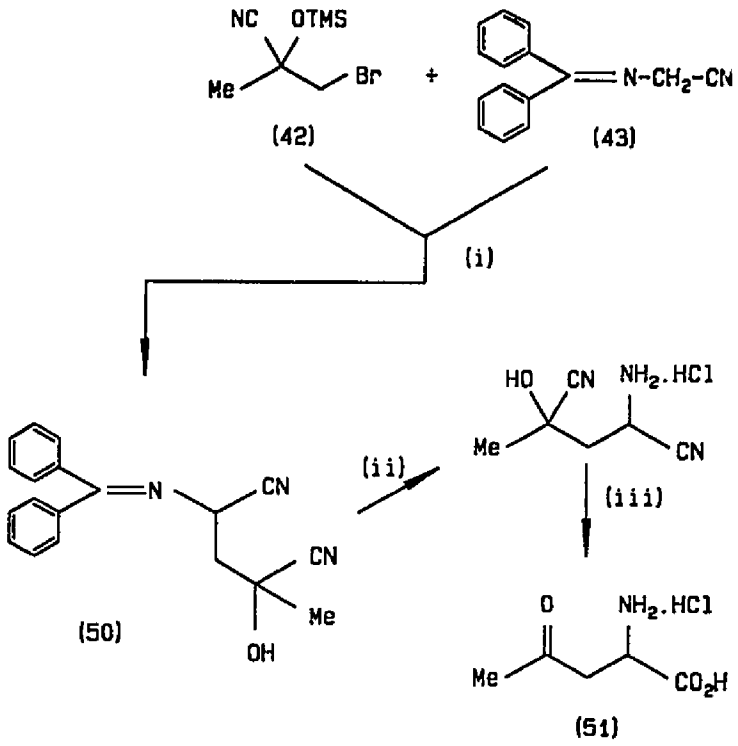
The removal of the excess of benzophenone used in the reactions proved to be a problem and an alternative approach involving a little-used transimination[133] reaction using diphenylketimine[134] (47) was employed. The reaction of diphenylketimine (47) formed by treatment of benzonitrile with phenylmagnesium bromide and the hydrochloride or sulphate[135] salt of glycinonitrile proceeded in average yield to give the required *N*-diphenylketimine glycinonitrile (43). Although both ethyl and methyl *N*-diphenylketimine glycinate (44 and 45, respectively) could be prepared in a similar transimination procedure[136] using the corresponding esters, the yield was lower and in the case of the methyl ester the product is involved in a dimerization reaction to form the protected glycylglycine derivative (49).



(49)

The availability of the *O*-trimethylsilyl derivative of the bromoacetonecyanohydrin synthon (42) and *N*-diphenylketimine glycinonitrile (43) enabled a study of the carbon-carbon bond formation step in the synthesis leading to 4-hydroxy-4-methylglutamic acid (28) (Scheme 20). The linking of the two synthons occurred in low yield by nucleophilic displacement of the bromine atom of (42) by the carbanion formed by abstraction of an acidic proton from the methylene group of (43) using lithium diisopropylamide. Subsequent acid hydrolysis of the ketimine and cyano groups of the product (50) occurred with concomitant loss of the trimethylsilyl protecting group to generate the unstable cyanohydrin which, under the reaction conditions, formed the 2-amino-4-oxo-pentanoic acid (51).

Scheme 20



Reagents: (i) LDA, HMPA-THF (ii) 1M HCl (iii) 5M HCl, reflux.

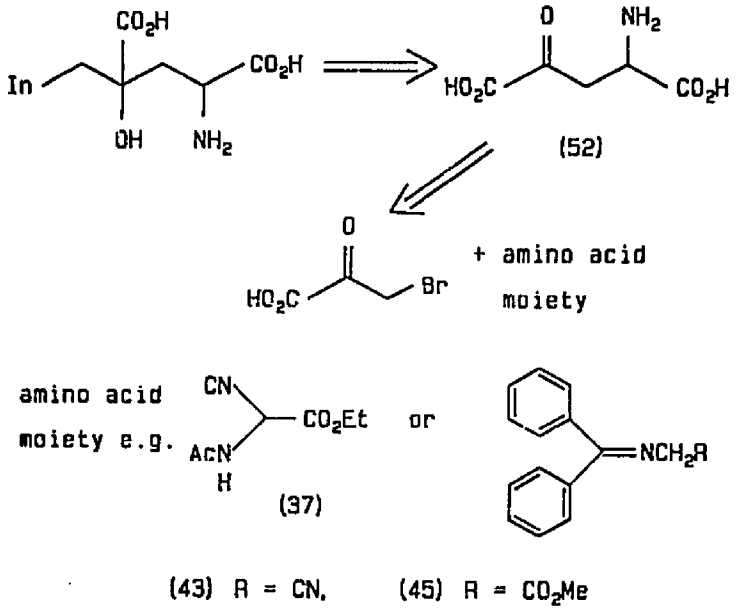
As a result of the repeated problems experienced with the carbon-carbon bond formation as well as the retro-cyanohydrin reaction, this approach towards the synthesis of 4-hydroxy-4-methylglutamic acid was abandoned.

3.2.2 APPROACH 2

The antithetic cleavage of the C-10--C-11 bond of monatin (**18**) as indicated in Scheme 21 generates a synthon, 4-oxo-glutamic acid (**52**), which on reaction with the appropriate Grignard reagents could be transformed into a number of monatin analogues. The disconnection of the C-12--C-13 carbon bond of (**52**) leads to the readily available bromopyruvic acid[137] and the protected and/or masked amino acid moieties such as (**37**),

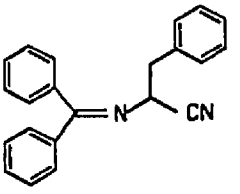
(43) and (45), (*cf* 3.2.1. Approach 1), as potential starting materials for a chemical synthesis.

Scheme 21



In the synthetic direction the procedure would entail the nucleophilic displacement of the bromine atom of bromopyruvic acid by the carbanion of a protected or masked amino acid moiety.

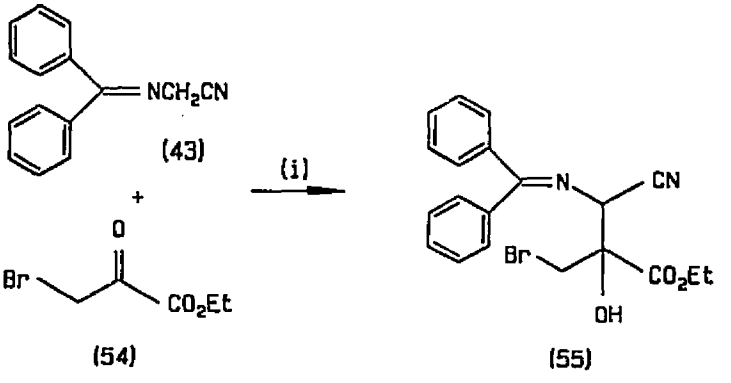
The feasibility of this synthetic approach was first tested in model studies using diphenylglycinonitrile ketimine (43) as the masked/protected amino acid moiety. Treatment of (43) with lithium diisopropylamide generated the carbanion which on reaction with benzylbromide and subsequent hydrolysis of the reaction product (53) leads to the formation of phenylalanine.



(53)

The same reaction sequence using ethyl bromopyruvate (54) failed. Although the carbanion could be generated using different bases such as lithium diisopropylamide, sodium hydride or sodium hydroxide solution under phase transfer conditions (triethylbenzylammonium chloride), the nucleophilic displacement reaction of the bromine atom from ethyl bromopyruvate does not occur. However when using the weaker base, potassium carbonate, nucleophilic attack occurs in poor yield at the keto carbonyl to yield the bromohydrin (55) (Scheme 22).

Scheme 22



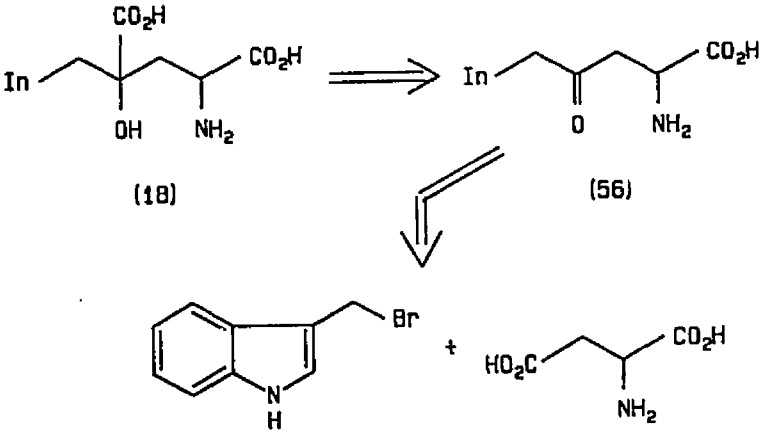
Reagents: (i) K_2CO_3/benzene, triethylbenzylammonium chloride

The above approach also failed using a different masked/protected amino acid moiety, ethyl acetamidocyanoacetate (37).

3.2.3 APPROACH 3

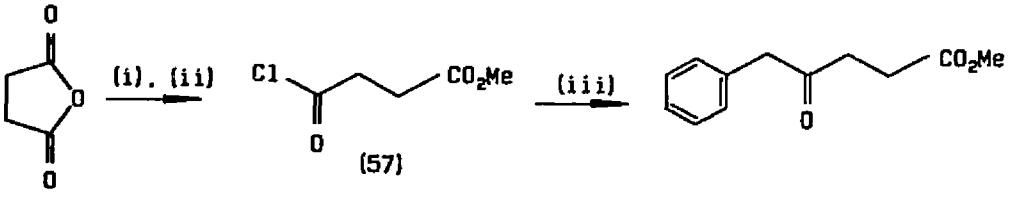
Application of a functional group based strategy for devising a retrosynthetic pathway for monatin involves the C-10 hydroxy- and carboxy-group functionalities and generates a synthetic target molecule containing a ketone function (**56**). The strategic disconnection of the C-4--C-5 bond identifies aspartic acid and 3-(bromomethyl)indole as synthetic targets.

Scheme 23



The proposed coupling of the two synthetic target molecules is based on palladium chemistry[138] and was initially investigated using simpler model compounds. Thus no difficulties were experienced in the reaction of methyl succinoyl chloride[139] (**57**) with benzyl bromide in the presence of $Pd(PPh_3)_2Cl_2$[140] and the expected product, methyl 4-oxo-5-phenylpentanoate, was formed in fair yield.

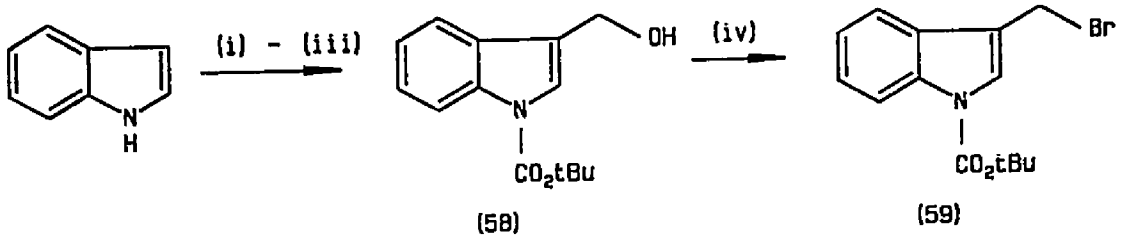
Scheme 24



Reagents: (i) 1 equivalent MeOH (ii) $SOCl_2$ (iii) $Pd(PPh_3)_2Cl_2$, Benzyl bromide.

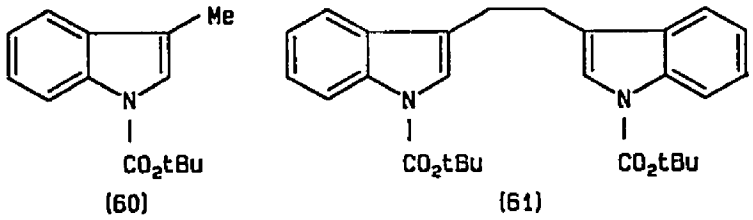
The protected 3-(bromomethyl)indole synthon (**59**) was prepared in a simple fourstep sequence from indole as shown in Scheme 25.

Scheme 25



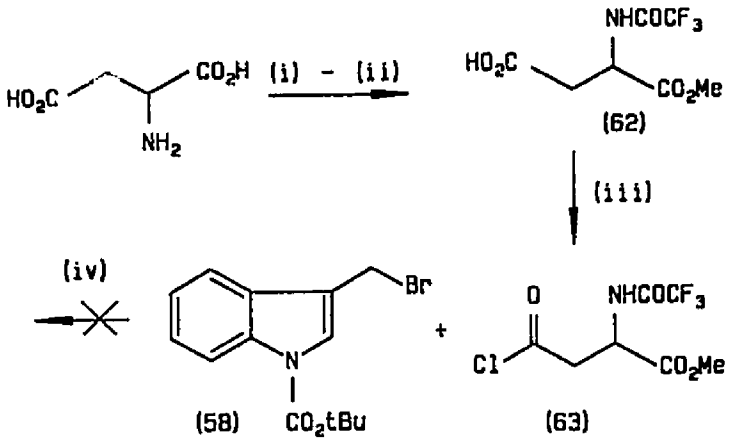
Reagents: (i) DMF, $POCl_3$ (ii) di-t-butyl-carbonate (iii) $NaBH_4$ (iv) Br_2, PPh_3.

The desired coupling reaction of (**59**) and the acid chloride of succinic acid monomethyl ester (**57**) in the presence of $Pd(PPh_3)_2Cl_2$ proved difficult and only two reduction products, *N*-t-Boc-skatole (**60**) (10% yield) and 1,2-di-(*N*-t-Boc-3-indolyl)ethane (**61**) (5% yield), were obtained.



Attempts to effect the palladium-mediated coupling between the protected 3-(bromomethyl)indole (**59**) and the protected acid chloride derived from aspartic acid (**63**) as shown in Scheme 26 were unsuccessful. No products were isolated and this approach towards the synthesis of monatin was abandoned.

Scheme 26

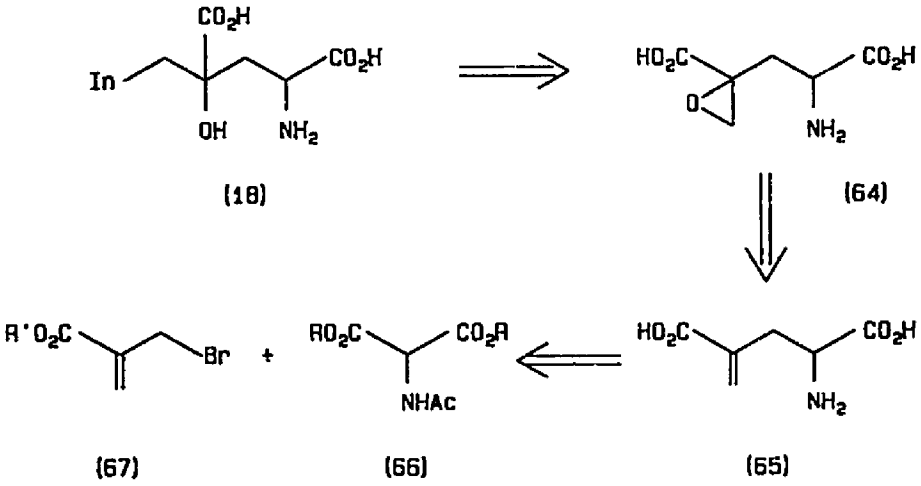


Reagents: (i) TFAA (ii) dry MeOH (iii) $SOCl_2$ (iv) $Pd(PPh_3)_2Cl_2$.

3.2.4 APPROACH 4

Retrosynthetic analysis of the monatin structure (**18**) was carried out by the concurrent use of several different strategies. In this fourth approach, depicted in Scheme 27, antithetic cleavage of the bond between the indole moiety and the multifunctional side-chain can be envisaged as leading to an epoxy amino acid (**64**) which is transformed by a functional group based strategy into 4-methylene-glutamic acid (**65**), a known amino acid.[144]

Scheme 27



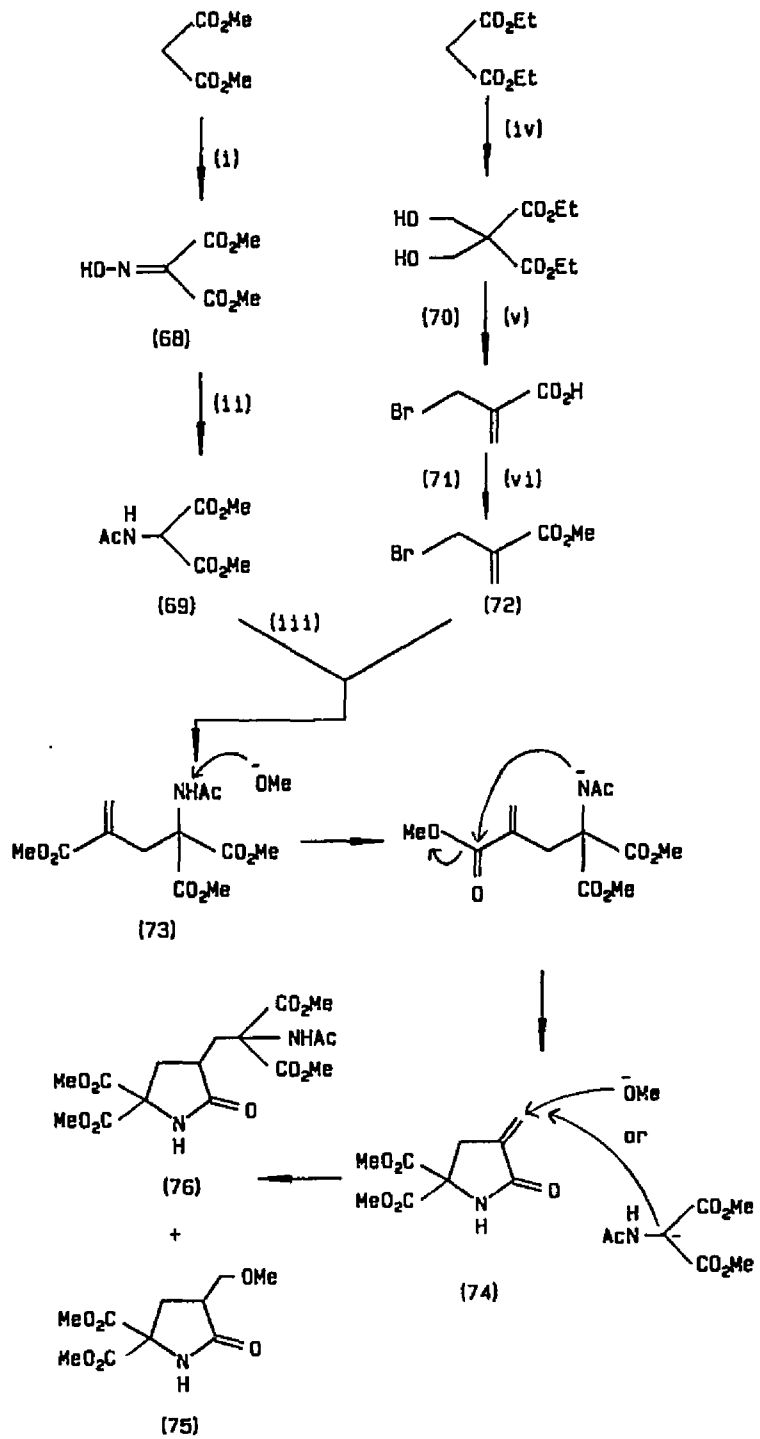
The reaction of the epoxide amino acid with appropriate Grignard reagents can only result in the formation of analogues of monatin e.g. the phenyl analogue, as 3-indole Grignard reagents are unknown.

The synthons (**66**) and (**67**) required for the synthesis of the 4-methyleneglutamic acid (**65**) are readily available from the diester of malonic acid.[145-147] The amino acid itself is the product of a nucleophilic displacement of the bromine atom of synthon (**67**) by the carbanion of synthon (**66**), produced by the treatment of (**66**) with sodium methoxide in methanol.

The addition sequence of the two synthons plays a pivotal role in the formation of the 4-methyleneglutamic acid structure. If the (bromomethyl)acrylic ester (**72**) is added dropwise to a solution of the carbanion produced from treatment of (**69**) with sodium methoxide, the desired S_N2' reaction does occur but the initially-formed product (**73**) is transformed into a number of unwanted by-products as a result of of various base catalysed addition reactions. The complete sequence of reactions is summarized in Scheme 28.

The carbanion of dimethyl acetamidomalonate (**69**) is expected to be in equilibrium with the methoxide ion in the methanol reaction mixture. Abstraction of the acidic amide proton from (**73**) by methoxide followed by nucleophilic attack on the terminal carbomethoxy group (lactam formation) and subsequent base hydrolysis of the amide functionality results in the formation of 4-methylene lactam (**74**). Michael addition of either methoxide or the anion derived from dimethyl acetamidomalonate (**69**) leads to the formation of an additional two by-products (**75**) and (**76**).

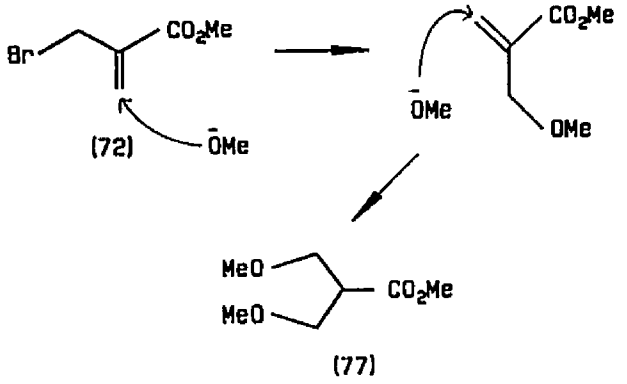
Scheme 28



Reagents: (i) $NaNO_2$, HOAc (ii) Zn, HOAc (iii) NaOMe, MeOH (iv) HCHO (v) aqueous HBr (vi) MeOH, TsOH.

