1
Amino Acids

BY G. C. BARRETT

1 Introduction

The literature on amino acids, taken as a whole, includes elegant lessons in biology for the chemist, and conversely provides insights for the biologist into relationships between molecular structure and properties. It will please this Reviewer if some sense of this duality continues to be conveyed in this Chapter, which has, as usual, been confined to the occurrence, chemistry and analysis of amino acids. The Chapter is arranged into sections as used in all previous Volumes of this Specialist Periodical Report.

Access to the literature for creating this Chapter has been by way of Chemical Abstracts (to Volume 110, issue 9) and Biological Abstracts (to issue 8 of Volume 85), supplemented by scanning major journals so as to cover adequately the literature of 1988. The abstracts coverage also nets a few citations published in 1987, and these are included to give continuity for the topic over the years in this series of Specialist Periodical Reports.

2 Textbooks and Reviews

One of the Tetrahedron 'Symposia-in-Print' series (which comprise collections of original research papers), is devoted to α-amino acid synthesis, describing current themes and practice in this field.[1] Reviews have appeared on methionine sulphoxide[2] and cross-linking amino acids in proteins,[3] the latter in a Volume of that covers other amino acids in the protein context. Further reviews are cited in the relevant sections later in this Chapter.

A Russian language text[4] originates from a research group active in the amino acids field.

3 Naturally Occurring Amino Acids

3.1 Isolation of Amino Acids.- While this would be thought of as a routine topic, there is a salutary lesson in the comparison of five different extraction methods for Serpula lacrimans;[5] the amino acid profile determined for this fungus varies widely, depending on the extraction procedure.

The adsorption of N-acetyl cysteine from solution on to activated carbon has been described.[6]

3.2 Occurrence of Known Amino Acids.- There is a vast and continuing literature on familiar amino acids in familiar biological contexts, and this is almost entirely excluded from this Chapter. This Section is restricted, as in previous Volumes of this Specialist Periodical Report, to the occurrence of unusual amino acids, and other significant relevant observations.

Amino acids present in carbonaceous chondrite meteorites have been reviewed.[7] The content of ornithine in fossil bones increases with age;[8] in samples of known age (1 100 - 37 000 years), reasonable linearity of correlation of ornithine content with age has been established, which suggests a useful method for fossil dating, as an alternative to enantiomer-ratio age determination (see Section 6.1).

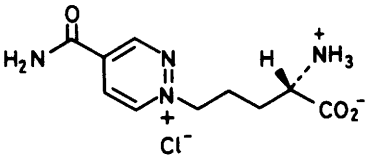
The simplest amino acids continue to be found as their betaines in natural sources. Glycine betaine occurs in Echinacea purpurea and angustifolia, then finds its way into pharmaceutical preparations;[9] L-alanine betaine occurs in the marine green alga Cladophora.[10] Hydroxylated analogues of simple amino acids include β-hydroxy-L-valine in fruiting bodies of Pleurocybella porrigens,[11] and β-hydroxyaspartic acid and N^{δ}-hydroxyornithine (with homoserine and citrulline) in pyoverdins and azotobactins.[12] The isolation of (S)-(+)-α-methylserine in Sphagnum palustre represents the first observation of the occurrence of this amino acid in plants.[13] α-Hydroxymethylserine occurs (with L-citrulline) in "tianhuafen", the root tuber of Trichosanthes kirilowii.[14]

The continuing fascination of phycobiliproteins (see Vol.20, p.1) from cyanobacterium Mastigocladus laminosus is expressed in the report[15] that these contain three N^{β}-methylasparagine residues. Occurrences of other relatively simple α-amino acids and analogues include 1-(malonylamino)cyclopropane-1-carboxylic acid in soya bean (Glycine soja) seedlings,[16] N^{α}-malonyl-D-tryptophan as the only D-amino acid that accumulates during wilting of tomato leaves,[17] (S)-4-chloro-tryptophan in seed protein of the pea plant (Pisum sativum),[18] and 4-amino-anthranilic acid in Streptomyces flocculus.[19] The last-mentioned 'β-amino acid', not previously observed in Nature, is an important discovery as a part of a new shikimate pathway. Another "first observation" is of 2-acetylamino-3-hydroxy-4-methyloct-6-enoic acid in Neocosmospora vasinfecta E.F.Smith;[20] the amino acid itself is well known as a constituent of the peptide cyclosporin A (which is also produced by this fungus).

A careful study[21] has established the absence (contrary to previous reports) of β-leucine in human blood.

The report[22] of the presence of 1-amino-2-propanol in Onopordum acanthium (ca 400 mg g^{-1} fresh weight) and 11 other Compositae is worth recording in this Chapter (it is not an amino acid, but is at least, a close relative).