The nucleophilic displacement of the bromide ion from (72) by methoxide, followed by Michael addition of methoxide to the intermediate ethoxy ester, constitutes the formation of a fourth by-product (77).

Scheme 29



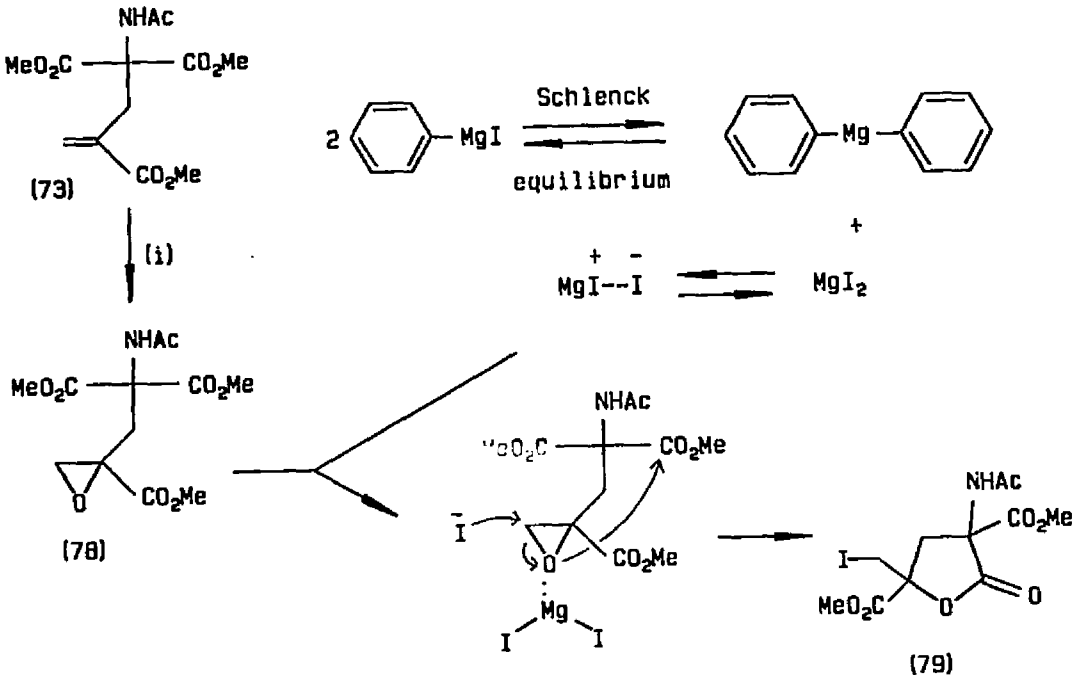
(77)

When the addition sequence was reversed *i.e.* dropwise addition of the anion of dimethyl acetamidomalonate (**69**) to a solution of the (bromomethyl)acrylate (**72**) the required protected putative 4-methyleneglutamic acid (**73**) was formed in moderate yield (40%).

Three procedures were investigated for the subsequent epoxidation of the double bond in (**73**). However neither *m*-chloroperbenzoic acid[148] nor hydrogen peroxide in the presence of sodium tungstate and phosphoric acid[149] was able to effect this transformation. Epoxidation of (**73**) did occur in low yield using hydrogen peroxide under alkaline conditions[150,151] (6M sodium hydroxide). However partial saponification of the esters of both the starting material and the product occured. In the course of the reaction workup (partitioning between chloroform and water) the sodium salt is transferred to the aqueous phase and subsequently lost. It was found that unreacted starting material could be recovered if the reaction was allowed to proceed until about 20% of the starting material had been converted to the epoxide (Scheme 29). The use of potassium carbonate, a weaker base, to circumvent the problem of saponification, was a failure as no epoxidation occurred under these conditions.

The conversion of the epoxide (78) into an analogue of monatin by reaction of an appropriate Grignard reagent, phenylmagnesium iodide, failed and instead an iodolactone (79) was obtained. The rationale behind the formation of (79) is the fact that Grignard reagents. e.g. phenylmagnesium iodide, exist as Schlenk equilibrium mixtures of the reagent on the one hand and diphenylmagnesium and magnesium iodide on the other hand (Scheme 30). The magnesium iodide acts as a Lewis acid and nucleophilic opening of the epoxide ring by iodide results in the formation of the lactone (79).

Scheme 30



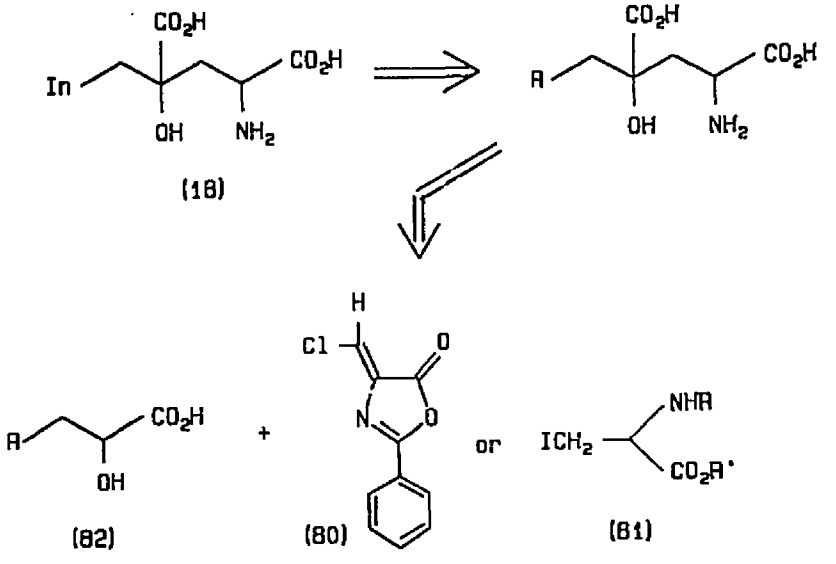
Reagent : (i) NaOH, H_2O_2

As a result of this undesirable reaction this approach was also abandoned.

3.2.5 APPROACH 5

The approaches towards the retrosynthetic analysis of the monatin structure which have been described so far have all concentrated on the cleavage of the C-10--C-11 bond. In the present approach attention is focused on the strategic disconnection of the C-11--C-12 bond which identifies an α-hydroxy- or α-keto-acid and once again a masked, protected amino acid as synthetic targets. The retrosynthetic interchange of the indolyl group with an alkyl group R, implies that analogues of monatin can be synthesized by the simple expedient of changing the nature of the R group of the α-hydroxy or α-keto-acid. The structural requirements for the amino acid synthetic target are satisfied by either of the two compounds (80) or (81) shown in Scheme 31.

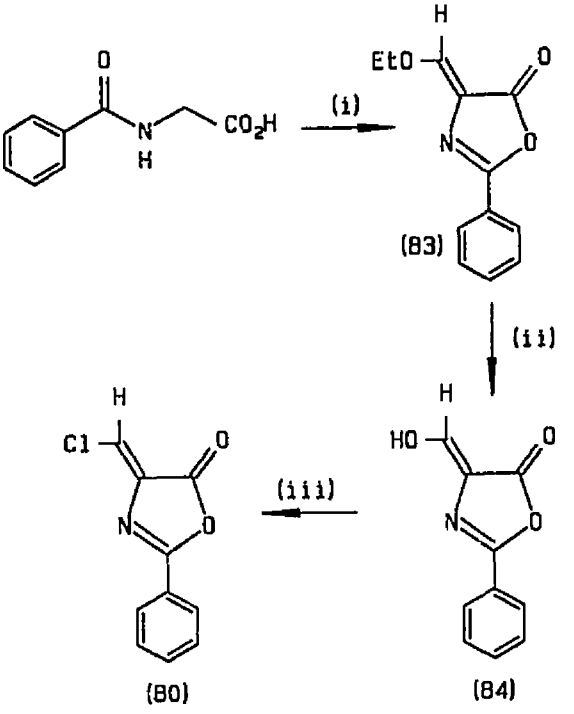
Scheme 31



Bergbreiter *et al.* has shown that lactic and mandelic acid esters can be α-alkylated by treatment of the ester with two equivalents of lithium diisopropylamide and quenching of the dianion with an appropriate alkyl halide.[152]

The azlactone (**80**) is readily prepared from hippuric acid.[153-156] (Scheme 32).

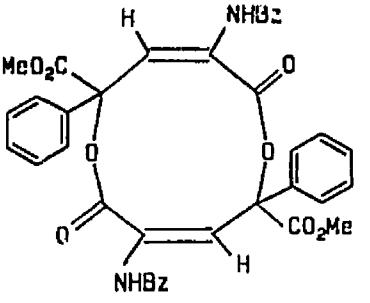
Scheme 32



Reagents: (i) Ethyl orthoformate (ii) HCl (iii) $SOCl_2$.

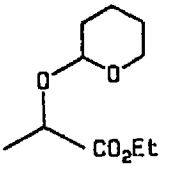
Initial attempts to quench the dianion of ethyl mandelate[157] (**85**) using this azlactone failed as nucleophilic substitution of the chlorine atom by diisopropylamine occurred. This result necessitated the use of lithium 2,2,6,6-tetramethylpiperidide, a compound which, as a result of steric constraints, acts as a non-nucleophilic base. In this manner the desired reaction does occur. However the lithium salt of the hydroxy function of the product reacts immediately with the azlactone carbonyl group of a second molecule to give a dimer which cyclizes to form the 10-membered bislactone (**86**).

It is evident that protection of the hydroxy functionality of the hydroxy ester synthon (**84**) is required in order to eliminate its involvement in unwanted side reactions.



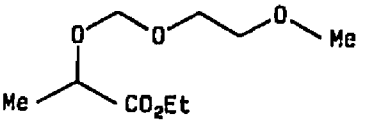
(86)

Protection of ethyl lactate as the *O*-tetrahydropyranyl (THP) ether (87) was achieved using pyridinium *p*-toluenesulphonate (PPTS) as catalyst[158] instead of the more commonly used catalysts such as hydrochloric, *p*-toluenesulphonic[159] or phosphoric acid. The use of the THP protecting group introduces an additional chiral centre into the molecule and the products are thus a mixture of diastereomers which would probably complicate matters in subsequent reactions.



(87)

An alternative protecting group which avoids the stereochemical complications of the THP-derivatives is the methoxyethoxymethyl (MEM) protecting group developed by Corey *et al.*[160] Treatment of ethyl lactate with methoxyethoxymethylchloride (88) and sodium hydride resulted in the formation of the *O*-MEM derivative (89) in moderate yield (49%).



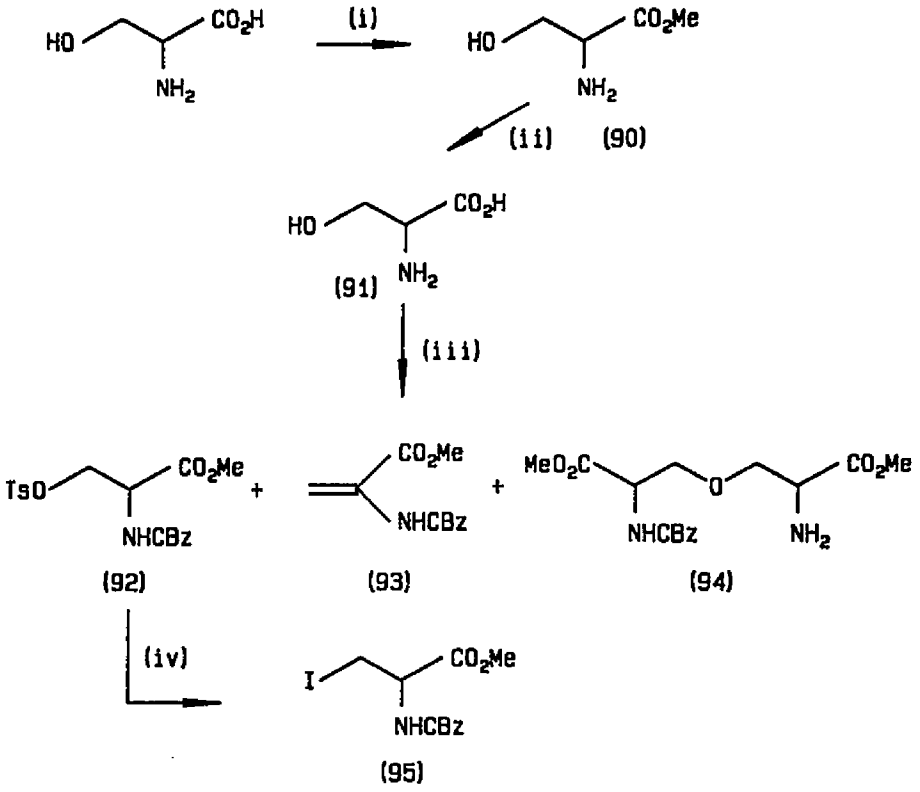
(89)

The generation of the carbanion of the protected ethyl lactate was investigated using three bases *viz.* sodium hydride, LDA and LTMP. The extent to which carbanion formation occurred was established by quenching the reaction mixture with deuterium oxide and monitoring the relevant resonance signals in the ^{1}H n.m.r. spectrum of the deuterated compound: the intensity of the methine proton resonance which appears as a quartet at δ_{II} 4,11 and the change of the methyl resonance at δ_{II} 1,78 from a doublet to a singlet.

In a typical procedure for deuterium exchange ethyl lactate was treated with LTMP at -78° C and the generated anion quenched with deuterium oxide. The n.m.r. spectrum of the product lacked any discernible signal at δ_{II} 4,11 and showed the methyl resonance as a singlet at δ_{II} 1,78.

At this stage the coupling of the protected hydroxy acid, ethyl 2-methoxy-ethoxymethoxypropanoate (**89**), with a protected amino acid moiety, methyl *N*-Cbz-iodoalanine (**95**), could be investigated. Methyl *N*-Cbz-*O*-*p*-toluenesulphonylserine (**92**) was prepared from serine as indicated in Scheme 31. The major product formed during the tosylation step turned out to be the 2,3-didehydro amino acid analogue (**93**) (40%) and not the *O*-tosyl derivative (**92**) (21%). Treatment of (**92**) with potassium iodide in acetone resulted in the nucleophilic displacement of the tosyl group by iodide to give methyl *N*-Cbz-iodoalanine (**95**).

Scheme 33

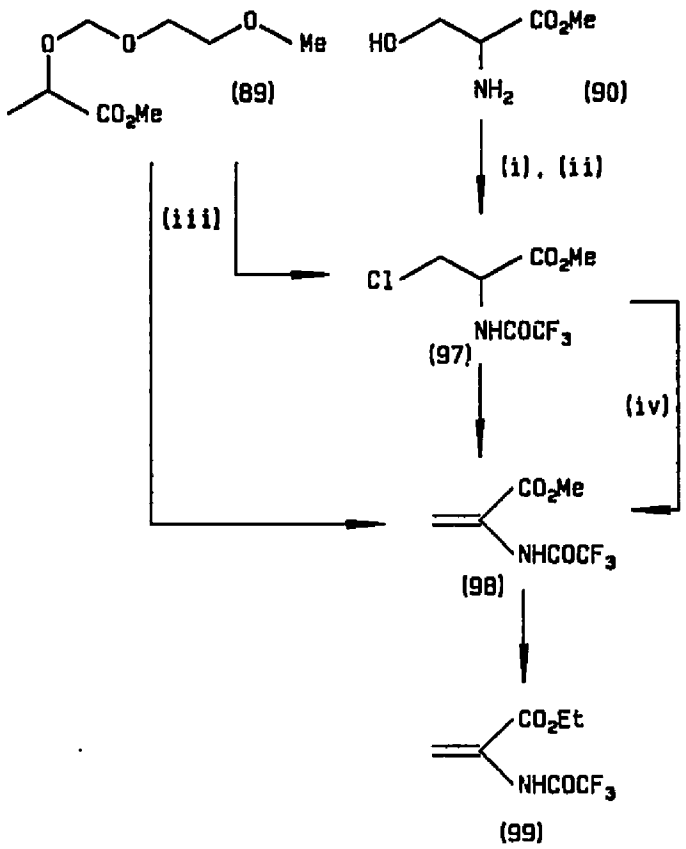


Reagents: (i) MeOH, HCl (ii) benzylchloroformate (iii) tosylchloride (iv) KI, acetone.

However when the carbanion of (**89**) was treated with the protected iodoalanine (**95**) bond formation did not occur and instead the 2,3-didehydro amino acid (**93**) was isolated. Reaction of the 2,3-didehydro amino acid (**93**) with the carbanion of (**89**) resulted in the formation of the ethyl ester derivative of (**93**) as a result of transesterification.

The introduction of the stronger electron-withdrawing *N*-trifluoroacetyl group in methyl chloroalanine as indicated in Scheme 34, had little effect on the carbon-carbon bond formation with the anion of (**89**).

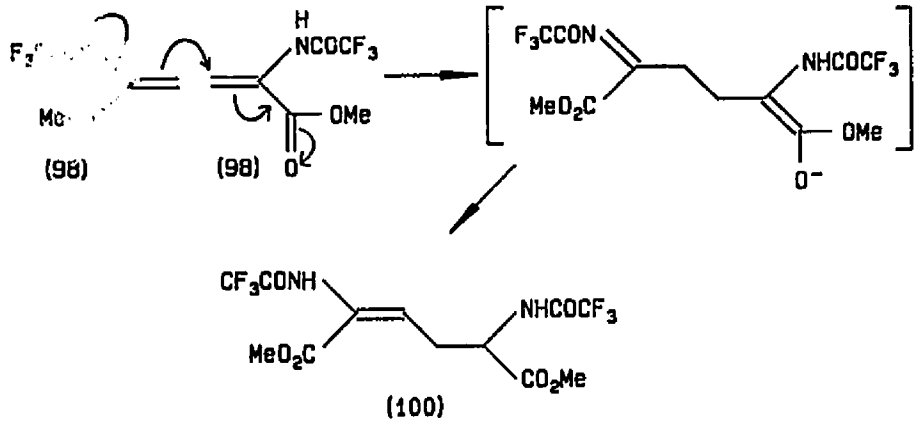
Scheme 34



Reagents: (i) PCl_5 (ii) TFAA (iii) NaH (iv) Quinoline.

Once again dehydrohalogenation occurred to give the 2,3-didehydro amino acid **(98)**. Addition of methyl *N*-trifluoro-acetyl-2,3-didehydroalanine **(98)** to a solution of the carbanion of **(89)** results in abstraction of the amide proton and subsequent Michael reaction with a second molecule of **(98)** to give the Michael addition product **(100)**.

Scheme 35



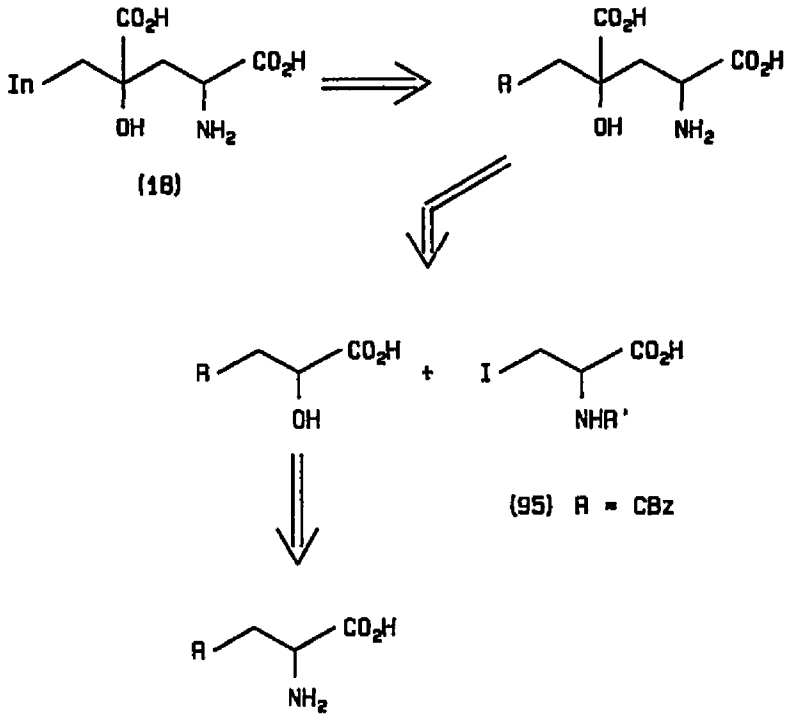
At this stage the hydroxy acid approach was terminated.

3.2.6 APPROACH 6

The retrosynthetic analysis of the previous approach led to the identification of an α-hydroxy- or α-keto-acid as a synthetic target for a monatin-type molecule. An additional functional group transformation identifies α-amino acids as the starting material for the synthesis of monatin and its analogues. The sequence of the analysis is shown in Scheme 36.

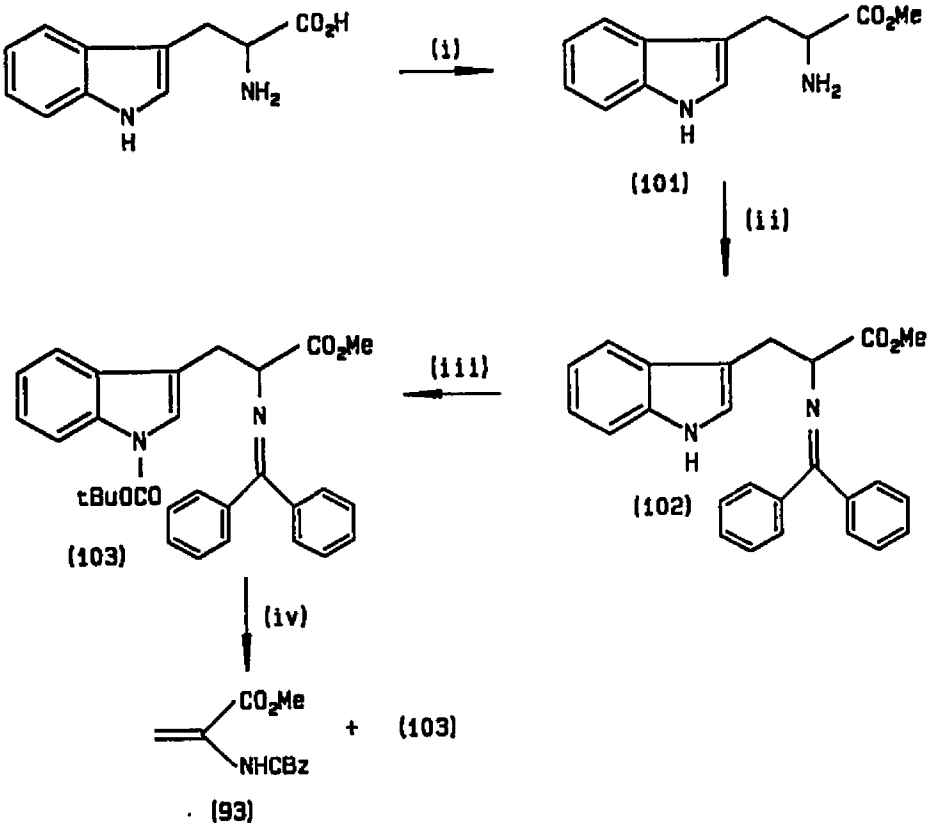
The use of tryptophan as starting material to generate the hydroxy acid moiety would lead to the formation of monatin. The key strategy of this approach involves the α-alkylation of tryptophan. The feasibility of this approach has been demonstrated by Braña *et al.*[166] by quenching the anion of the benzaldimine of methyl tryptophan, generated by treatment of this compound with LDA, with various alkyl halides such as methyl iodide, ethyl iodide and benzyl iodide.

Scheme 36



In the present approach (see Scheme 37) the amino group of tryptophan was protected as the more stable diphenylketimine derivative and in addition the indole nitrogen was protected using the t-butyloxycarbonyl group. α-Alkylation of the protected tryptophan **(103)** was initiated by generating the C-2 carbanion using LDA. The subsequent carbon-carbon bond formation using the protected iodoalanine **(95)** failed and, as was the case in the previous approach, dehydrohalogenation of the substrate occurred to give the 2,3-didehydro amino acid derivative **(93)**.

Scheme 37

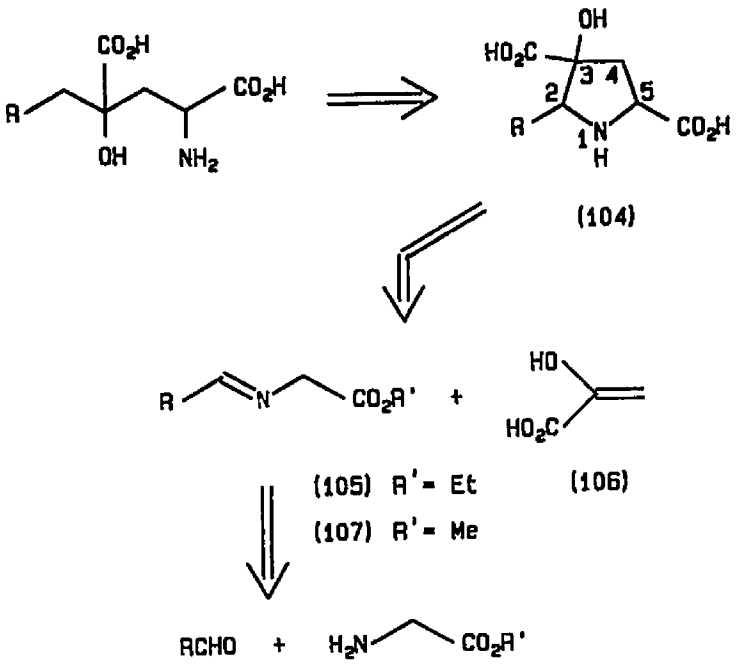


Reagents: (i) MeOH, HCl (ii) diphenylketimine (iii) di-tert-butylcarbonate (iv) lithium diisopropylamide, (95).

3.2.7 APPROACH 7

In the preceding approaches towards the synthesis of monatin the retrosynthetic analyses of the molecule involved the identification of specific strategic bond disconnections. In the present approach as outlined in Scheme 38 the retrosynthetic interchange of the indolyl group with an R group would allow once again the synthesis of a number of analogues of monatin. The general monatin-type structure is subsequently converted via an antithetic bond formation step into a substituted pyrrolidine structure which posesses the keying elements for a 1,3-dipolar cycloaddition transform. Application of this transform identifies a protected glycine moiety (105) and the α-hydroxy acid (106) as starting materials for the synthesis.

Scheme 38



In the synthetic direction the proposed pathway is critically dependent on the 1,3-dipolar cycloaddition and the subsequent cleavage of the C-2 nitrogen bond of the pyrrolidine ring in **(104)**.

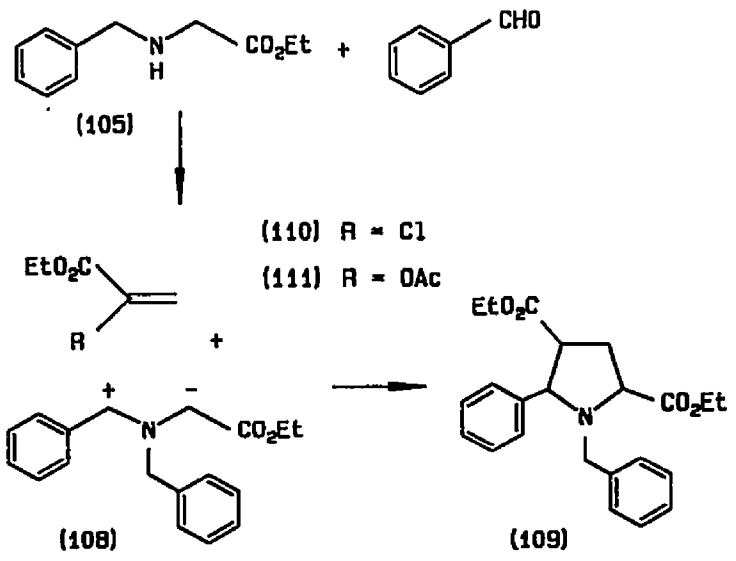
The synthesis of pyrrolidines and indolizidines by way of 1,3-dipolar cycloaddition reactions has been investigated by Joucla *et al.*[167] In practice benzaldehyde, appropriately substituted alkenes and α-amino esters were reacted in refluxing benzene or toluene to give 1,3-dipolar cycloaddition products.

The feasibility of this approach for the synthesis of monatin was first investigated using ethyl *N*-benzylglycinate[168] **(105)** and an unsubstituted acrylic acid synthon, ethyl acrylate, as the dipolarophile.

The resulting pyrrolidine (**109**) (Scheme 39) lacks the C-3 hydroxy group necessary for the eventual generation of the monatin type side-chain. The introduction of this functionality requires the use of 2-substituted acrylic acids such as methyl 2-chloro- (**110**)[169] or 2-acetoxy-acrylate (**111**)[170] as dipolarophiles in the 1,3-cycloaddition reaction with the glycine synthon (**107**).

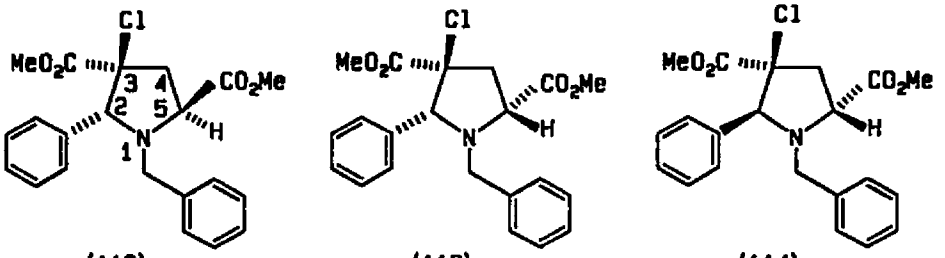
Methyl 2-chloroacrylate (**110**) is readily prepared by addition of chlorine to the double bond of methyl acrylate followed by dehydrochlorination of the methyl 2,3-dichloro-propionate under basic conditions.[169]

Scheme 39



The second synthon, methyl *N*-benzylglycinate (**107**) (see Table 10 in Chapter 4), is usually prepared in poor yield (25%) from methyl chloroacetate and benzylamine.[168] An alternative route, which entails the formation of the benzaldimine of methyl glycinate and the reduction of the carbon-nitrogen sp^2 bond using sodium borohydride, proceeds in fair (53%) yield.

The actual cycloaddition reaction which proceeded as indicated in Scheme 39 followed the method as described by Joucla *et al.*[167], using equivalent amounts of the reagents methyl α-chloroacrylate (**110**), methyl *N*-benzylglycinate (**107**) and benzaldehyde.



Three racemic compounds (**112**), (**113**) and (**114**), in the ratio 15:2:3 were isolated from the reaction mixture. The elemental analysis of each compound satisfied the empirical formula $C_{21}H_{22}ClNO_4$. The e.i. mass spectrum showed the typical molecular ion cluster at *m/e* 387/389 due to the presence of the ^{35}Cl and ^{37}Cl isotopes. The structure and relative configuration of these diastereomers was established by single crystal *X*-ray crystallographic analysis of the pyrrolidine (**114**) and ^1H n.O.e. n.m.r. studies of each diastereomer. Compound (**114**) crystallized from diethyl ether-hexane as monoclinic crystals, m.p. 111-112°C, space group $P2_1$, a = 11,709(2), b = 5,938(2), c = 14,074(2) Å, ß = 92,36° and Z = 2. A perspective view of the crystal is shown in Figure 15.

Figure 15. Perspective View of the Crystal Structure of Compound (114)

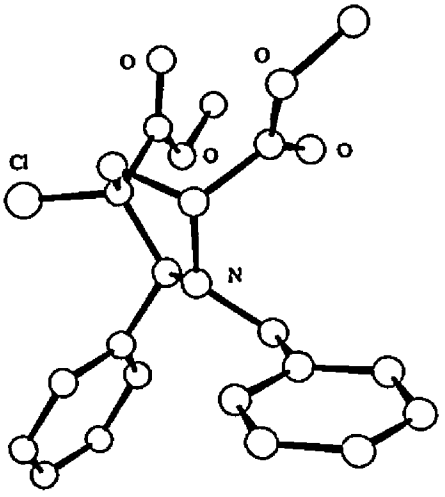


Table 7: **^{1}H N.M.R. Data for the Pyrrolidine Diastereomers (112), (113) and (114).**

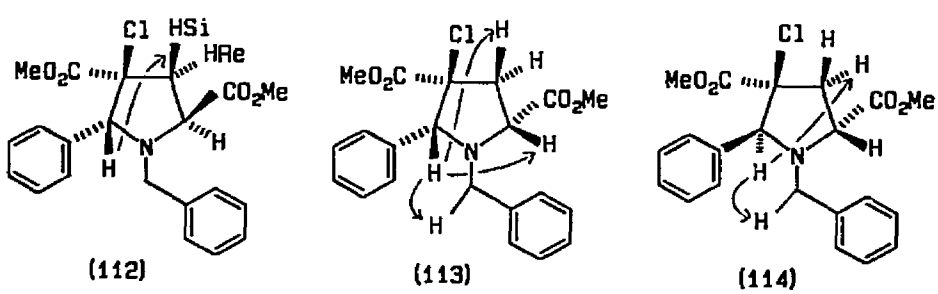
Compound	Atom no	δ_H	J (HH) Hz
	5	3,99dd	9,6; 2,0
	4	2,49dd	15,8; 2,0
		3,44dd	15,8; 9,6
112	2	4,80s	
	benzylic	3,80s	
	C-5 CO_2Me	3,70s	
	C-3 CO_2Me	3,19s	
	5	3,99dd	6,1; 10,3
	4	2,41dd	13,7; 6,1
		3,09dd	13,7; 10,3
113	2	4,43s	
	benzylic	3,90d	13,6
		4,00d	13,6
	C-5 CO_2Me	3,60s	
	C-3 CO_2Me	3,25s	
	5	3,90dd	8,3; 2,9
	4	2,70dd	13,7; 8,3
		3,15dd	13,7; 2,9
114	2	5,01s	
	benzylic	3,55d	13,5
		3,80d	13,5
	C-5 CO_2Me	3,81s	
	C-3 CO_2Me	3,64s	

The relevant ^{1}H n.m.r. data for (**114**) are collated in Table 7. The methylene protons of the *N*-benzyl group form an AB spin system (*J* 13,5 Hz) and resonate at δ_H 3,55 and 3,80, respectively whereas the benzylic methine proton, 2-H, appears as a singlet at δ_H 5,01. The signals at δ_H 2,70dd (*J* 13,7, 8,3 Hz) and 3,14dd (*J* 13,7, 2,9 Hz) are assigned to the C-4 prochiral diastereotopic methylene protons which together with the C-5 proton [δ_H 3,90 (*J* 8,3, 2,9 Hz)] form a three-spin ABX system. The protons of the two methyl ester groups appear as singlets at δ_H 3,81 and 3,64.

The assignment of the δ_H 3,15 resonance to the 4*Re* proton followed from the n.O.e. observed for this proton upon irradiation of the 2-H resonance in a homonuclear ^{1}H-{^{1}H} n.O.e. experiment (see Figure 16). The fact that an n.O.e. is observed for one of the benzylic protons (δ_H 3,55) but not for the C-5 proton confirms the relative configuration as obtained from the *X*-ray study: the C-2 phenyl group is *trans* to the C-5 carbomethoxy group.

The assignment of the protons of the carbomethoxy groups is based on the two- and three-bond (H,C) connectivity pattern determined in ^{13}C-{^{1}H}SPI experiments. Irradiation of the C-2 proton transitions in a SPI experiment affected *inter alia* the carbonyl carbon resonance at δ_C 170,27S. The same resonance was also affected upon irradiation of the methyl ester protons at δ_H 3,81 whereas irradiation at δ_H 3,64 affected the δ_C 173,17S resonance.

Figure 16



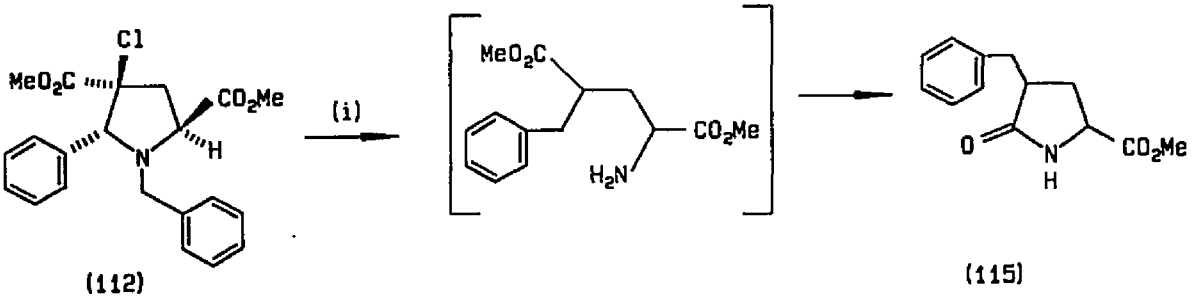
The relative configuration of the diastereomeric compounds (**112**) and (**113**) was deduced from the proton chemical shift values and the results obtained by irradiation of the C-2 proton in homonuclear ^{1}H-{^{1}H} n.O.e. experiments. In the case of compound (**112**) an n.O.e. was observed for the 4*Si* proton which resonates at δ_{II} 2,49, but not for the C-5 proton. The chemical shift of the 4*Si* proton indicates that it is located *cis* with respect to the chlorine atom. As a consequence the C-2 phenyl group is *cis* orientated to the C-3 carbomethoxy group. This orientation explains the chemical shift value of δ_{II} 3,19 for the methyl protons of the C-3 carbomethoxy group. The upfield shift is the result of diamagnetic shielding by the phenyl ring. The assignment of the resonances of the protons of the carbomethoxy group is based once again on the two- and three-bond (C,H) connectivity pattern established by SPI experiments. The results indicate that the carbonyl carbon atom which resonates at δ_C 169,04S is three-bonds removed from both the C-2 proton (δ_{II} 3,80) and the methyl ester protons which resonate at δ_{II} 3,19. Irradiation of the δ_{II} 3,70 methyl ester protons affected the resonance at δ_C 173,38S (C-5 carbomethoxy group).

In the case of the diastereomer (**113**) n.O.e.s are observed between 2-H and both the C-5 proton and the 4*Si* proton which resonates at δ_{II} 2,41. The chemical shift value of the 4*Si* proton is indicative of its *cis* orientation with respect to the C-3 chlorine atom. The chemical shift value of the methyl protons of the C-3 carbomethoxy group (δ_{II} 3,25) indicates that this group and the C-2 phenyl ring have the *cis* orientation.

Upon standing it was found that compound (**112**), the kinetic product of the reaction, is slowly transformed into the diastereomeric pyrrolidine (**114**) through epimerization of the C-2 and C-5 chiral centres.

The subsequent steps in the synthesis towards the phenyl analogue of monatin require the cleavage of the C-2-carbon-nitrogen bond and the replacement of the chlorine substituent with a hydroxy group. The removal of the benzyl protecting group and the reductive cleavage of the C-2 carbon-nitrogen bond was performed by catalytic hydrogenation at 100°C and 5 atm H_2 pressure using palladium on carbon as catalyst. A serious drawback of the procedure is the loss of the chlorine substituent and the formation of a 2-pyrrolidone (**115**), ν_{max} 1755 (ester CO) and 1697 (lactam CO) cm^{-1}, as a result of nucleophilic attack of the newly-formed amino group on the C-3 carbomethoxy moiety (Scheme 40).

Scheme 40

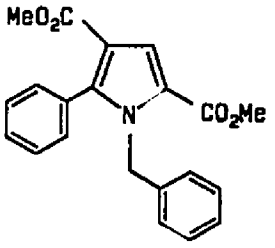


(112) (115)

Reagents: (i) H_2 (5 atm), Pd/C, 100°C, MeOH.

This result indicated that substitution of the chlorine substituent by either a hydroxy or an acetoxy group should precede the reductive ring cleavage step. Several procedures to effect the displacement of the chlorine substituent compound (**112**) were attempted but as was to be expected of chlorine on a tertiary substituted carbon atom all were unsuccessful. No reaction occurred upon refluxing compound (**112**) (a) in anhydrous dimethyl-formamide with sodium acetate, (b) in aqueous dioxane (1:1 v/v) solution with silver nitrate or (c) by irradiation of an aqueous dioxane (1:1 v/v) solution at 254 nm. Treatment of an acetic anhydride-acetic acid (1:3 v/v) solution of (**112**) with zinc powder resulted in dehydro-halogenation and formation of a substituted pyrrole (**116**). The

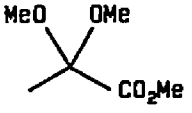
formation of the pyrrole was deduced from the mass spectrum which exhibited the molecular ion at *m/e* 349. The ^{1}H n.m.r. spectrum showed the C-4 pyrrole proton at δ_H 7,60.



(116)

As efforts to replace the chlorine atom in the pyrrolidine **(112)** with a hydroxy group were unsuccessful attention was turned to the use of 2-acetoxyacrylate as a more suitable dipolarophile. Methyl 2-acetoxyacrylate[170] **(111)** is readily prepared (in 52% yield) by the trapping of the enol form of methyl pyruvate with acetic anhydride and a catalytic amount of dimethylaminopyridine to give **(111)**.

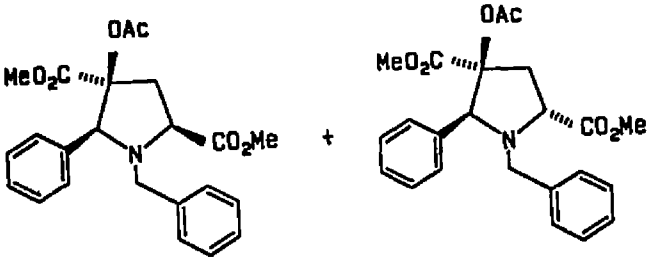
The use of anhydrous methanol as well as the rigorous removal of the water formed during the esterification of pyruvic acid should be avoided as ketalisation of the keto carbonyl group to give compound **(117)**[171] causes a significant drop in the yield of the desired methyl ester.



(117)

The cycloaddition reaction using methyl 2-acetoxyacrylate **(111)** as the dipolarophile was executed using benzaldehyde and methyl *N*-benzylglycinate **(107)** in refluxing toluene. The cycloaddition reaction gave a mixture of two inseparable diastereomers **(118)** as was

evident from the four methyl ester resonances at δ_{II} 3,50, 3,68, 3,72 and 3,74 as well as two acetoxy resonances at δ_{II} 1,45 and 1,48 in the ^1H n.m.r. spectrum. Since these methyl ester signals are in the region where methyl esters normally resonate (δ_{II} 3,5-3,9) it was deduced that the C-2 phenyl group and the C-3 carbomethoxy group for both compounds are *trans* relative to each other. However, the C-3 acetoxy groups resonate at δ_{II} 1,45 and 1,48 respectively, which is much higher upfield than the region of δ_{II} 2,0-2.1, the usual chemical shift of acetates. The position of this chemical shift can be explained by the diamagnetic shielding effect that the C-2 phenyl groups have on their respective acetoxy groups which is only possible if these groups are *cis* orientated. This leaves the C-5 carbomethoxy as the only other centre where the stereochemistry of the two compounds in the mixture (**118**) could differ.