3.3 New Natural Amino Acids.- The L-ornithine-based α-amino acid (1) is a new antifungal agent (from Streptomyces violaceoniger griseofuscus Tü 2557.[23] It is



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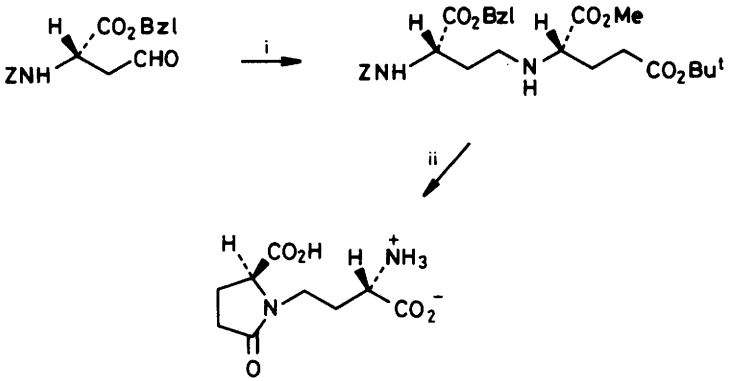

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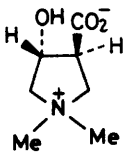
Reagents: i, H·Glu(OBu^t)OMe and $NaBH_3CN$; ii, routine elaboration

Scheme 1



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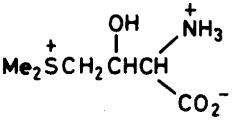

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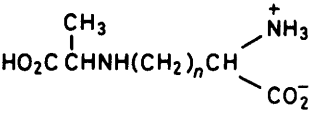
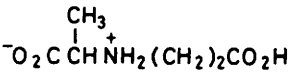


(5; a: $n = 3$; b: $n = 4$)



(6)

the first pyridazine discovered among microbial secondary metabolites. A compound from the mushroom Lactarius piperatus[24] that is, at first sight, similar to (1) is, however, on closer inspection, clearly shown to be an N-alkylated L-glutamic acid (2 in Scheme 1). The n.m.r. structure determination of (2) has been verified through its straightforward synthesis from γ-t-butyl-L-glutamic acid.[24]

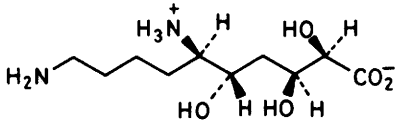
Further new amino acids showing similarities with (1), on the basis of their betaine or mixed zwitterion structures, are the hydroxyproline relative (3) and the L-methionine derivative (4).[25] Both (3) and (4) are from marine algae; (3) from Grateloupia proteus, and (4) from Lophocladia lallemandi.[25]

Making comparisons between amino acids on this superficial basis is not intended to indicate similarities in biosynthetic pathways, but the link between (2) and the opines [N-(α-carboxyalkyl)- α-amino acids] is less tenuous. New opines continue to be reported more frequently than many other types of α-amino acid; can a rational explanation for the ubiquity of this class of compound be the subject of much longer gestation? In addition to the recently-discovered ornithine analogue (5a) produced by Streptococcus lactus cultured on an arginine-deficient medium, (2S,8S)-N^6-(1-carboxyethyl)lysine (5b) has now been detected.[26] Among further new opines are β-alanopine, N-[(R)-1-carboxyethyl]-β-alanine (6), from the adductor muscle of the blood shell Scarpharca broughtonii;[27] and vitopine (details absent from Chemical Abstracts source of this citation.[28]

Full details are available for galantinamic acid (7), a component of galantin, shown to be (2R,3S,5S,6R)-6,10-diamino-2,3,5-trihydroxydecanoic acid.[29,214]

3.4 New Amino Acids from Hydrolysates.- New amino acids that have been discovered as residues in peptides and proteins are described in this Section, whether or not they are actually liberated as such by hydrolysis. Even so, the section would be substantial if it attempted to cover thoroughly, for example, newly-discovered compounds with amino acid side-chain - carbohydrate covalent links (as in glycoproteins); and no such comprehensiveness is intended. An example is asparagine, glycosidically-linked to rhamnose through the side-chain amide nitrogen atom, this moiety being released on hydrolysis of the surface glycoprotein of Bacillus stearothermophilus NRS 2004/3a.[30]

(2S,3R,5S)-3-Amino-2,5,9-trihydroxy-10-phenyldecanoic acid (8) is found (with other unusual amino acids) in hydrolysates of scytonemin A$_1$, a novel calcium antagonist from the blue-green alga Scytonema.[31] It is interesting to note the structural similarity between this new amino acid and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (9), present in cyanoginosin-LA, a cyclic heptapeptide toxin from Microcystis aeruginosa.[32]