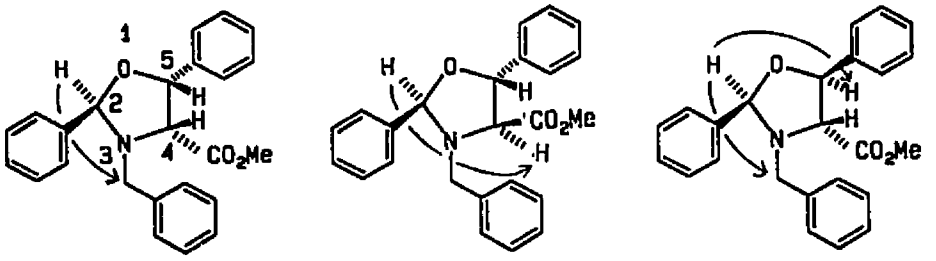
(**118**)

Reductive hydrogenolysis of the diastereomeric mixture (**118**) using 10% palladium on carbon at 100°C and 5 atm H_2 pressure results only in *N*-debenzylation. No cleavage of the C-2--N bond occurred. The use of zinc in acetic acid or sodium in liquid ammonia (Birch reduction) was even less effective and only starting material was isolated.

The 2-acetoxyacrylate dipolarophile (**111**) proved to be less reactive than 2-chloroacrylate (**110**) in the 1,3-cyclo-addition reaction. As a result the unreacted benzaldehyde in the reaction mixture acted as a competitive dipolarophile and three diastereomeric oxazolines (**119**), (**120**) and (**121**) were isolated as low yield by-products

in a ratio of 2:9:3. The elemental analysis of all three compounds agreed with the empirical formula $C_{23}H_{21}NO_3$ and the e.i. mass spectrum showed the molecular ion at *m/e* 359.

Figure 17. N.O.e. Connectivity Pattern for Oxazolines (119), (120) and (121) Established by Irradiation of the C-2 Proton



(119) (120) (121)

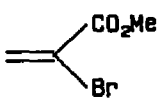
The relevant ^1H n.m.r. data for the three diastereomeric oxazolines are collated in Table 8. The chemical shifts of the methyl ester protons were once again used as a diagnostic indicator of the relative configuration of the C-4 and C-5 substituents.

Irradiation of the C-2 proton in a homonuclear ^1H-{^1H} n.O.e. experiment established the remainder of the stereochemical relationships in each of the three diastereomers. The results of the n.O.e. studies are shown in Figure 17.

In order to avoid the competing reaction of the benzaldehyde with the dipole generated from benzaldehyde and methyl *N*-benzylglycinate (**107**), the *N*-unprotected benzaldimine of methyl glycinate (**46**) was used in the 1,3-cycloaddition reaction with the three dipolarophiles methyl 2-chloro- (**110**), 2-acetoxy- (**111**) and 2-bromoacrylate (**122**).

Table 8 **N.M.R. Data of Oxazolines (114), (115) and (116)**

No	Proton	δ_H	J(HH)/Hz
	C-4 ester	3,17s	-
	H-4	4,09d	6,6
119	H-5	5,65d	6,6
	H-2	6,01s	-
	aromatic	7,1-7,8m	-
	C-4 ester	3,68s	-
	H-4	3,62d	8,1
120	H-5	5,36d	8,1
	H-2	5,49s	-
	aromatic	7,1-7,8m	-
	C-4 ester	3,64s	-
	H-4	3,87d	4,2
121	H-5	5,18d	4,2
	H-2	5,78s	-
	aromatic	7,1-7,8m	-

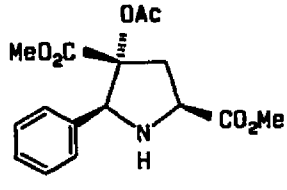


(122)

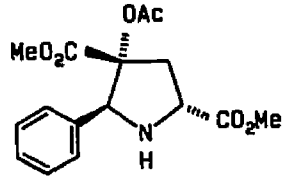
The use of 2-acetoxyacrylate **(111)** led in poor yield to the formation of a mixture of two diastereomeric pyrrolidines **(123)** and **(124)** (3% and 4,3% respectively) which both analysed for $C_{16}H_{19}NO_6$. This result is in agreement with the poor yields obtained previously using 2-acetoxyacrylate.

The chemical shift value of the protons of one of the carbomethoxy groups in both (**123**) (δ_H 3,17) and (**124**) (δ_H 3,17) is explained by diamagnetic shielding by the C-2 phenyl ring when the C-2 phenyl ring and the C-3 carbomethoxy group are *cis* orientated. As a consequence the two compounds must differ in their configuration at C-5. The singlet at δ_H 2,10 is assigned to the protons of the C-3 acetoxy group. The relative configuration at C-5 was not confirmed in either case by n.O.e. experiments.

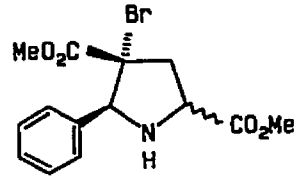
The corresponding bromo analogues (**125**) (a diastereomeric mixture which could not be separated and which proved to be too unstable for analysis) were formed when the 2-bromoacrylate synthon (**122**) was used. The *cis* relative configuration of the C-2 phenyl and C-3 carbomethoxy groups in both diastereomers was deduced once again from the proton chemical shift values of the two carbomethoxy groups. As a consequence these two diastereomers must also differ in their configuration at C-5.



(123)



(124)

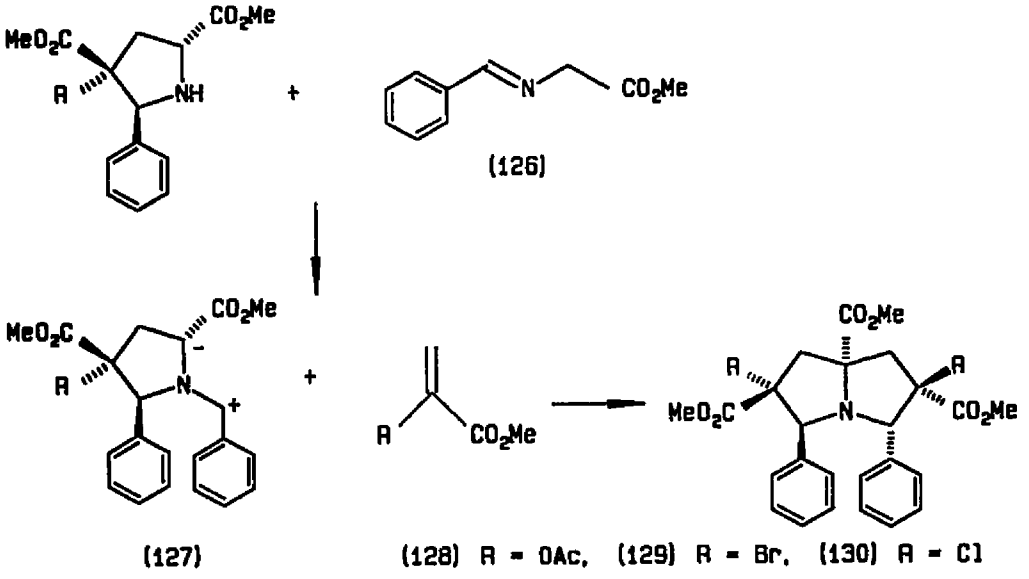


(125)

No substituted chloropyrrolidine was formed when methyl 2-chloroacrylate (**110**) was used as dipolarophile. However, in the reaction of each of the three different dipolarophiles with the unprotected dipole (**126**) an interesting by-product, a highly substituted pyrrolizidine e.g. (**128**), (**129**) and (**130**) differing only in the nature of the R group was formed.

The formation of the bicyclic pyrrolizidine derivatives can be readily explained by a phenomenon encountered earlier in Approach 1 (Scheme 19) namely the transimination[133] of glycinonitrile with diphenylketimine to give the diphenylketimineglycinonitrile. In the present instance the unreacted methyl *N*-benzaldimineglycinate (126) acts as the transiminating reagent for the newly-formed pyrrolidine which is thus converted to the dipole (127). The dipolar nature of (127) allows reaction with a second molecule of the appropriate dipolarophile, a 2-substituted acrylic ester, and leads to the formation of the pyrrolizidine structures (Scheme 41).

Scheme 41



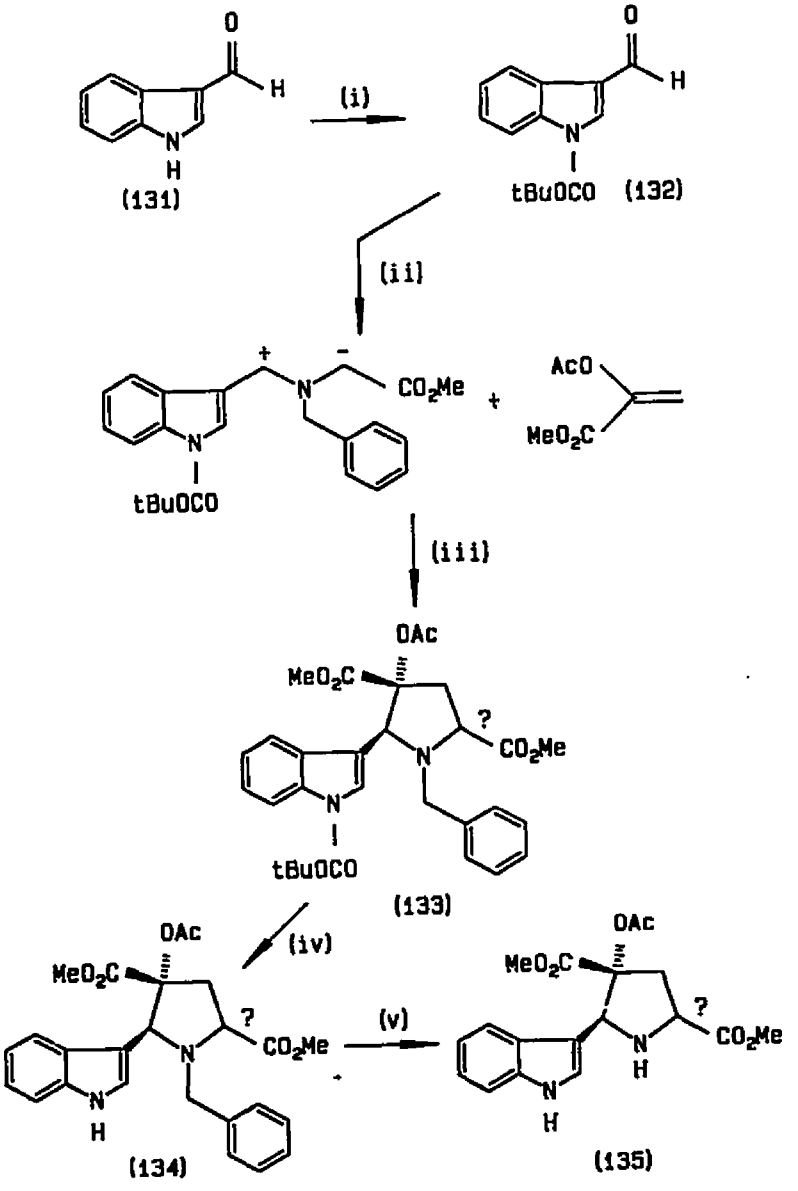
The relative stereochemistry of the chiral centres of these compounds was determined by a single crystal *X*-ray crystallographic study of the pyrrolizidine (129), which crystallized from chloroform-diisopropyl ether as monoclinic crystals, m.p. 182-183°C, space group $P2_1/n$, a = 11,542(2), b = 13,797(1), c = 17,863 Å, ß = 96,92(1)° and Z = 4 (see Figure 18).

In the next 1,3-cycloaddition experiment, use was once again made of methyl 2-acetoxyacrylate (111) as dipolarophile but in this instance the dipole was generated by

the reaction of methyl *N*-benzylglycinate (**107**) with *N*-t-Boc-indole-3-aldehyde[142] (**132**) which is readily available by N-t-Boc protection of 3-formylindole (**131**) (Scheme 42).

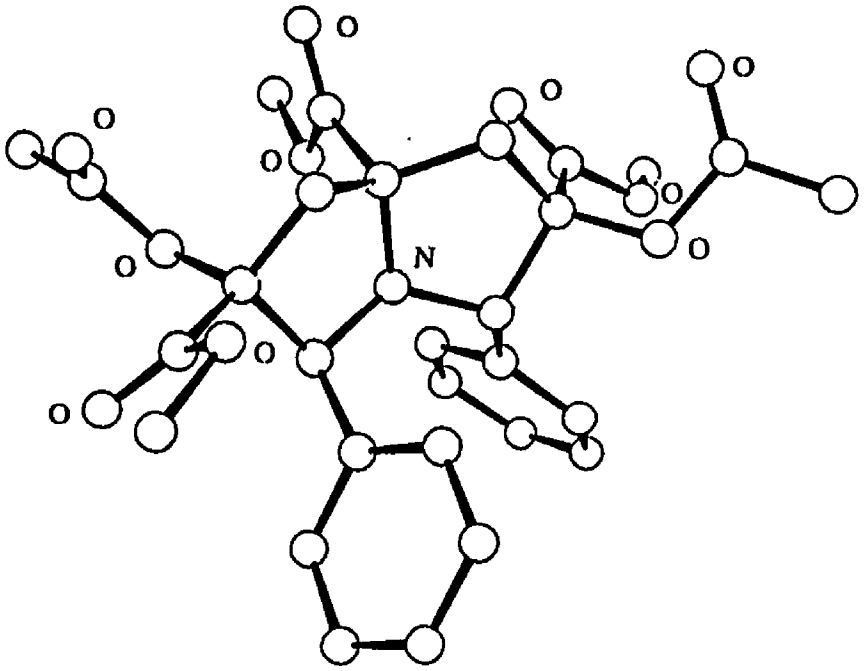
The cycloaddition reaction proceeded in low yield (18%) to give mainly the substituted pyrrolidine (**133**) which has all the requisite functionalities for conversion into the monatin structure.

Scheme 42



Reagents: (i) Di-tert-butyl carbonate, dimethylamino pyrridine (ii) (107) (iii) Reflux in toluene (iv) 180°C (v) H_2,Pd/C.

Figure 18. Perspective View of the Crystal Structure of Compound (129)



The elemental analysis of compound (**133**) agreed with the empirical formula $C_{30}H_{34}N_2O_8$. The e.i. mass spectrum showed the molecular ion at *m/e* 550 and the base peak at *m/e* 91 as a result of cleavage of the benzylic C-N bond. The ^1H n.m.r. spectrum was analysed as described earlier for the other pyrrolidines and indicates the presence of a *N*-benzyl moiety, two carbomethoxy groups, an acetoxy group, a CH_2-CH spin system (representing the C-4--C-5 unit of the pyrrolidine ring) and an isolated methine proton (2-H). The resonance of the protons of the t-Boc protective group of the indole moiety appeared at δ_H 1,65. The chemical shift values of the protons of the carbomethoxy groups (δ_H 3,04 and 3,68) indicated that the C-2 indolyl and the C-3 carbomethoxy groups have the *cis* orientation. The upfield chemical shift of the protons of the C-3 carbomethoxy group is, as was the case for the phenyl analogue, the result of diamagnetic shielding.

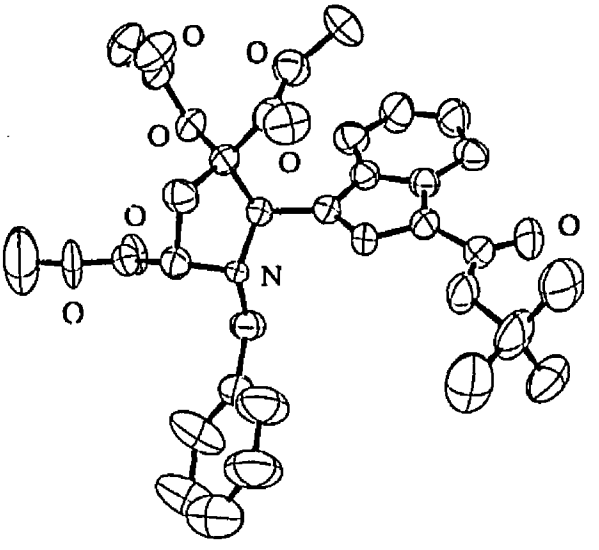
Table 9. ^{1}H N.m.r Data of the Pyrrolidine Ring of Compounds (133) and (134)

Compound	Proton	δ_H	J (HH)/Hz
	Acetyl CH_3	2,10s	-
	4-H_b	2,32dd	16,0 2,0
	C-3 CO_2Me	3,04s	-
133	4-H_a	3,46dd	15,9 10,2
	C-5 CO_2Me	3,68s	-
	5-H	3,95dd	10,1 2,0
	2-H	5,20s	
	Acetyl CH_3	2,10s	-
	4-H_a	2,36dd	16,2 2,0
	C-3 CO_2Me	2,97s	-
134	4-H_b	3,51dd	16,2 9,9
	C-5 CO_2Me	3,69s	-
	5-H	3,97dd	9,9 2,0
	2-H	5,20s	

A single crystal *X*-ray crystallographic analysis of monoclinic crystals of **(133)** space group $P2_1/n$, a = 17,837(2), b = 11,526(2), c = 15,931(1) Å, ß = 114,11(1)° and Z = 4, established the relative configuration as shown in Figure 19.

Removal of the t-Boc protective group to give compound **(134)** was effected by a thermal method[171] which proceeded in better yield (53%) than the acid hydrolysis procedure (35%). The *N*-debenzylation with palladium on carbon as catalyst using hydrogen at 20 atm pressure gave the pyrrolidine synthon **(104)** (27%) identified in the retrosynthetic analysis (Scheme 35) as the key to the synthesis of monatin. The elemental analysis of compound **(135)** agreed with the empirical formula $C_{25}H_{26}N_2O_6$. The ^{1}H n.m.r. spectrum confirmed the loss of both the t-butyl and *N*-benzyl groups.

Figure 19. A Perspective View of the Crystal Structure of Compound (133)



Efforts to effect the cleavage of the C-2 carbon-nitrogen bond, such as hydrogenation and Birch reduction, failed and this, combined with the poor yields encountered in the last cycloaddition approach towards the synthesis of monatin, caused the cycloaddition approach to be abandoned.

Although the synthesis of monatin has not been achieved to date either by the author or other workers in this research area, the synthetic approaches which were investigated provide a wealth of knowledge of importance to future synthetic endeavours as well as organic chemistry in general.

CHAPTER 4

EXPERIMENTAL

Melting points were determined on a Reichert Koffler hotstage and are uncorrected. Ultraviolet absorptions were measured on a Unicam SP 8-100 spectrometer. Infrared spectra were recorded on a Beckman Acculab 8 spectrometer as KBr discs or for solutions in chloroform. Mass spectra were recorded on a Varian MAT 212 double focussing mass spectrometer. N.m.r. spectra were recorded on a Varian EM 390, a Bruker WM-500 or a Bruker AM-300 spectrometer. Chemical shifts are reported to 0,01 δ units and coupling constants to 0,1 Hz. TMS was used as internal standard except for highfield spectra where the chemical shift of the solvent peak was used as internal standard. Optical rotations were measured at 24 °C on a Perkin-Elmer 241 polarimeter.

X-ray crystallographic data were collected on an Enraf Nonius CAD4 diffractometer operating in an environment maintained at 22 °C. The horizontal and vertical slits were set to 1,3 and 4,0 mm respectively, for all three data collections. A variable scan speed with a maximum of 2,35 ° min^{-1}, and a minimum that corresponded to 60 sec measuring time was used. Accurate unit cell parameters were obtained by the least-squares setting of 25 high-order reflections for each crystal. The relevant crystallographic details are given with the physical data of the compounds. Crystal stability and orientation were checked at regular intervals. Empirical absorbtion corrections were carried out on compound (**129**), using the standard EAC program of the Enraf Nonius system. The structures were solved using MULTAN80[173] and refined using SHELX76[174]. A σ^{-2} (F) weighting scheme was employed in all the refinements. All the hydrogen atoms for compounds (**114**), (**129**) and (**133**) were refined in experimentally determined positions, except for the hydrogen atoms of one methyl group of compound (**133**) which were refined in theoretical positions (C-H bond length: 1,08 Å, H-C-H dihedral angle: 109,5 °).

All reactions were monitored on Merck F254 precoated silica gel plates (0,25 mm thickness). Flash chromatography[175] was performed using Merck 230-400 mesh silica gel.

Most solvents used were of technical grade and were purified before use by fractional distillation. During the fractionation the fore- and after-run was discarded.

Table 10. Compounds Synthesized or Isolated in the Study

The postscript letter after the reference number under the heading Ref. has the following meaning:

S = The procedure as described in the literature was used.

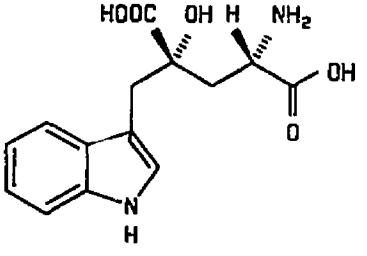
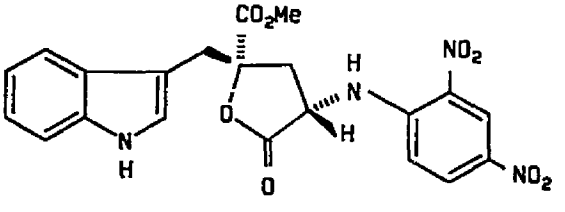
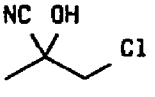
V = The procedure as described in the literature was used with slight variation(s).

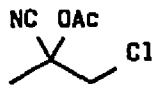
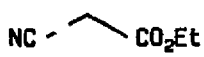
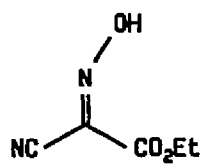
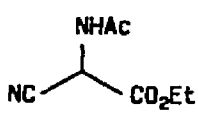
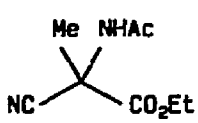
K = The compound is known but was prepared according to a different procedure.

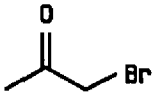
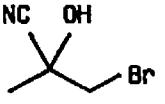
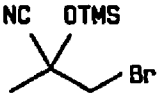
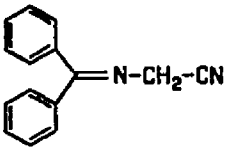
N = This compound has not been described before and in cases where a reference is cited it means that a procedure used to prepare another compound was applied here.

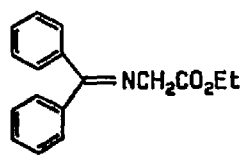
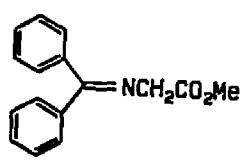
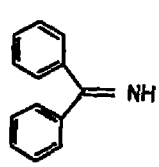
For the cases **V**, **K** and **N** the experimental procedure is described in detail.

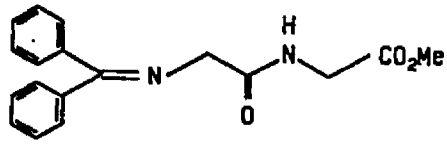
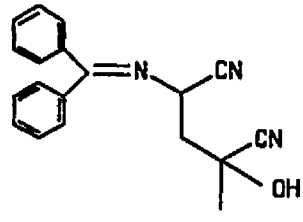
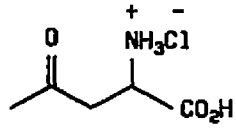
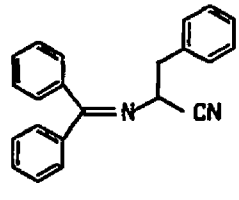
In the column b/m pt, b = boiling, m = melting, pt = point and mm = mm Hg.

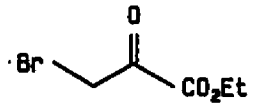
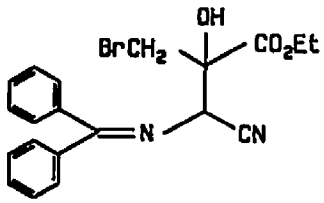
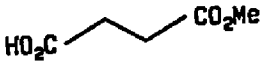
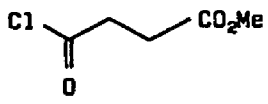
No. Name Structure	Ref	Yield (%) Own	 Lit	m/b pt (°C)
18 Monatin 4-Hydroxy-4-(3-indolylmethyl) glutamic acid 	**N**			m: 247- 264° dec
26 Methyl 4-hydroxy-4(3-indolylmethyl)-*N*-2,4-dinitrophenyl glutamate lactone 				m: 180- 180,5°
33 Chloroacetone cyanohydrin 	**123** **S**	67	73	b: 67° 0,6mm lit. 73-4°/ 1,5 mm

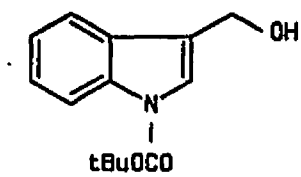
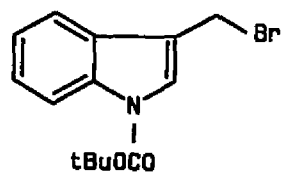
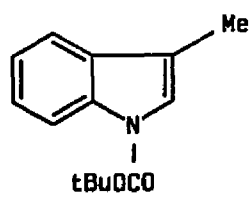
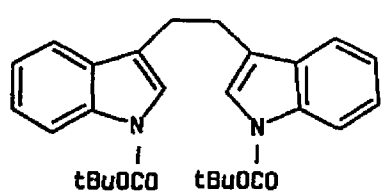
34 Chloroacetone cyanohydrin acetate 	123 S	57	59	b: 66°/ 0,4 mm lit. 57-9°/ 0,3 mm
35 Ethyl cyanoacetate 	124 S	53	77	b: 103-4°/ 16 mm lit. 97-8°/ 16 mm
36 Ethyl isonitrosocyano-acetate 	125 S	71	90	m: 133-4° lit. 131-3°
37 Ethyl acetamidocyano-acetate 	125 S	84	85	m: 128-9° lit., 129-30°
38 Ethyl 2-acetamido-2-cyano-propanoate 	126 S	33	94	m: 103-5° lit., 101-2°

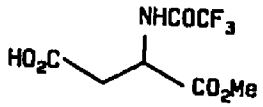
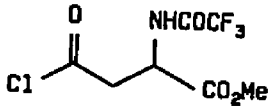
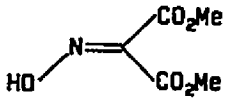
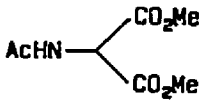
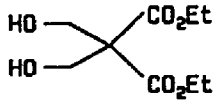
39	Bromoacetone 	127 **S**	50	43-4	b: 31°/ 11 mm lit 40-2°/ 13 mm
40	Bromoacetone cyanohydrin 	123 **N**	21	-	b: 105-12°/ 9 mm
41	Cyanotrimethylsilane Me_3SiCN	128 **S**	50	85	b: 112° lit., 112-7°
42	Bromoacetone cyanohydrin-trimethylsilane 	129 **N**	23	-	b: 93-4° at 90 mm
43	Diphenylketimine glycinonitrile 	130 **V**	32 and 47	not given	m: 84-5° lit. 85-7°

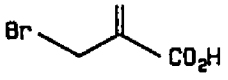
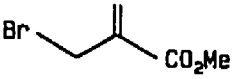
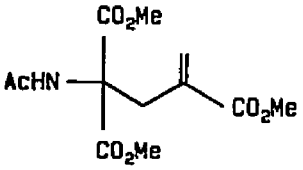
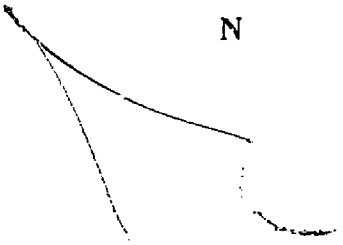
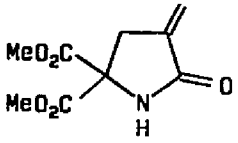
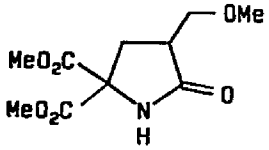
44	Ethyl diphenylketimine glycinate 	1312 S	23	82	oil m: lit. 49-50°
45	Methyl diphenylketimine glycinate 	130 V	17	91	b: 140/1 mm lit., m: 42,5-3° ref 128
46	Methyl glycinate 	132 V	88	not given	m: 180-1° gas evolution > 170° lit. 175-6
47	Diphenylketimine 	134 S	59	61-81	b: 113°/ 0,5 mm lit. 127-8°/ 3,5 mm
48	Aminoacetonitrile hydrogensulphate $HSO_4^-.NH_3^+CH_2CN$	135 S	71	66	m: 144-55° lit.177°

49	Methyl diphenylketimine glycylglycinate 	trace V	-		m: 97-8° lit.[136] 95-6°
50	2-Hydroxy-2-methyl-4-(diphenylketimino)-pentanedinitrile 	132 N	23	-	oil
51	2-Amino-4-oxopentanoic acid hydrochloride 	132 N	43	-	amorphous
53	Diphenylketimine phenylalaninonitrile 	132 V	47	90	unstable not given

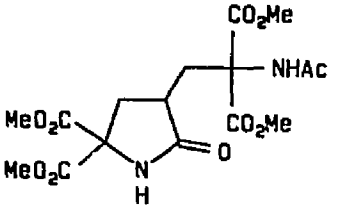
54	Ethyl bromopyruvate 	137 **S**	49	64	b: 60°/ 0,6 mm lit.,/ 83-8° 8 mm
55	Ethyl 2-bromomethyl-3-cyano-2-hydroxy-3-diphenylketimino propanoate 	 **N**	8	-	oil
56	Succinic acid monomethyl ester 	139 **S**	71	95	m: 55-7° lit. 57-8°
57	Methyl succinoyl chloride 	139 **S**	76	98	b: 102°/ 13 mm lit. 92-3°/ 18 mm

58 *N*-t-Butyloxycarbonyl-3-hydroxymethylindole 	141 142 S	91	100	m: 120-1 • lit. not given
59 *N*-t-Butyloxycarbonyl-3-bromomethylindole 	142 S	42	?	m: decompose lit. 97 • decompose
60 N-t-Butyloxycarbonyl-3-methylindole 	 V	10,3	-	oil
61 1,2-Di-(*N*-t-butyloxy-carbonyl-3-indolyl)ethane 	 N	5,5	-	m: 142,5-3 •

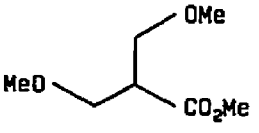
62	*N*-Trifluoroacetylaspartic acid α-methyl ester 	143 V			m: 113-6°
63	*N*-Trifluoroacetylaspartoyl chloride α-methyl ester 	143 V	100 nmr	-	unstable
68	Dimethyl isonitroso-malonate 	145 S	64	98	m: 67-9° lit. 71°
69	Dimethyl acetamidomalonate 	145 S	61	98	m: 128-9° sublimes > 116° lit., 128.5°
70	Diethyl di(hydroxy-methyl)malonate 	146 S	95	75	m: 45-7° lit. 48-50°

71 α-Bromomethyl acrylic acid 	147 S	68	43	m: 68-73° lit. 71-3°
72 Methyl α-bromomethyl-acrylate 	147 V N	52	-	b: 55° / 1 mm
73 Dimethyl *N*-acetyl-2-carbomethoxy-4-methyleneglutamate  	144 N	39	-	m: 72-3°
74 5,5-Dicarbomethoxy-3-methylene-2-pyrrolidone 	144 N	2,2		m: 104-5°
75 5,5-Dicarbomethoxy-3-methoxymethyl-2-pyrrolidone 	144 N	49	-	m: 90-1°

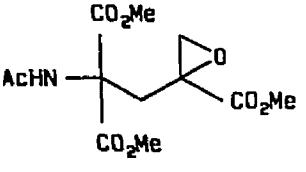
76 5,5-Dicarbomethoxy-3-(2,2-dicarbomethoxy-2-*N*-acetyl)ethyl-2-pyrrolidone		144 **N**	6	-	m: 64-5°



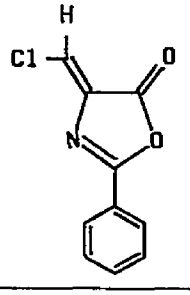
77 Methyl 3-methoxy-2-methoxymethylpropanoate		 **N**	33	-	oil

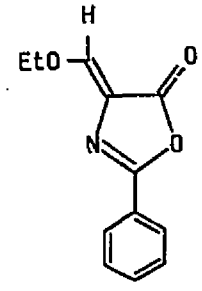
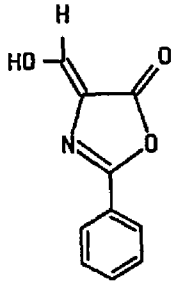
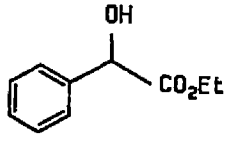
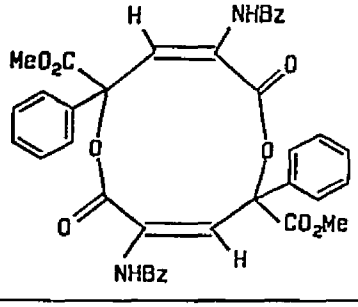


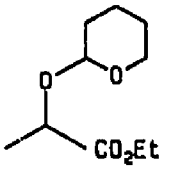
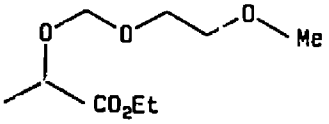
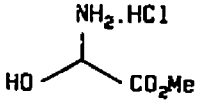
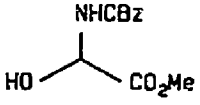
78 2-(2-Acetamido-2,2-di-carbomethoxy)ethyl-2-carbomethoxyoxirane		150 151 **N**	24		m: 93-5°

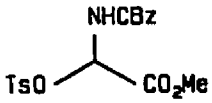
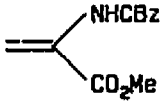
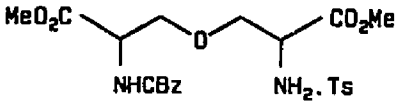
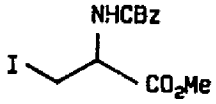


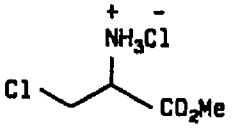
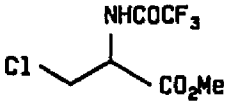
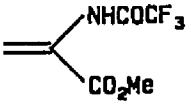
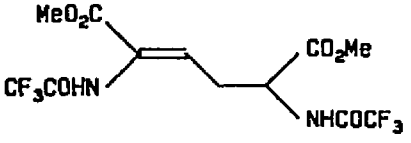
80 4-Chloromethylene-2-phenyloxazolin-5-one		154 156 **S**	63	73	m: 123-7° lit. 120-7°

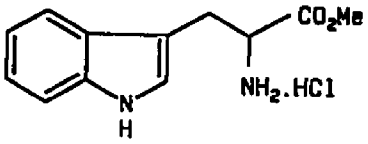
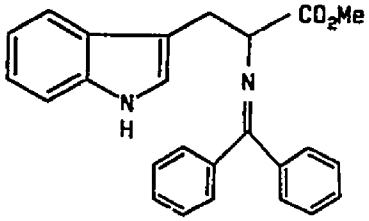
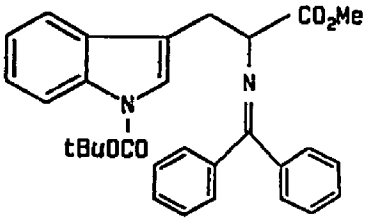
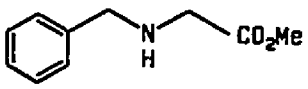


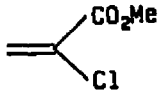
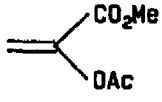
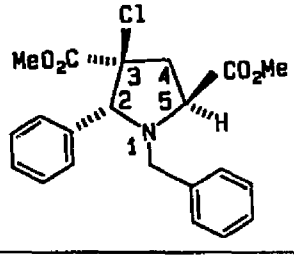
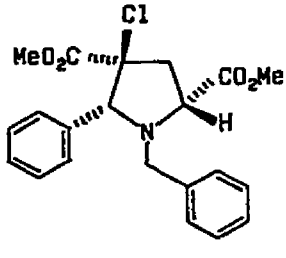
83	4-Ethoxymethylene-2-phenyloxazolin-5-one 	153 154 **S**	33	66	m: 96-8° lit. 97-8°
84	4-Hydroxymethylene-2-phenyloxazolin-5-one 	154 155 **S**	96	85-90	m: 148-50° lit. 152°
85	Ethyl mandelate 	157 **S**	81	84	b: 88°/ 0,7 mm lit 144-5/ 16 mm
86	Bislactone 	**N**	7,5	-	oil

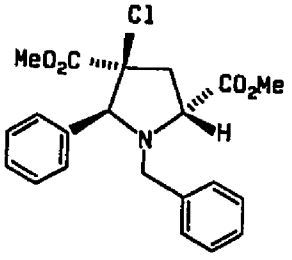
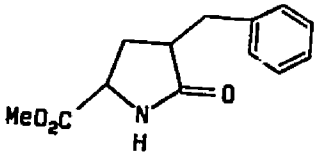
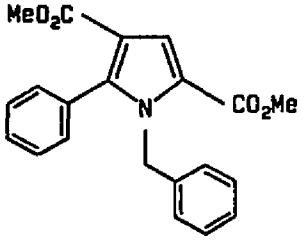
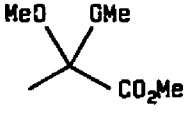
87	Ethyl *O*-pyranyllactate 	158 159 **S**	16	87	oil lit., 55°/ 0,01 mm
88	Methoxyethoxymethylchloride 	160 **S**	62	?	oil lit. ?
89	Ethyl (*S*)-2-methoxy-ethoxymethoxypropanoate 	160 **V**	49	90 nmr	b: 105°/ 0,1 mm lit. 75-75,6° /0,05mm
90	Serine methyl ester hydrochloride 	161 **S**	95	82	m: 162° gas evolution > 158° lit., 163-7°
91	*N*-Benzyloxycarbonylserine methyl ester 	162 **S**	63	85	m: 34-8° lit., 33-5°

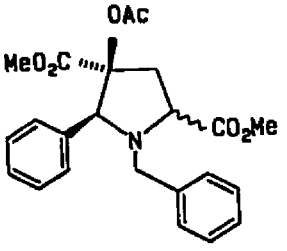
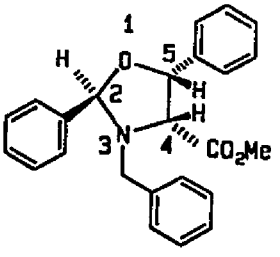
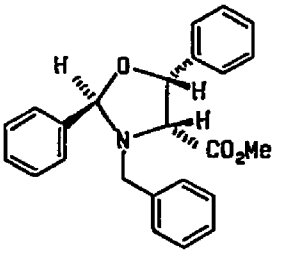
92 *O-p*-Toluenesulphonyl-*N*-benzyloxycarbonylserine methyl ester 	163 S	21	60	m: 120-1° ex MeOH lit., 117-9° ex iPrOH
93 Methyl α-*N*-benzyloxycarbonyl-2-aminoacrylate 	163 N	40,5	-	oil
94 Dimethyl 6-amino-2-benzyloxycarbonylamino-4-oxaheptanedioate tosylate 	163 N	9.5	-	m: 56-7°
95 *N*-Benzyloxycarbonyl-ß-iodoalanine methyl ester 	163 S	56	86	m: 68-70° lit., 67-8°

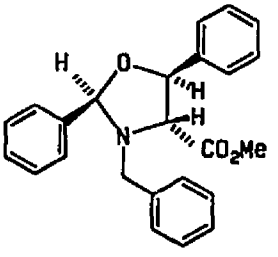
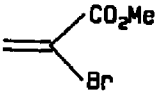
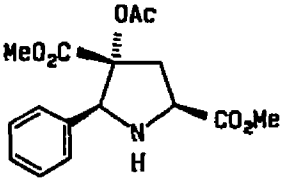
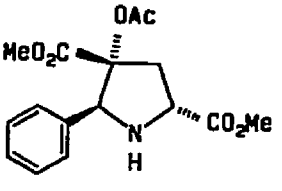
96 ß-Chloroalanine methyl ester 	164 **S**	83	58	m: 151-3° lit., 150°
97 *N*-Trifluoroacetyl-ß-chloroalanine methyl ester 	164 **S**	79	93	m: 61-61,5° lit. 46°
98 Methyl α-*N*-trifluoroacetyl-acrylate 	164 **S**	70	77	b: 70° lit., 48-52°/ 2 mm
100 Dimethyl 2,5-di(trifluoro-acetylamino)-2-hexenedioate 	164 **N**	14	-	m: 97-8°

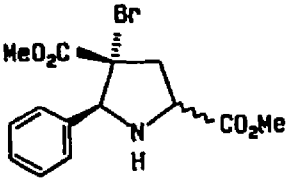
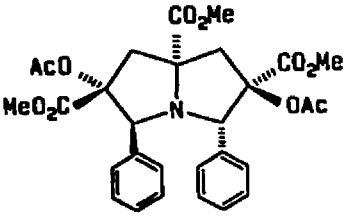
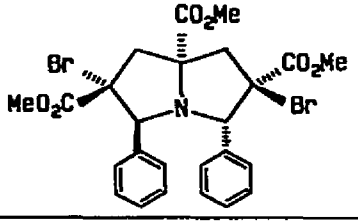
101 DL-Tryptophan methyl ester 	165 **S**	77	72	m: 219-25° with gas evolution lit., 214
102 DL *N*-Diphenylketimine-tryptophan methyl ester 	136 **S**	20	91	oil L-amino acid m: 114-5°
103 Indole-*N*-t-butyloxycarbonyl *N*-diphenylketimine-tryptophan methyl ester 	142 **N**	69	-	foam
107 Methyl *N*-benzylglycinate 	168 **S** **K**	24 53	60 -	b: 120-30°/ 0,5 mm lit., 150-2°/ 12 mm

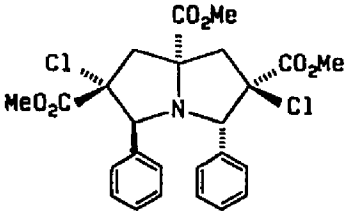
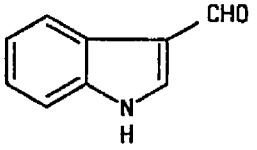
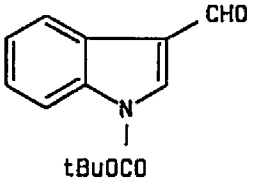
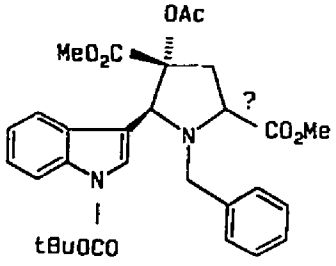
110	Methyl α-chloroacrylate  	169 **S**	78	73	b: 51-2°/ 52 mm lit., 57-9°/ 55 mm
111	Methyl 2-acetoxyacrylate  	170 **V**	52	37	b: 85°/ 20 mm lit., 67-9° 20 mm
112	(2*R**,3*S**,5*S**)-*N*-Benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidine  	 **N**	48	-	m: 105,5-6°
113	(2*R**,3*S**,5*R**)-*N*-Benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidine  	 **N**	6,6	-	oil

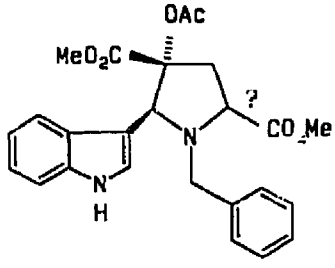
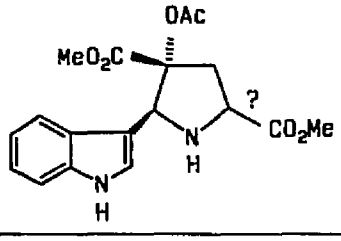
114 (2*S**,3*S**,5*R**)-*N*-Benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidine 	N	9,8		m: 111-2°
115 Methyl 4-benzylglutamate lactam 	N	13,5	-	m: 93-4°
116 *N*-Benzyl-3,5-dicarbomethoxy-2-phenylpyrrole 	N	24 ex O_2-*v* 51 ex Zn	-	m: 109-9,5°
117 Methyl 2,2-dimethoxypropanoate 	171 K	34	68	b: 46° / 1mm lit., 62-3° / 12 mm