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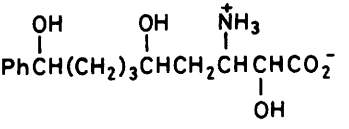

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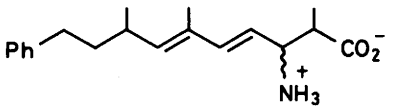

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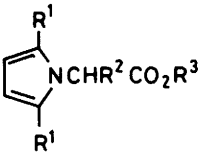

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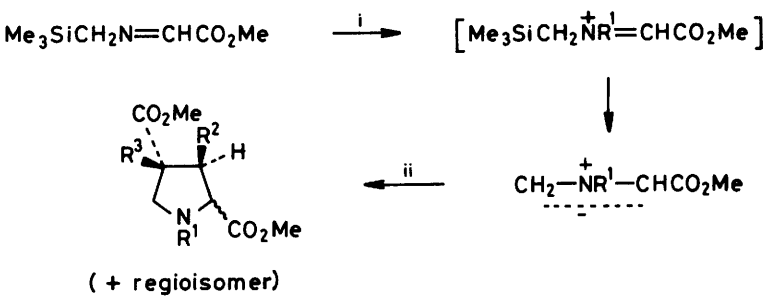
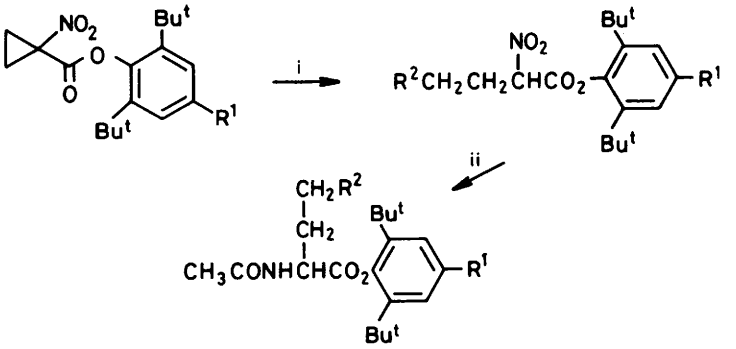


Reagents: i, R^1Br, or TFAA (catalytic) ⟶ $R^1 = H$; ii, $R^2CH{=}CR^3CO_2Me$

Scheme 2



Reagents: i, R^2X (^-CN, $^-CH(CO_2R)_2$, N_3^-, *etc.*); ii, Zn–AcOH/Ac_2O, reflux 4h

Scheme 3

4 Chemical Synthesis and Resolution of Amino Acids

4.1 General Methods for α-Amino Acid Synthesis. - The full range of standard methods of α-amino acid synthesis[33] continues to be gainfully employed, and in some cases, usefully developed. Later sections of this Chapter, particularly the next one (4.2 'Asymmetric Synthesis') and that (6.3) covering modifications of amino acid side chains (by which one amino acid can be prepared from another) are relevant for readers seeking an overall view of the recent literature on this topic.

The hydroformylation - amidoalkylation procedure has been reviewed,[34] and has been given a thorough mechanistic study using $C_6F_5CH{=}CH_2$ and $Co_2(CO)_8$-$Rh_6(CO)_{16}$ as the test-rig.[35] Distantly related amination procedures include reaction of α-hydroxyalkanoic acid esters with hydrazoic acid, diethyl azodicarboxylate, and Ph_3P,[36] and ammonolysis of 2-bromo-3-methyl-5,5,5-trifluoropentanoic acid for the synthesis of 5,5,5-trifluoro-DL-isoleucine and DL-alloisoleucine.[37]

α-Alkylation of glycine derivatives services most of the routine syntheses, diethyl 2-acetamidomalonate[38-41] continuing to be favoured; among these is a notable study[39] describing phase transfer-catalyzed alkylation 'without solvent' (although using solid KOH containing up to 15% water as base). Imines $Ph_2C{=}NCH_2CO_2R$ have been converted into α-methylene derivatives $Ph_2C{=}NC(=CH_2)CO_2R$ to be used as synthons for β-substituted alanines (Michael addition or Lewis acid-catalyzed addition).[42] A related approach[43] employs radical addition [RHgX to $CF_3CONHC(=CH_2)CO_2Me$]. The carbon radical approach [irradiation of N-hydroxy-pyridine-2-thione esters of N-protected amino acids (Vol.20, p.12, ref.159) in the presence of alkenes] has been used for the synthesis of ω-carboxyalkyl-α-amino acids.[44]

Alkylation of 2-(1-pyrrolyl)acetates (10) using an alkyl halide and lithium di-isopropylamide is a new glycine alkylation approach;[45] however, the alkyl group introduced in this way needs to be ozone-friendly if it is to survive 'deprotection'. The 'other-way-round' imines , e.g. $Me_3SiCH_2N{=}CHCO_2Me$,[46] are the source of azomethine ylides suitable for 1,3-dipolar cycloaddition reactions (e.g. in a synthesis of substituted prolines, Scheme 2).