118	3-Acetoxy-*N*-benzyl-3,5-dicarbomethoxy-2-phenylpyrrolidine 	N	7,9	-	oil
119	(2*R**,4*R**,5*R**)-*N*-Benzyl-4-carbomethoxy-2,5-diphenyl-oxazoline 	N	1,8	-	oil
120	(2*R**,4*S**,5*R**)-*N*-Benzyl-4-carbomethoxy-2,5-diphenyl-oxazoline 	N	8,3	-	m: 68-68,5°

121 ($2R^*,4R^*,5S^*$)-*N*-Benzyl-4-carbomethoxy-2,5-diphenyl-oxazoline 	N		3,2	-	oil
122 Methyl α-bromoacrylate 	S	161	76	82	b: 70-3°/ 70 mm lit. 72-4°/ 78 mm
123 3-Acetoxy-3,5-dicarbo-methoxy-2-phenylpyrrolidine 	N		3	-	oil
124 3-Acetoxy-3,5-dicarbo-methoxy-2-phenylpyrrolidine 	N		4,2		oil

125 3-Bromo-3,5-dicarbo-methoxy-2-phenylpyrrolidine 	N	19,7	-	oil
128 (2*S**,3*R**,7*R**,8*S**)-3,7-Diacetoxy-3,5,7-tricarbo-methoxy-2,8-diphenyl-pyrrolizidine 	N	4,6	-	m: 182-3°
129 (2*S**,3*R**,7*R**,8*S**)-3,7-Dibromo-3,5,7-tricarbo-methoxy-2,8-diphenyl-pyrrolizidine 	N	5	-	m: 191,5 -191°

130 (2*S**,3*R**,7*R**,8*S**)-3,5,7-Tricarbomethoxy-3,7-dichloro-2,8-diphenyl-pyrrolizidine 	N	7	-	m: 202-202,5°
131 3-Formylindole 	141 S	58	97	m: 196-8° sublime >185 lit. 196-7°
132 *N*-t-Butyloxycarbonyl-3-formylindole 	142 S	91	?	m: 124-6° lit. 122°
133 3-Acetoxy-*N*-benzyl-2-(*N*-t-butyloxycarbonyl-3-indolyl)-3,5-dicarbo-methoxypyrrolidine 	N	18	-	oil

134	3-Acetoxy-*N*-benzyl-3,5-dicarbomethoxy-2-(3-indolyl)pyrrolidine 	172 N	53	-	oil
135	3-Acetoxy-3,5-dicarbomethoxy-2-(3-indolyl)-pyrrolidine 		27	-	oil

Isolation of Monatin (18).- Roots of *Schlerochiton ilicifolius* (160 kg) were collected in northern Transvaal, air dried in a cool place and milled in a farm-type hammermill to give ground material (83 kg). Portions of the ground material (10 kg) were stirred overnight with water (25 l) and the resulting slurry pressed in a mechanical press to obtain an aqueous extract which was filtered to remove the fine particles. This extract was stirred in portions (2 l) with Bio-Rad cation resin (AG50WX-8, 500 ml) in the H^+-form. The resin was washed with water and the basic components removed from the resin by stirring with an ammonia solution (5%, 1 l). The combined ammonia extracts were freeze-dried to give the crude basic components (658 g). The basic components were dissolved in water (2 l) and portions (50 ml) were applied to a Bio Gel P2 gel filtration column (100 x 6 cm) and eluted with water at a flow rate of 5 ml/min. The sweet tasting fractions were combined and freeze-dried to give a crude product (143 g). Portions of this product were applied to a Sephadex G10 gel filtration column (200 x 2,2

cm) and eluted with water at a flow rate of 2 ml/min. Once again the sweet tasting fractions were combined and freezedried to give impure sweet tasting product (9,4 g). This product was again applied to a Sephadex G10 column but in smaller portions (300 mg) and eluted with water at the same flow rate as previously. The combined sweet tasting fractions were combined and freeze-dried to give pure *monatin* (**18**, 1,73 g) as a mixture of salts, $[\alpha]_D$ -49,6° (*c* 1,00 in H_2O).

The free amino acid was obtained in two ways:

1. Direct treatment with acetic acid.- Monatin salts (100 mg) were dissolved in water (1 ml) and glacial acetic acid (1 ml) added. Ethanol (96%, 5 ml) was added and upon standing overnight at room temperature, fine rosettes of needle-like crystals of *monatin* (**18**) formed (35 mg), m.p. 216-20°C (with gas formation).

2. *Elution from an anion resin*.- Strong basic anion exchange resin (Dowex 1 X-8, 200-400 mesh 20 ml) was prepared in its acetate form and packed in a short column. Monatin (100 mg) was dissolved in water (2 ml), loaded onto the column and eluted with a 2M acetic acid solution. Fractions (10 ml) were collected and upon standing at room temperature for eight days crystals of monatin formed in the fraction tubes. The crystals were collected by filtration, washed with ethanol and air-dried to give pure *monatin* (**18**) (9 mg), m.p. 247-264°C, (No mass spectrum could be obtained, even with FAB techniques), $\nu_{max.}$ (KBr) 3396 (NH_2), 3020, 1580 (acid) and 1540 cm^{-1}, λ_{max} (0,1 N NaOH) 279nm (ϵ 5355) (Found: C, 56,59; H, 5,49; N, 9,31%. $C_{14}H_{16}N_2O_5.¼\ H_2O$ requires: C, 56,66; H, 5,56 and N, 9,44%). ^1H and ^{13}C N.m.r. data are collated in Table 4, Chapter 2.

Methyl N-2,4-dinitrophenyl-4-hydroxy-4-(3-indolylmethyl)-glutamate lactone (**26**).- Monatin (**18**) (60 mg) and sodium hydrogen carbonate (20 mg) was stirred for 10 min in an ethanol-water mixture (1:1 v/v). A solution of fluoro-2,4-dinitrobenzene (200 mg) in

ethanol (1 ml) was added and the reaction mixture stirred overnight at room temperature. The reaction mixture was acidified (6M HCl, 2 drops) and extracted twice with diethyl ether. The ethereal extract was treated with an ethereal solution of diazomethane (generated from 2,14 g Diazald) and after 30 min the excess of diazomethane was decomposed by the addition of a few drops of acetic acid. The ether was evaporated in a stream of nitrogen and the crude product purified by flash chromatography using ethyl acetate-hexane (1:3 v/v) as eluent. Crystallization from ethyl acetate-hexane yielded crystals of (**26**) (25 mg, 27%), m.p. 174-5°C, ν_{max} 3350 (NH), 1788 (lactone) and 1741 (ester) cm^{-1}, $[\alpha]_D$ +0,2° (*c* 1,00 in $CHCl_3$) (Found: C, 55,72; H, 4,04; N, 11,94%; M^+ 454,1114. $C_{21}H_{18}N_2O_8$ requires C, 55,51; H, 3,96 and N, 12,33%; *m/e* 454,1115); ^1H and ^{13}C n.m.r. data are collated in Table 7, Chapter 2.

Bromoacetone cyanohydrin (**40**).[123]-Bromoacetone[127] (**39**) (68,5 g, 0,5 mol) was added dropwise to a stirred solution of sodium metabisulphite (63 g) in water (160 ml) kept below 35°C. The mixture was cooled to below 25°C and diethyl ether (200 ml) added. A solution of sodium cyanide (25 g) in water (50 ml) was added dropwise with stirring to the reaction mixture while the temperature was maintained between 25-30°C. The ethereal phase was separated and the aqueous phase extracted once with ether. The combined ethereal extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give *bromoacetone cyanohydrin* (**40**) (17 g, 21%), b.p. 105-112°C at 9 mmHg; δ_H ($CDCl_3$) 1,75 (3H, s, CH_3), 3,45 (1H, s, OH), 3,58 (2H, s, $-CH_2-Br$).

Bromoacetone cyanohydrintrimethylsilane (**42**).[129]- Bromoacetone[128] (**39**) (13,7 g, 0,1 mol) was stirred in a two- necked flask fitted with a cooler and a rubber septum. Cyanotrimethylsilane (**41**) (10,9 g, 0,11 mol) and zinc iodide (20 mg) were introduced with a syringe into the flask. After standing overnight the mixture had turned black. The product was distilled from the black mass (b.p. 190-206°C) and redistilled under reduced pressure to give (**42**) (5,4 g, 23%) b.p. 93-4°C at 90 mmHg; δ_H ($CDCl_3$) 0,27 (9H, s,

$Si(CH_3)_3$), 1,73 (3H, s, CH_3), 3,40 (1H, *J* 10,2 Hz), 3,55 (1H, *J* 10,2 Hz) (Found: C:H ratio = 6,27 and C:N ratio = 6,02. $C_7H_{14}BrNSi$ requires C:H ratio = 6,0 and C:N ratio = 6,0).

Diphenylglycinonitrileketimine (**43**).[130]-Diphenylketimine[134] (**47**) (16,5 g, 85 mmol) and aminoacetonitrile hydrochloride (7,0 g, 76 mmol) were refluxed in acetonitrile (200 ml) until the reaction was complete. The precipitate which had formed was filtered off and the solvent evaporated under reduced pressure. The crude product was then partitioned between chloroform and water. The chloroform solution was dried over anhydrous calcium chloride and then evaporated under reduced pressure. The crystals which formed in the resulting syrup were filtered off and recrystallized from chloroform-hexane to give (**43**) (8,32 g 49%), m.p. 84-85 °C; δ_{II} ($CDCl_3$) 4,33 (2H, s, CH_2), 7,2-7,7 (10H, m, 2x phenyl).

Methyl diphenylketimine glycinate (**45**) *and methyl diphenylketimine glycylglycinate* (**49**).[130]-Diphenylketimine[134] (**47**) (28 g, 0,155 mol), acetic acid (9 g, 0,155 mol) and methylglycinate hydrochloride (**46**) (18,9 g, 0,155 mol) were refluxed in acetonitrile (200 ml) for 8 h, the mixture was partitioned between chloroform and water, the chloroform washed twice with water and then dried over anhydrous calcium chloride. After evaporation of the solvent under reduced pressure, the products were separated on a silica gel column by first eluting with a ethyl acetate-hexane (1:6 v/v) to give benzophenone (10,5 g, 37% based on diphenylketimine) followed by the first ninhydrin positive compound *methyl diphenylketimine glycinate* (**45**)[130] (6,47 g, 17% based on glycine), b.p. 140 °C at 1 mmHg; δ_{II} ($CDCl_3$) 3,73 (3H, s, OCH_3), 4,22 (2H, s, -CH_2-), 7,1-7,95 (10H, m, 2x phenyl), n_D = 1,5957.

Subsequent elution with ethyl acetate yielded a second ninhydrin-positive compound *methyl diphenylketimine glycylglycinate* (**49**) which was recrystallized from ethyl acetate-hexane to give (**49**) (140 mg 0,1%), m.p. 97-98 °C δ_{II} ($CDCl_3$) 3,78 (3H, s, OCH_3), 3,99 (2H, s, -CH_2-), 4,16 (2H, d, *J* 4,8 Hz, NH$\underline{CH_2}$), 7,03-7,73 (10H, m, 2x phenyl), 7,95 (1H, d,

J 4,8 Hz, -NH-) (Found: C, 70,75; H, 6,25; N, 9,32%; *m/e* 310. $C_{18}H_{18}N_2O_3$ requires C, 69,67; H, 5,80 and N, 9,03%; *m/e* 310).

Methyl glycinate (**46**).[132]- Anhydrous methanol (Mg/I_2 procedure, 500 ml) was added to glycine (75 g, 1 mol) and dry hydrogen chloride gas bubbled through the reaction mixture until the end of the bubbler tube started to clog with crystals. The reaction mixture was cooled in an icebath during the gas addition. Dry hydrogen chloride gas (55 g) was bubbled through anhydrous methanol (1 l). This solution was added to the reaction mixture and left overnight at room temperature. The excess methanol was evaporated under reduced pressure and the product recrystallized from methanol to give *methyl glycinate* (**41**) (109 g, 88%), m.p. 180-181°C (bubbles >170°C); δ_{II} (methanol-d_4) 3,85 (3H, s, OCH_3), 3,87 (2H, s, CH_2), 4,83 (2H, broad, NH_2).

Diphenylketiminepentanenitrile-4-one cyanohydrin (**50**).[131]- Lithium diisopropylamide was generated in hexamethylphosphoric triamide-tetrahydrofuran (1:5 v/v) solution (20 ml) from butyllithium solution (1 ml 15% solution in hexane, 2,34 mmol) and diisopropylamine (0,227 g, 2,24 mmol). The reagent was cooled to -78°C, diphenylketimineglycinonitrile[130] (**43**) (440 mg, 2 mmol) added and stirred for 45 minutes. Bromoacetonecyanohydrin trimethylsilane[129] (**42**) (600 mg, 2,5 mmol) was added, the mixture allowed to reach room temperature and stirred for 24 hours. The solvent was evaporated under reduced pressure and the mixture partitioned between chloroform and water. The chloroform was washed with water (3x), dried over anhydrous calcium chloride and evaporated under reduced pressure. The product was first chromatographed on silica gel using chloroform as eluent to retain the hexamethylphosphoric triamide on the column. After evaporation of the solvent the residue was rechromatographed on silica gel using ethyl acetate-hexane (1:8 v/v) as eluent to give (**50**) (140 mg 23%) oil; δ_{II} ($CDCl_3$) 2,16 (3H, s, CH_3), 3,15 (1H, *J* 7,8 Hz, *J* 15,0 Hz), 3,40 (1H, *J* 6,3 Hz, *J* 15,0 Hz), 4,68 (1H, dd, *J* 6,3 Hz, *J* 7,8 Hz), 7,2-7,73 (11H,

m, 2x phenyl and OH) (Found: C, 75,59; H, 5,88; N, 13,60%. $C_{19}H_{17}N_3O$ requires C, 75,26; H, 5,62; N, 13,86%).

2-Amino-4-oxopentanoic acid hydrochloride (**51**).[131]- Compound (**50**) (250 mg, 0,77 mmol) in 1M HCl (10 ml) to which hexane (10 ml) had been added, was stirred at room temperature for 3 h. The hexane phase was separated and evaporated under reduced pressure to give benzophenone (148 mg, 100%). Hydrochloric acid (11M, 5 ml) was added to the aqueous phase and the reaction mixture refluxed overnight. The water was evaporated under reduced pressure, the product redissolved in water and freeze-dried to give (**51**) (74 mg) as an amorphous powder; δ_H (D_2O) 2,26 (3H, s, $-CH_3$), 3,19 (1H, dd, *J* 19,3 Hz, *J* 4,1 Hz), 3,25 (1H, dd, *J* 19,3 Hz, *J* 7,3 Hz), 3,99 (1H, dd, *J* 4,1 Hz, *J* 7,3 Hz); δ_C (D_2O) 31,95Q (C-5), 45,44T (C-3), 52,83D (C-2), 176,20S (C-1), 213,40S (C-4).

Diphenylketimine phenylalaninonitrile (**53**).[131]- Diphenylketimine glycinonitrile[130] (**43**) (500 mg, 2,25 mmol), triethylbenzylammonium hydroxide (70 mg) and sodium hydroxide solution (50% 550 mg, 6,8 mmol) were mixed in toluene. The mixture was stirred magnetically at 0°C, benzyl bromide (430 mg, 2,5 mmol) was added and the reaction stirred for 2 h at 0°C. After stirring for 24 h at room temperature the mixture was partitioned between water and dichloromethane. The water was extracted with dichloromethane (3x), the combined organic phases washed with water (3x) and once with saturated sodium chloride solution. The dichloromethane solution was dried ($MgSO_4$) and evaporated under reduced pressure. The resulting oil was purified on a silica gel column (1 m x 1 cm) using chloroform-hexane (1:1 v/v) as eluent to give (**53**) (324 mg 47%) as an oil; δ_H ($CDCl_3$) 3,25 (2H, d, *J* 6,3 Hz), 4,42 (1H, t, *J* 6,3 Hz, CH), 6,75-7,75 (15H, m, 3x phenyl).

*Ethyl 2-bromomethyl-3-cyano-2-hydroxy-3-*N*-diphenylketimino propanoate* (**55**).- Diphenylketimine glycinonitrile[130] (**43**) (660 mg, 3 mmol), ethyl bromopyruvate[137] (**54**) (600 mg, 3 mmol), triethylbenzylammonium chloride (20 mg) and potassium

carbonate (500 mg) were stirred and refluxed in a hexamethyl-phosphoric triamide-benzene mixture (1:5 v/v, 50 ml) with azeotropic removal of the water in a Dean-Stark trap. The solvent was evaporated under reduced pressure. The high-boiling hexamethylphosphoric triamide was removed from the mixture using a silica gel column with two deadvolumes of ethyl acetate-hexane (2:1 v/v). The remainder of the product was eluted from the column with two deadvolumes of ethyl acetate. The solvent was evaporated under reduced pressure and the product rechromatographed on a silica gel column using chloroform-benzene (1:1 v/v) as eluent to give (**55**) (100 mg, 8%) as an oil; δ_{II} ($CDCl_3$) 1,42 (3H, t, *J* 7,8 Hz, $OCH_2\underline{CH_3}$), 3,59 (1H, d, *J* 10,8 Hz, $BrCH_2$), 3,82 (1H, d, *J* 10,8 Hz, $BrCH_2$), 4,00 (1H, s, OH), 4,50 (2H, q, *J* 7,8 Hz, $O\underline{CH_2}CH_3$), 4,53 (1H, s, CH-CN), 7,22-7,75 (10H, m, 2x phenyl) (no elemental analysis was obtained as the compound was unstable).

N-*t-butyloxycarbonyl-3-methylindole* (**60**).-*N*-t-Butyloxycarbonyl-3-bromomethyl-indole[142] (**59**) (624 mg, 2 mmol), 3-carbomethoxypropionyl chloride[139] (**57**) (300 mg, 2 mmol), dichloro-bis-triphenylphosphine palladium(0) (70 mg, 0,1 mmol) and zinc powder (260 mg, 4 mmol) were ultrasonicated for 1 h in dry dimethoxyethane (20 ml). The catalyst was filtered off and the solvent evaporated under reduced pressure. The reaction mixture was partitioned between chloroform and hydrochloric acid (1M). The chloroform solution was washed with water and dried over anhydrous calcium chloride. The products were purified by flash chromatography using diethyl ether-hexane (1:40 v/v) as eluent to give

N-*t-butyloxycarbonyl-3-methylindole* (**60**) (49 mg, 10,3%) as an oil; δ_{II} ($CDCl_3$) 1,65 (9H, s, 3x t-butyl CH_3's), 2,25 (3H, d, *J* 1,8 Hz, CH_3), 7,17-7,6 and 8,03-8,23 (5H, m, indole nucleus) and

*1,2-di-(*N*-t-butyloxycarbonyl-3-indolyl)ethane* (**61**) (29 mg, 5,5%) crystals from cyclohexane-hexane, m.p. 142,5-143 °C, ν_{max} 1726 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 1,65 (18H, s,

6x t-butyl CH_3's), 3,08 (4H, s, CH_2CH_2), 7,15-7,60 and 8,05-8,20 (10H, m, 2x indole nuclei) (Found: C, 73,14; H, 7,01; N, 6,29%. $C_{28}H_{32}N_2O_4$ requires C, 73,04; H, 6,94 and N, 6,09%).

N-*Trifluoroacetylaspartic acid α-methyl ester* (**62**).- *N*-Trifluoroacetyl aspartic anhydride[143] (obtained from 9,8 g aspartic acid, 82 mmol) was refluxed for 2 h in dry methanol (80 ml). The solvent was evaporated under reduced pressure and the products purified by flash chromatography making use of two solvent systems. The crude mixture was applied to the column and eluted with chloroform to give the *dimethyl ester*. The column was then scrubbed with ethyl acetate and the solvent evaporated under reduced pressure. The residual crude product was rechromatographed using hexane-ethyl acetate-acetic acid (25:5:2 v/v/v) as eluent to give *α-monomethyl ester* (**62**) (4,3 g, 24%), m.p. 114-116°C (lit.[143] m.p. 102-3°).

Methyl α-(bromomethyl)acrylate (**72**).[147]- α-(Bromomethyl)acrylic acid[147] (**71**) (50 g, 0,303 mol), dry methanol (100 ml), dihidroquinone (2 g) and *p*-toluenesulphonic acid (2 g) were refluxed for 3 h in benzene (200 ml). The water formed in the reaction was removed azeotropically using a Dean-Stark apparatus by refluxing the solution overnight. Water (250 ml) was added to the reaction mixture and dry sodium hydrogen carbonate added portionwise until effervescence ceased. The mixture was extracted with chloroform, the chloroform dried over anhydrous calcium chloride and evaporated under reduced pressure. The residual material was distilled *in vacuo* (b.p. 55°C at 1 mmHg) to give (**72**) (28 g, 51,5%), ν_{max} 2950, 1725 (ester) and 1625 cm^{-1}; δ_H ($CDCl_3$) 3,83 (3H, s, OCH_3), 4,21 (2H, s, $BrCH_2$), 5,99 (1H, d, *J* 0,9 Hz, $=CH_2$), 6,39 (1H, d, *J* 0,9 Hz, $=CH_2$).

Dimethyl N-acetyl-2-methoxycarbonyl-4-methyleneglutamate (**73**).[145]- Sodium (2,3 g, 0,1 mol) was reacted with dry methanol (200 ml). Dimethyl acetamidomalonate[141] (**69**) (28,3 g, 0,15 mol) was added to this solution and the reaction stirred for 30 min at room temperature. This solution was added dropwise to a solution of methyl α-(bromomethyl)acrylate[147] (**72**) (19.3 g, 0,108 mol) in methanol (200 ml) at 0°C over a period of 1 h and stirred at room temperature for another 2 h. Chloroform (200 ml) and water (400 ml) were added and the reaction mixture partitioned between the two phases. The chloroform phase was dried over anhydrous calcium chloride and then evaporated under reduced pressure. The residual oil was chromatographed on a silica gel column (15 x 7 cm) using ethyl acetate-hexane (1:1 v/v) as eluent. The ninhydrin positive fractions (which were visualised after first spraying the t.l.c. plate with concentrated hydrochloric acid followed by heating at 120°C for 15 minutes) were retained. After evaporation of the solvent the product was crystallized from ethyl acetate-hexane to give (**73**) (28,9 g, 39%), m.p. 72-3°C, ν_{max} 3260, 1745 (ester), 1725 (ester) and 1640 cm^{-1}; δ_{H} ($CDCl_3$) 2,01 (3H, s, $COCH_3$), 3,43 (2H, s, CH_2), 3,73 (3H, s, OCH_3), 3,82 (6H, s, 2x OCH_3), 5,63 (1H, d, *J* 1,8 Hz, $=CH_2$), 6,32 (1H, d, *J* 1,8 Hz, $=CH_2$) (Found: C, 50,17; H, 6,31; N, 4,91%. $C_{12}H_{17}NO_7$ requires C, 50,17; H, 5,92 and N, 4,88%).

Products of the reversed addition of compound (**72**)[147] *to compound* (**69**).[145]- Sodium (3,1 g, 0,135 mol) was reacted with dry methanol (100 ml), dimethyl acetamidomalonate[145] (**69**) (26 g, 91 mmol) was added and the reaction mixture stirred at room temperature for 30 min. After this, methyl bromoacrylate[147] (**72**) (24 g, 0,124 mol) was added dropwise over 30 min and the reaction mixture then kept at room temperature for 2 h. At this stage a t.l.c. analysis revealed three ninhydrin positive products. A one-fifth aliquot of this mixture was taken and the solvent evaporated under reduced pressure. The mixture was chromatographed on a silica gel column (30 x 5 cm) using ethyl acetate-hexane (1:1 v/v) as eluent to give four compounds *viz.*:

5,5-Dicarbomethoxy-3-methylene-2-pyrrolidone (**74**)[144]- crystals from ethyl acetate-hexane (120 mg, 2,2%), m.p. 104-105°C, ν_{max} 3170, 1755 (ester), 1745 (ester), 1710 (amide) cm^{-1}, δ_{II} ($CDCl_3$) 3,37 (2H, t, *J* 3,0 Hz, CH_2), 3,83 (6H, s, 2x OCH_3), 5,48 (1H, m, $=CH_2$), 6,1 (1H, m, $=CH_2$), 7,03 (1H, s, NH) (Found: C, 50,51; H, 5,19; N, 6,46%. $C_9H_{11}NO_5$ requires C, 50,70; H, 5,16 and N, 6,57%),

5,5-dicarbomethoxy-3-methoxymethyl-2-pyrrolidone (**75**)[144], crystals from ethyl acetate-hexane, (3,10 g, 49%) m.p. 90-91°C, ν_{max} 3200, 1760 (ester), 1740 (ester), 1695 (amide) cm^{-1}, δ_{II} ($CDCl_3$) 2,5-2,96 (3H, m, $-CH_2CH-$), 3,33 (3H, s, ether OCH_3), 3,6 (2H, d, OCH_2), 3,80 (3H, s, ester OCH_3), 3,83 (3H, s, ester OCH_3), 6,64 (1H, s, NH), (Found: C, 48,97; H, 6,16; N, 5,17%. $C_{10}H_{15}NO_6$ requires C, 48,97; H, 6,12 and N, 5,17%),

*5,5-Dicarbomethoxy-3-(2,2-biscarbomethoxy-2-*N*-acetyl)ethyl-2-pyrrolidone* (**76**)[144], Crystals from ethyl acetate-hexane (0,53 g, 6%), m.p. 64-65°C, ν_{max} 3390, 3320, 1738 (ester), 1712 (ester), 1655 cm^{-1} (amide); δ_{II} ($CDCl_3$) 2,05 (3H, s, $COCH_3$), 2,3-3,05 (5H, m, $-CH_2-CH-CH_2-$), 3,81 (12H, s, 4x OCH_3), 6,68 (1H, s, lactam NH), 7,24 (1H, s, amidic NH) (Found: C, 47,82; H, 5,61; N, 6,93%. $C_{10}H_{22}N_2O_{10}$ requires C, 47,76; H, 5,47 and N, 6,97%) and

methyl 3-methoxy-2-methoxymethylpropanoate (**77**), an oil (1,5 g, 33%), n_D 1,4209, ν_{max} 2890 and 1740 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 2,8-3,06 (1H, m,-CH-), 3,33 (6H, s, 2x etheric OCH_3), 3,60 (2H, d, *J* 6,06 Hz,CH_2O), 3,61 (2H, d, *J* 6,7 Hz, $-OCH_2$), 3,73 (3H, s, ester OCH_3) (Found: C, 51,95; H, 8,35. $C_7H_4O_4$ requires C, 51,85 and H, 8,64%).

2-(2-Acetamido-2,2-dimethoxycarbonyl)ethyl-2-methoxycarbonyloxirane (**78**).[150,151]- The epoxidation reagent was prepared by first mixing hydrogen peroxide solution (30%, 2ml) and acetone (10 ml) followed by the addition of 6M sodium hydroxide solution (6 drops). After 20 minutes at room temperature the substrate (**73**)[144] (200 mg, 0,696 mmol) was added and left at room temperature for 1 h. The reaction mixture was partitioned

between chloroform and water. The chloroform phase was washed twice with water, dried over anhydrous calcium chloride and evaporated under reduced pressure. The products were separated by silica gel chromatography using ethyl acetate-hexane (1:2 v/v) as eluent to give starting material (**73**) (94 mg, 57% recovery) and epoxide (**78**) (51 mg, 24%), crystals from ethyl acetate-hexane, m.p. 93-95 °C, ν_{max} 3350, 1735 (ester), 1672 (amide) cm^{-1}; δ_{H} ($CDCl_3$) 2,02 (3H, s, $COCH_3$), 2,61 (1H, *J* 15,3 Hz, $-CH_2-$), 3,54 (1H, *J* 15,3 Hz, $-CH_2-$), 2,81 (1H, *J* 9,0 Hz, epoxide CH_2), 2,96 (1H, *J* 9,0 Hz, epoxide CH_2), 3,73 (3H, s, OCH_3), 3,78 (3H, s, OCH_3), 3,81 (3H, s, OCH_3), 6,9 (1H, s, NH) (Found: C, 47,29; H, 5,60; N, 4,63%. $C_{12}H_{17}NO_8$ requires C, 47,52; H, 5,61 and N, 4,62%).

Dimer or bislactone (**86**).- Lithium diisopropylamide was prepared from diisopropylamine (0,32 ml, 2,24 mol) and butyllithium solution (1 ml 15% in hexane, 2,34 mmole) in dry tetrahydrofuran. The lithium diisopropylamide solution was cooled to -78 °C and ethyl mandelate[157] (**85**) (180 mg, 1 mmol) in dry tetrahydrofuran (5 ml) was added at 0 °C and stirred for 1,5 h. The reaction mixture was cooled to -78 °C and 4-chloromethylene-2-phenyloxazoline-5-one[153-156] (**82**) (207,5 mg, 1 mmol) in anhydrous tetrahydrofuran (10 ml) was added. The reaction mixture was stirred at -78 °C for 1 h and then for 4 h at room temperature. The reaction was terminated by the addition of a 10% hydrochloric acid solution (5 ml) and then extracted with ethyl acetate. The ethyl acetate was washed once with a 10% hydrochloric acid solution and with a saturated sodium chloride solution. The ethyl acetate was dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The products were purified by medium pressure silica gel chromatography using ethyl acetate-hexane (1:4 v/v) as eluent to give the *dimer or bislactone* (**86**), (27 mg, 7,5%) as an oil; δ_{H} ($CDCl_3$) 1,13 (3H, t, *J* 7,2 Hz, $OCH_2\underline{CH_3}$), 4,10 (2H, dq, *J* 1,8 and *J* 7,2 Hz, $O\underline{CH_2}CH_3$), 5,51 (1H, s, C=CH), 7,16-7,87 (11H, m, 2x phenyl and 1x NH) (Found: C, 65,94; H, 4,68; N, 3,77, *m/e* 702. $C_{40}H_{34}N_2O_{10}$ requires C, 66,67; H, 4,72 and N, 3,89%, *M* 702).

Ethyl O-*pyranyllactate* (**87**).[158,159]- A solution of ethyl lactate (11,8 g, 0,1 mol), dihydropyran (9,24g, 0,11 mol) and pyridinium-*p*-toluenesulphonate[158] (250 mg, 10 mmol) in dichloromethane (100 ml) was stirred for 3 h at room temperature. The reaction mixture was diluted with diethyl ether (200 ml) and washed twice with a sodium chloride solution. The solvent was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The products were purified by medium pressure chromatography on silica gel using ethyl acetate-hexane (1:9 v/v) as eluent to give two stereoisomers of (**87**) as oils (2,46 g, 13% and 1,9 g, 6,2% respectively), each with ν_{max} 2945, 1754 (ester) cm^{-1}; δ_{H} ($CDCl_3$) 1,26 (3H, t, *J* 7,4 Hz, $OCH_2\underline{CH_3}$), 1,43 (3H, d, *J* 7,2 Hz, $\underline{CH_3}$-CH), 1,45-1,9 (6H, m, H-3', H-4', H-5' of pyranyl ring), 3,3-4,1 (2H, dm, H-5' of pyranyl ring), 4,22 (2H, q, *J* 7,4 Hz, $O\underline{CH_2}CH_3$), 4,43 (1H, q, *J* 7,2 Hz, CH_3-$\underline{CH}$), 4,65-5,1 (1H, dm, H-2' of pyranyl ring).

Ethyl (S)-2-2-methoxyethoxymethoxy propanoate (**89**).[160]- Ethyl lactate (11,8 g, 0,1 mol) was added to a suspension of sodium hydride (2,4 g, 0,1 mol) in dry tetrahydrofuran at 0°C over a period of 30 min. Methoxyethoxy chloride[160] (**88**) (11,6g, 0,1 mol) in dry tetrahydrofuran was added and the mixture stirred at 0°C for 1 h. The solvent was evaporated under reduced pressure and the product distilled under vacuum, b.p. 105°C at 0,1 mmHg, to give (**89**) (10,0 g, 49%) as a liquid; δ_{H} ($CDCl_3$) 1,25 (3H, t, *J* 7,3 Hz, $OCH_2\underline{CH_3}$), 1,40 (3H, d, *J* 6,6 Hz, $\underline{CH_3}CH$), 3,32 (3H, s, OCH_3), 3,45-3,8 (4H, dm, OCH_2CH_2O), 4,30 (2H, q, *J* 7,3 Hz, $O\underline{CH_2}CH_3$), 4,7 (2H, s, OCH_2O).

Tosylation of N-*benzyloxycarbonylserine methyl ester* (**91**).[163]- A solution of methyl *N*-benzyloxycarbonylserine[162] (**91**) 12 g, 47,5 mmol) in pyridine (25 ml) was cooled to 5°C. *p*-Toluene-sulphonyl chloride (11,5 g, 60 mmol) was added in portions and the reaction stirred overnight at 5°C. The precipitate was filtered off, the filtrate diluted with methanol (100 ml) and the solvent evaporated under reduced pressure. On t.l.c. (acetone-hexane, 1:2 v/v as eluent) three ninhydrin-positive spots were detected corresponding to compounds (**92**), (**93**) and (**94**). The filtrate was partitioned between

diethyl ether and water (4x) and after evaporation of the ether under reduced pressure the products were separated on a medium pressure silica gel column (100 x 2,5 cm) using acetone-hexane (1:5 v/v) as eluent to give

O-p-*toluenesulphonyl*-N-*benzyloxycarbonylserine methyl ester* (**92**) (4,1 g, 21%), m.p. 120-121 °C (crystals from methanol), $[\alpha]_D$ -9,9 ° (*c* 1,00 in DMF) [lit.[163] -10,27 ° (*c* 1,1 in DMF)],

methyl α-N-*benzyloxycarbonylamino acrylate* (**93**) (4,5 g, 40,5%) as an oil; δ_{II} ($CDCl_3$) 3,80 (3H, s, OCH_3), 5,15 (2H, s, benzylic protons), 5,8 (1H, d, *J* 1,8 Hz, *E* vinylic proton), 6,27 (1H, s, *Z* vinylic proton), 7,28 (1H, broad, NH), 7,38 (5H, s, aromatic protons) (Found: C, 60,72; H, 5,64; N, 5,86%. $C_{12}H_{13}NO_4$ requires C, 60,28; H, 5,53 and N, 5,96%) and

dimethyl 6-amino-2-benzyloxycarbonylamino-4-oxaheptanedioate tosylate (**94**) (1,1 g, 9,5%), crystals from diethyl ether-hexane, m.p. 56-7 °C, $[\alpha]_D$ +39,1 ° (*c* 1,01 in $CHCl_3$); ν_{max} 3340 (amine), 1758 (ester) and 1694 (amide) cm^{-1}; δ_{II} ($CDCl_3$) 3,82 (3H, s, OCH_3), 3,87-4,00 (2H. m, O-CH_2), 4,79 (1H, m, -CH-), 5,17 (2H, s, benzylic protons), 7,40 (5H, s, phenyl protons) (Found C, 52,23; H, 5,31; N, 5,06%. $C_{23}H_{30}N_2O_{10}S$ requires C, 52,47; H, 5,70 and N, 5,32%).

Dimethyl 2,5-di(N-trifluoroacetylamido)-2-hexenedioate (**100**).[164]- Ethyl *O*-methoxy-ethoxymethyl lactate[161] (**93**) (348mg, 2,0 mmol), methyl 2-*N*-trifluoroacetyl-2-propenoate (**98**) (400 mg, 2,02 mmol) and anhydrous sodium carbonate (20 mg) in anhydrous dimethylsulphoxide (5 ml) was stirred overnight at room temperature. Water (100 ml) was added and the mixture extracted with chloroform (3x). The chloroform was washed with water and then dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the product purified by flash chromatography using ethyl acetate-hexane (1:2 v/v) as eluent. The main product crystallized from chloroform-hexane to give (**100**) (55 mg, 14%), m.p. 97-98 °C, ν_{max} 3260, 1760 (ester), 1735 (ester), 1710 (amide), 1655, 1540 cm^{-1}; δ_{II} ($CDCl_3$) 2,75 (2H, m, CH_2), 3,82 (3H, s, OCH_3), 3,87

(3H, s, OCH_3), 4,71 (1H, m, CH), 6,72 (1H, dd, *J* 7,3 Hz, and *J* 8,7 Hz, C=CH), 7,71 (1H, d, *J* 9,0 Hz, NH-CH), 8,00 (1H, s, NH-C=C) (Found: C, 36,50; H, 2,92; N, 6,55%. $C_{12}H_{12}F_6N_2O_6$ requires C, 36,55; H, 3,05 and N, 7,11%)

Indole-N-*t-butyloxycarbonyl*-N-*diphenylketiminetryptophan methyl ester* (**103**).[142]- A solution of DL-*N*-diphenylketimine-tryptophan methyl ester[136] (**102**) (1,85g, 5 mmol), dimethylaminopyridine (61 mg, 0,5 mmol) and di-t-butyl-carbonate (1,2 g, 5,5 mmol) in dry acetonitrile (20 ml) was stirred at room temperature. After 1 h the solvent was evaporated under reduced pressure and the product purified by flash chromatography using diethyl ether-hexane (1:7 v/v) as eluent to give (**103**) (1,56 g, 69%), ν_{max} 2980, 1732 (ester), 1380, 1100 cm^{-1}; δ_H ($CDCl_3$) 1,58 (9H, s, t-butyl), 3,36 (2H, *J* 4,8 Hz and *J* 9,0 Hz), 3,74 (3H, s, OCH_3), 4,45 (1H, *J* 4,8 Hz and *J* 9,0 Hz), 6,66-8,2 (15H, m, 2x phenyl, 1x indole) (Found: C, 74,36; H, 5,99; N, 5,93%. $C_{30}H_{30}N_2O_4$ requires C, 74,69; H, 6,22 and N, 5,81%).

Methyl 2-acetoxyacrylate (**111**) A solution of dimethylaminopyrridine (2,1 g), triethylamine (24 ml) and acetic anhydride (50 ml) was added dropwise to a stirred solution of methyl pyruvate (16,6 g, 0,163 mol)in acetic anhydride (50 ml) while the temperature was maintained at 0-5 °C. After 1 h the reaction mixture was poured onto ice. After 6 h the mixture was extracted with diethyl ether and a few crystals of hydroquinone (a radical scavenger used as stabilizer) were added. The solvent was evaporated under reduced pressure and the residual material purified by distillation under reduced pressure (b.p. 85 °C at 20 mmHg), into a flask containing a few crystals of hydroquinone to give (**111**) (12,1 g 52%); δ_H ($CDCl_3$) 2,21 (3H, s, CO-CH_3), 3,78 (3H, s, ester OCH_3), 5,44 (1H, d, *J* 1,3 Hz, *E*-vinylic proton), 6,02 (1H, d, *J* 1,3 Hz, *Z*-vinylic proton).

Methyl N-*benzylglycinate* (**107**).[168]- Methyl phenylaldimine glycinate (3,74 g, 21,1 mmol) in methanol was cooled to 0 °C. Sodium borohydride (1,32 g, 20 mmol) was added in

portions and the mixture stirred for 30 min (when effervescence ceased). Water was added and the mixture extracted with diethyl ether. The organic solvent was dried (Na_2SO_4) and evaporated under reduced pressure. The product was purified by distillation (120°C at 0,5 mmHg) using a Kugelruhr apparatus to give (**107**) (2,0 g, 53%); δ_{II} ($CDCl_3$) 1,90 (1H, s, NH), 3,42 (2H, s, NH-CH_2), 3,70 (3H, s, OCH_3), 3,80 (2H, s, benzylic protons), 7,32 (5H, s, aromatic protons).

N-*Benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidines* (**112-114**).-Methyl *N*-benzyl glycinate[168] (**107**) (1,79 g, 10 mmol), methyl 2-chloroacrylate[169] (**110**) (1,2 g, 10 mmol) and benzaldehyde (1,06 g, 10 mmol) were refluxed for 18 h in toluene with azeotropic removal of the formed water using a Dean-Stark trap. The solvent was evaporated under reduced pressure to give a mixture of three compounds which was separated by flash chromatography using diethyl ether-hexane (1:5 v/v) as eluent to give

(2R*,3S*,5S*)-N-*Benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidine* (**112**), crystals from diethyl ether-hexane (1,81 g, 48%), m.p. 105-106°C, ν_{max} 1738 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 2,49 (1H, dd, *J* 15,8 Hz and *J* 2,0 Hz, 4-H), 3,19 (3H, s, C-3 CO_2CH_3), 3,44 (1H, dd, *J* 15,8 Hz and *J* 9,6 Hz, 4-H), 3,70 (3H, s, C-2 CO_2CH_3), 3,80 (2H, s, benzylic protons), 3,99 (1H, dd, *J* 9,6 Hz and *J* 2,0 Hz, 5-H), 4,80 (1H, s, 2-H), 7,24 (5H, s, phenyl), 7,30 (5H, s, phenyl) [Found: C, 65,25; H, 5,81; N, 3,76%, M^+ 387 and 389 (ratio 3:1). $C_{21}H_{22}ClNO_4$ requires C, 65,03; H, 5,67 and N, 3,61%, *M* 387 and 389 (ratio 3:1)].

(2R*,3S*,5R*)-N-*benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidine* (**113**), obtained as an oil (248 mg, 6,6%), ν_{max} 1740 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 2,41 (1H, *J* 13,7 Hz and *J* 6,1 Hz, 4-H), 3,09 (1H, *J* 13,7 Hz and *J* 10,3 Hz, 4-H), 3,25 (3H, s, C-3 CO_2CH_3), 3,60 (3H, s, C-5 CO_2CH_3), 3,90 (1H, *J* 13,6 Hz, benzylic proton), 3,99 (1H, dd, *J* 6,1 Hz and *J* 10,3 Hz,

5-H), 4,00 (1H, *J* 13,6 Hz), 4,43 (1H, s, 2-H), 7,11-7,46 (10H, m, 2x phenyl) [Found: C, 64,75; H, 5,50; N, 3,64%, M^+ 387 and 389 (ratio 3:1). $C_{21}H_{22}ClNO_4$ requires C, 65,03; H, 5,67 and N, 3,61%, *M* 387 and 389 (ratio 3:1)] and

(2S*,3S*,5R*)-N-*benzyl-3,5-dicarbomethoxy-3-chloro-2-phenylpyrrolidine* (**114**), crystals from diethyl ether-hexane (370 mg, 9,8%), m.p. 111-112°C, ν_{max} 1730 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 2,70 (1H, *J* 13,7 Hz and *J* 8,3 Hz, 4-H), 3,15 (1H, *J* 13,7 Hz and J 2,9 Hz, 4-H), 3,55 (1H, *J* 13,5 Hz, benzylic proton), 3,64 (3H, s, C-3 CO_2CH_3) 3,80 (1H, *J* 13,5 Hz, benzylic proton), 3,81 (3H, s, C-5 CO_2CH_3), 3,90 (1H, dd, *J* 8,3 Hz and *J* 2,9 Hz, 5-H), 5,01 (1H, s, 5-H), 7,2-7,56 (10H, m, 2x phenyl) [Found: C, 65,33; H, 5,46; N, 3,58%, M^+ 387 and 389 (ratio 3:1). $C_{21}H_{22}ClNO_4$ requires C, 65,03; H, 5,67 and N, 3,61%, *M* 387 and 389 (ratio 3:1)]. *Crystal data.*- Monoclinic and space group $P2_1$, a = 11,709(2), b = 5,938(2), c = 14,074(2) Å, ß = 92,36(1)°, V 978 $Å^3$, λ = 0,7107 A, Z = 2. Crystal dimensions 0,24x0,25x0,30 mm. Intensity measurements were made with Mo-*K*α radiation (λ = 0,7107 Å; monochromator) in a four circle diffractometer in the w - 2Θ mode with 3 ≤ Θ ≤30, at an angle of 0,67 + 0,34 tanΘ. A total of 6513 unique reflections were measured of which 2678 were regarded as observed [$I > 2\sigma(I)$].Convergence with anisotropic thermal parameter, for all non-hydrogen atoms and a common isotropic thermal parameter for the hydrogen atoms, was reached at R_W = 0,026 (R = 0,042).

Methyl 4-benzylglutamate lactam (**115**).- Compound (**112**) (700 mg, 1,86 mmol) was hydrogenated in methanol (30 ml) at 100°C for 3 h at 550 KPa using palladium on carbon (10%, 60 mg) as catalyst. The catalyst was filtered off and the solvent evaporated under reduced pressure. Flash chromatography of the product using ethyl acetate as eluent gave unreacted compound (**112**) (560 mg, 80% recovery) and (**115**) (57 mg, 13,5%), crystals from diisopropyl ether-hexane m.p. 93-94°C, ν_{max} 3210, 1755 (ester), 1698 (amide) cm^{-1}; δ_{II} ($CDCl_3$) 2,02-2,33 (2H, m, CH_2), 2,60-2,97 (2H, d on m, *J* 9,3 Hz, benzylic proton and 3-H), 3,28 (1H, d, *J* 9,3 Hz, benzylic proton), 3,72 (3H, s, OCH_3),

4,06 (1H, dd, *J* 7,8 Hz and *J* 5,1 Hz, 5-H), 7,18 (1H, s, NH), 7,26 (5H, s, phenyl protons) (Found: C. 67,20; H, 6,73; N, 6,14, M^+ 233. $C_{13}H_{15}NO_3$ requires C, 66,95; H, 6,43 and N, 6,14%), *M* 233).

N-*Benzyl-3,5-dicarbomethoxy-2-phenylpyrrole* (**116**).- *Procedure 1* A solution of (**112**) (200 mg, 0,53 mmol) in chloroform was irradiated for 2 h with u.v. light (254 nm) while air was bubbled through the solution. The solvent was evaporated under reduced pressure and the products isolated by flash chromatography with chloroform as eluent. Two products were obtained *viz.* starting material (**112**) (141 mg, 70% recovery) and compound (**116**) (44 mg, 23%).

Procedure 2. Compound (**112**) (130 mg, 0,345 mmol) and zinc powder (88 mg) were stirred in a mixture of acetic anhydride and acetic acid (1:1 v/v, 10 ml) for 30 min. The solvent was evaporated under reduced pressure and the product purified by flash chromatography using chloroform as eluent. Crystallization from diethyl ether-hexane afforded crystals of (**116**) (60 mg, 51%), m.p. 109-109,5 °C, ν_{max} 2940, 1715 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 3,66 (3H, s, C-5 CO_2CH_3), 3,77 (3H, s, C-3 CO_2CH_3), 5,47 (2H, s, benzylic protons), 6,66-6,90 and 7,11-7,60 (11H, m, aromatic protons), 7,60 (1H, s 3-H) (Found: C, 72,77; H, 5,48; N, 4,09%. $C_{21}H_{19}NO_4$ requires C, 72,21; H, 5,44 and N, 4,01%).

Methyl 2,2-dimethoxypropanoate (**117**).- Pyruvic acid (88,6 g, 1 mol) and *p*-toluenesuphonic acid (5 g) in dry methanol (600 ml) were refluxed overnight while the condensed methanol was percolated through a column of molecular sieves (3Å). The methanol was evaporated under reduced pressure to about 20% of its original volume and the acid neutralized by the addition of solid sodium hydrogen carbonate. The mixture was partitioned between diethyl ether and water. The ether was dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure. The

product was distilled under reduced pressure (b.p. 46°C at 1 mmHg) to give (**117**) (50 g, 34%); δ_{II} ($CDCl_3$) 1,52 (3H, s, CH_3), 3,29 (6H, s, 2x ether OCH_3), 3,80 (3H, s, ester OCH_3). (Found: C, 48,93; H, 8,34%. $C_6H_{12}O_4$ requires C, 48,65 and H, 8,10%).

N-*Benzyl-3-acetoxy-3,5-dicarbomethoxy-2-phenylpyrrolidine* (**118**) *and diastereomers of* N-*benzyl-4-carbomethoxy-2,5-diphenyl-oxazoline* (**119**), (**120**) *and* (**121**).- Methyl 2-acetoxyacrylate[170] (**111**) (4,32 g, 30 mmol), methyl *N*-benzylglycinate[168] (**107**) (5,37 g, 30 mmol) and benzaldehyde (3,18 g, 30 mmol) in toluene (100 ml) were refluxed overnight with azeotropic removal of the formed water using a Dean- Stark trap. The solvent was evaporated under reduced pressure and the mixture flash chromatographed using diethyl ether-hexane (1:6 v/v) as eluent to give unreacted methyl 2-acetoxyacrylate (320 mg, 5,3%) as well as

N-*Benzyl-3-acetoxy-3,5-dicarbomethoxy-2-phenylpyrrolidin*e (**118**) (970 mg, 7,9%) as an oil, which contained a mixture of two diastereomers (ratio 1:1); δ_{II} 1,45 (3H, s, $COCH_3$), 1,48 (3H, s $COCH_3$), 3,50, (3H, s, CO_2CH_3), 3,68 (3H, s, CO_2CH_3), 3,72 (3H, s, CO_2CH_3), 3,74 (3H, s, CO_2CH_3), 4,08 (2H, s, benzylic CH_2), 4,40 (2H, s, benzylic CH_2), 5,27 (1H, s, 2-H), 5,93 (1H, s, 2-H), 7,1 -7,83, m, 4x phenyl),

(2R*,*4*R*,*5*R*)-N-*Benzyl-4-carbomethoxy-2,5-diphenyloxazoline* (**119**).- crystals from cyclohexane-hexane (98 mg, 1,8%), m.p. 110-115°C, ν_{max} 3030, 2850, 1736 cm^{-1} (ester); δ_{II} ($CDCl_3$) 3,16 (3H, s, CO_2CH_3), 3,75 (2H, s, benzylic protons), 4,07 (1H, d, *J* 6,6 Hz, 4-H), 5,65 (1H, d, *J* 6,6 Hz, 5-H), 6,05 (1H, s, 2-H), 7,1-7,83 (15H, m, aromatic protons) (Found: C, 76,7%; H, 5,81, N, 3,76%, M^+ 359. $C_{23}H_{21}NO_3$ requires C, 76,88; H, 5,84 and N, 3,89%, *M* 359).

(2R*,*4*S*,*5*R*)-N-*Benzyl-4-carbomethoxy-2,5-diphenyloxazoline* (**120**), crystals from cyclohexane-hexane (468 mg, 8,3%), m.p. 68-68,5°C, ν_{max} 3030, 2850, 1750 (ester) cm^{-1}; δ_{II} ($CDCl_3$) 3,37 (3H, s, C_2OCH_3), 3,63 (1H, d, *J* 8,05 Hz, 4H), 3,68 (1H, *J* 15,2 Hz,

benzylic proton), 3,99 (1H, d, *J* 15,2 Hz, benzylic proton), 5,36 (1H, d, *J* 8,05 Hz, 5-H), 5,49 (1H, s, 2-H), 7,22 (5H, s, aromatic protons), 7,33 (5H, s, aromatic protons), 7,06-7,5 and 7,56-7,83 (5H, m, aromatic protons) (Found: C, 76,78; H, 5,56; N, 3,49%, M^+ 359. $C_{23}H_{21}NO_3$ requires C, 76,88; H, 5,84 and N, 3,89%, *M* 359),

(2R*,4R*,5S*)-*N-Benzyl-4-carbomethoxy-2,5-diphenyloxazoline* (**121**) (165 mg, 3,2%) as an oil; δ_{II} ($CDCl_3$), 3,65 (3H, s, CO_2CH_3), 3,72 (2H, s, benzylic protons), 3,87 (1H, d, *J* 4,5 Hz, 4-H), 5,19 (1H, d, *J* 4,5 Hz, 5-H), 5,78 (1H, s, 2-H), 7,11 and 7,2-7,76 (15H, s and m, aromatic protons) (Found: C, 76,59; H, 5,65; N, 3,82, M^+ 359. $C_{23}H_{21}NO_3$ requires C, 76,88; H, 5,84 and N, 3,89%, *M* 359).

3-Acetoxy-3,5-dicarbomethoxy-2-phenylpyrrolidines (**123**) *and* (**124**) and (2S*,3R*,7R*,8S*)-*3,7-diacetoxy-3,5,7-tricarbomethoxy-2,8-diphenylpyrrolizidine* (**128**).- A solution of methyl 2-acetoxyacrylate[170] (**111**) (2,9 g, 20 mmol) and methyl *N*-benzaldimineglycinate (3,54 g, 20 mmol) in benzene (200 ml) were refluxed in a nitrogen atmosphere for 48 h. The solvent was evaporated under reduced pressure and the products purified by flash chromatography using diethyl ether-hexane (1:1 v/v) as eluent to give

3-Acetoxy-3,5-dicarbomethoxy-2-phenylpyrrolidine (**123**) as an oil (216 mg, 3%); δ_{II} ($CDCl_3$) 2,06 (3H, s, $COCH_3$), 2,46 (1H, dd, *J* 16,3 Hz and *J* 4,1 Hz, 4-H), 3,17 (3H, s, C-3 CO_2CH_3), 3,25 (1H, dd, *J* 16,3 Hz and *J* 10,5 Hz, 4-H), 3,76 (3H, s, C-2 CO_2CH_3), 4,23 (1H, dd, *J* 4,1 Hz and *J* 10,5 Hz, 5-H), 4,69 (1H, s, 2-H), 6,97 (1H, broad, NH), 7,23 (5H, s, aromatic protons) (Found: C, 60.10; H, 6,23; N, 5,46%. $C_{16}H_{19}NO_6$ requires C, 59,81; H, 5,91 and N, 5,36%),

3-Acetoxy-3,5-dicarbomethoxy-2-phenylpyrrolidine (**124**) (257 mg, 4,2%) as an oil; δ_{II} ($CDCl_3$) 2,10 (3H, s, $COCH_3$), 2,50 (1H, dd, *J* 15,9 Hz and *J* 9,3 Hz, 4-H), 2,88 (1H, s, NH), 3,07 (1H, dd, *J* 15,9 Hz and *J* 9,2 Hz, 4-H), 3,17 (3H, s, C-3 CO_2CH_3), 3,80 (3H, s,

C-5 CO_2CH_3), 4,06 (1H, dd, *J* 9,3 Hz, 5-H), 4,52 (1H, s, 2-H), 7,33 (5H, s, aromatic protons) (Found: C, 59,61; H, 5,88; N, 5,00%. $C_{16}H_{19}NO_6$ requires C, 59,81; H, 5,91 and N, 5,36%) and

(2S*,3R*,7R*,8S*)-*3,7-Diacetoxy-3,5,7-tricarbomethoxy-2,8-diphenylpyrrolizidine* (**128**).- (255 mg, 4,6%) crystals from chloroform-diisopropyl ether,m.p. 182-183°C, ν_{max} 2960, 1753 (ester), 1725 (ester) cm^{-1}; δ_{H} ($CDCl_3$), 2,06 (3H, s, C-3 ester OCH_3), 2,11 (3H, s, C-7 $COCH_3$), 2,46 (1H, d, *J* 14,0 Hz, 4-H), 2,95 (1H, d, *J* 14,8 Hz, 6-H), 3,03 (3H, s, C-3 CO_2CH_3), 3,10 (1H, d, *J* 14,8 Hz, 6-H), 3,18 (3H, s, C-7 CO_2CH_3), 3,74 (1H, d, *J* 14,0 Hz, 4-H), 3,83, (3H, s, C-5 CO_2CH_3), 4,69 (1H, s 2-H), 4,78 (1H, s, 8-H), 7,04-7,20 (10H, m, aromatic protons) (Found: C, 62,74; H, 5,21; N, 2,51. $C_{29}H_{31}NO_{10}$ requires C, 62,92; H, 5,60 and N, 2,53%). *Crystal data*.- Monoclinic space group $P2_1$, a = 11,452(2), b = 13,797(1), c = 17,863(2) Å, ß = 96,92(1)°, V 2804(1) $Å^3$, *and* Z = 4. Crystal dimensions 0,28x0,28x0,55 mm. Intensity measurements were made with Cu-$K\alpha$ radiation (λ = 1,5418 Å; monochromator) on a four-circle diffractometer in the w -2θ mode with $3 \leq \theta \leq 75$, at an angle of 0,54 + 0.34tanθ. A total of 6024 unique reflections were measured of which 5011 were regarded as observed [$I > 2\sigma(I)$]. Convergence, with anisotropic thermal parameters for all non-hydrogen atoms and a common isotropic thermal parameter for the hydrogen atoms, was reached at R_W = 0,053 (R = 0,064).

3-Bromo-3,5-dicarbomethoxy-2-phenylpyrrolidine (**125**) and

(2S,3R*,7R*,8S*)-3,7-Dibromo-3,5,7-tricarbomethoxy-2,8-diphenylpyrrolizidine* (**129**).-A solution of methyl 2-bromoacrylate[169] (**122**), (3,3 g, 20 mmol) and methyl phenylaldimine glycinate (3,54 g, 20 mmol) were refluxed overnight in benzene (100 ml). The formed precipitate was removed by filtration and the filtrate evaporated under reduced pressure. The reaction products were separated by flash chromatography using diethyl ether-hexane (1:5 v/v) as eluent to give three major products *viz.*

3-Bromo-3,5-dicarbomethoxy-2-phenylpyrrolidine (**125**).-(1,4 g, 20,5%) an unstable oil, as a mixture; δ_{H} 3,31 (3H, s, ester OCH_3), 3,36 (3H, 2, ester OCH_3), 3,70 (3H, s, ester OCH_3), 3,73 (3H, s, ester OCH_3), 4,83 (2H, s, 2-H), 7,23 (10H, s, phenyl) and

(2S*,3R*,7R*,8S*)-*3,7-Dibromo-3,5,7-tricarbomethoxy-2,8-diphenylpyrrolizidine* (**129**), crystals from chloroform-hexane (300 mg, 5%), m.p. 190,5-191°C, ν_{max} 2950, 1762 (ester), 1731 (ester) cm^{-1}; δ_{H} ($CDCl_3$) 2,92 (1H, d, *J* 13,3 Hz, 6-H), 3,253 (3H, s, C-3 CO_2CH_3**), 3,254 (3H, s, C-7 CO_2CH_3**), 3,32 (2H, s, C4 protons), 3,80 (1H, d, *J* 13,3 Hz, 6-H), 3,88 (3H, s, C-5 CO_2CH_3), 4,67 (1H, s, C2 proton), 4,97 (1H, s, 8-H), 6,91-7,28 (10H, m, aromatic protons) (Found: C, 50,52; H, 4,22; N, 2,38%. $C_{25}H_{25}Br_2NO_6$ requires C, 50,42; H, 4,20 and N, 2,35%).

** These chemical shifts can be interchanged.

(2S*,3R*,7R*,8S*)-*3,5,7-Tricarbomethoxy-3,7-dichloro-2,8-diphenylpyrrolizidine* (**130**).- A solution of methyl 2-chloroacrylate[169] (**110**), (6 g, 50 mmol) and methyl benzaldimineglycinate (8,85 g, 50 mmol) were refluxed for 48 h in toluene (150 ml) while the condensate was percolated through a column containing molecular sieves (3Å). The solvent was evaporated under reduced pressure and the main compound isolated by flash chromatography using diethyl ether-hexane (1:5 v/v) as eluent. The impure material was rechromatographed on a medium pressure silica gel column using the same solvent system and crystallized from chloroform-hexane to give (**130**) (885 mg, 7%), m.p. 202-202,5°C, ν_{max} 2970, 1761 (ester), 1732 (ester) cm^{-1}; δ_{H} ($CDCl_3$) 2,84 (1H, d, *J* 13,3 Hz, 4-H), 3,19 (6H, s, C-3 and C-7 CO_2CH_3), 3,24 (2H, s, 6-H), 3,74 (1H, d, *J* 13,3 Hz, 4-H), 3,89 (3H, s, C-5 CO_2CH_3), 4,60 (1H, s, 2-H), 4,90 (1H, s, 8-H), 6,87-7,07 and 7,14-51 (10H, m, aromatic protons) (Found: C, 59,16; H, 4,72; N, 2,76%. $C_{25}H_{25}Cl_2NO_6$ requires C, 59,28; H, 4,94 and N, 2,77%).

3-Acetoxy-N-*benzyl-2-(*N-*t-butyloxycarbonyl-3-indolyl)-3,5-dicarbomethoxypyrrolidine* (**133**).- A mixture of methyl 2-acetoxyacrylate[170] (**111**) (3,0 g, 21 mmol), methyl *N*-benzylglycinate (**107**) (3,6 g, 20 mmol) and *N*-t-butyl-oxycarbonylindole-3-aldehyde (**132**) (4,9 g, 20 mmol) in toluene (50 ml) containing molecular sieves (3Å) (5 g) was refluxed overnight. The solution was decanted from the molecular sieves and the solvent evaporated under reduced pressure. The main product was isolated by flash chromatography using ethyl acetate-hexane (1:5 v/v) as eluent. The product crystallized from diisopropyl ether-hexane to give (**130**) (2,0 g 18%), m.p. 156-156,5°C; δ_{H} ($CDCl_3$) 1,65 (9H, s, CMe_3), 2,10 (3H, s, $COCH_3$), 2,30 (1H, dd, *J* 16,0 Hz and *J* 2,0 Hz, 4-H), 3,04 (3H, s, C-3 CO_2CH_3), 3,46 (1H, dd, *J* 16,0 Hz and *J* 10,2 Hz, 4-H), 3,68 (3H, s, C-5 C_2OCH_3), 3,90 (2H, s, benzylic protons), 3,95 (1H, dd, *J* 2,0 Hz and *J* 10,2 Hz, 5-H), 5,11 (1H, s, 2-H), 7,33-8,20 (10H, m, phenyl and indole protons) (Found: C, 65,21; H, 6,48; N, 5,09%. $C_{30}H_{34}N_2O_8$ requires C, 65,45; H, 6,18 and N, 5,09%). *Crystal data*.- Monoclinic space group $P2_1/n$, a = 17,837(2), b = 11,526(2), c = 15,931(1) A, ß = 114,11(1)°, V 2990(1) Å^3, and Z = 4. Crystal dimensions 0,30x0,34x0,35 mm. Intensity measurements were made with Cu - $K\alpha$ radiation (λ = 1,5418 Å; graphite monochromator) on a four circle diffractometer in the w - 2Θ mode with $3 \leq \Theta \leq 78$, at an angle of 0,43 + 0,34tanΘ. A total of 6618 unique reflections were measured of which 5349 were regarded as observed [$I > 2\sigma(I)$]. Convergence, with anisotropic thermal parameters for all non-hydrogen atoms and a common isotropic thermal parameter for the hydrogen atoms was reached at R_W = 0,064 (R = 0,069).

3-Acetoxy-N-*benzyl-3,5-dicarbomethoxy-2-(3-indole)pyrrolidine* (**134**).- The t-butyl-oxycarbonyl deblocking of compound (**133**) was done using two procedures.

1. Hydrolytic procedure.- Compound (**133**) (200 mg, 0,36 mmol) was stirred overnight in a 8M HCl solution in methanol (10 ml). The solvent was evaporated under reduced pressure and the product purified by flash chromatography using ethyl acetate-hexane (1:3 v/v) as eluent to give (**133**) (58 mg, 35%) as an oil.

2. *Thermal procedure.*[171]- Compound (**133**) (550 mg, 10 mmol) was heated in a nitrogen atmosphere in an oil bath to 180°C and maintained at that temperature until effervescence ceased (± 20 min). The mixture was cooled and then flash chromatographed using ethyl acetate-hexane (1:3 v/v) as eluent to give (**134**) (240 mg, 53%) as an oil; δ_{H} ($CDCl_3$) 2,10 (3H, s, $COCH_3$), 2,36 (1H, dd, *J* 16,2 Hz and *J* 2,0 Hz, 4-H), 2,97 (3H, s, C-3 CO_2CH_3), 3,51 (1H, dd, *J* 16,2 Hz and *J* 9,9 Hz, 4-H), 3,69 (3H, s, C-5 CO_2CH_3), 3,79 (1H, d, *J* 15,6 Hz, benzylic proton), 3,97 (1H, d, *J* 15,6 Hz, benzylic proton), 3,97 (1H, dd, *J* 2,0 Hz and *J* 9,9 Hz, 5-H), 5,20 (1H, s, 2-H), 7,03-7,6 and 7,77-8,00 (10H, m, phenyl and indolyl protons), 8,37 (1H, s, indole NH) (Found: C, 66,07; H, 5,61; N, 5,92%. $C_{25}H_{26}N_2O_6$ requires C, 66,67; H, 5,78 and N, 6,22%).

3-Acetoxy-3,5-dicarbomethoxy-2-(-3-indolyl)-pyrrolidine (**135**).- Compound (**134**) (65 mg, 0,15 mmol) in methanol (20 ml) was stirred at 105°C in a hydrogen atmosphere (20 atm pressure) in the presence of a palladium on carbon catalyst (10%, 10 mg). The catalyst was filtered off and the product purified by flash chromatography using ethyl acetate-hexane (1:1 v/v) as eluent to give (**135**) (13 mg, 27%) as an oil; δ_{H} 2,10 (3H, s, $COCH_3$), 2,60 (1H, m, 4-H), 3,13 (3H, s, C-3 CO_2CH_3), 3,43 (1H, dd*, 4-H), 3,78 (3H, s, C-5 CO_2CH_3), 4,21 (1H, dd*, 5-H), 5,05 (1H, s, 2-H), 7,1 - 8,4 (5H, m, indole).

* Due to background noise coupling constants could not be measured.

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