The classical route based on alkylation of 2-phenyloxazol-5(4H)-one (formed *in situ* from hippuric acid) is widely useful (e.g. in synthesis of β-di- and -trifluoromethylphenylalanines by condensation with corresponding phenyl fluoromethyl ketones, followed by reduction and hydrolysis[47]).

α-Alkylation of an alkyl α-nitroacetate, in the various ways described above for analogous glycine derivatives,[18,48,49] is the starting point for another general amino acid synthesis. Reduction using H_2-Pt[48] or Zn-AcOH[49] completes the process, which, in the latter case, is part of an overall route to γ-substituted N-

acetylbutyrines through nucleophilic ring-opening of aryl 1-nitrocyclopropane-1-carboxylates (Scheme 3).

Substitution of the halogen atom of N-benzoyl-α-bromoglycine methyl ester[80] (see also ref.72) and the corresponding reaction with an α-methoxy amino acid,[81] illustrate other general methods. Radical conditions with 2-functionalized allylstannanes are used in the former example, leading to γδ-unsaturated α-amino acids (Scheme 4), and intramolecular electrophilic substitution in the latter.

A non-routine variant is employed in a single-step Strecker synthesis of methionine hydantoin (acrolein, ammonium carbonate, MeSH, HCN),[82] and a classical version (a ketone R^1COR^2, ammonium hydroxide or chloride, NaCN; then methacryloyl chloride, NaOH; then 12N-sulphuric acid) has been reported for the synthesis of N-(methacryloyl)amino acids $CH_2=CMeCONHCR^1R^2CO_2H$.[83]

4.2 Asymmetric Synthesis of α-Amino Acids.- Most of the methods employed are developments of standard general routes, though there seems to be no end to novelties of planning and execution. A review, with title identical with that of this Section, has appeared.[84] Later sections of this Chapter feature specific asymmetric syntheses, using both routine and novel methods.

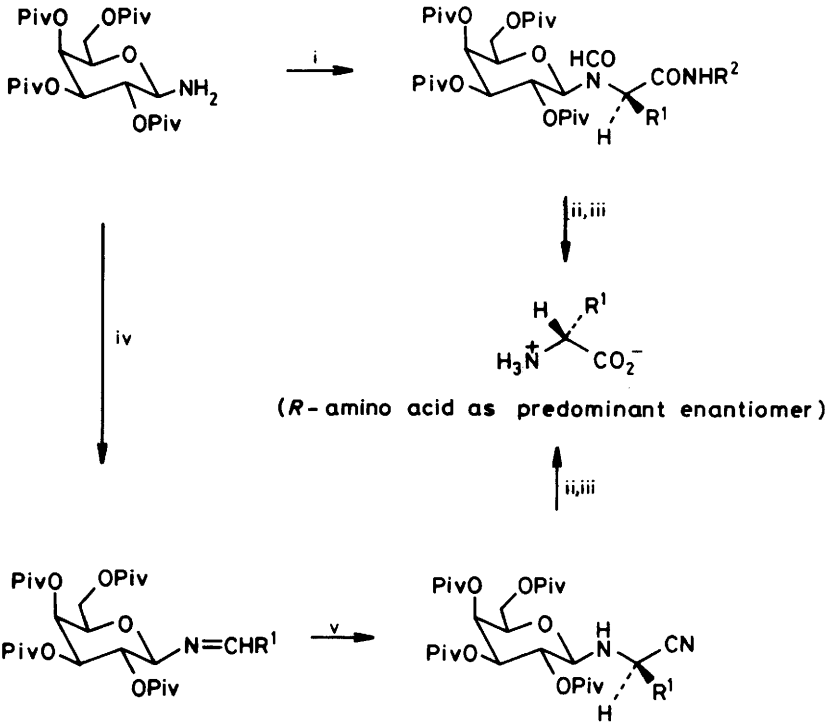
A rare example, amounting to asymmetric α-carboxylation of an amine, involves anodic α-methoxylation (cf.Vol.20,p.18) of an N-protected (S)-phenylethylamine, followed by substitution by Me_3SiCN and hydrolysis, to give a mixture of L-isoleucine and D-alloisoleucine in modest optical yield.[85] Poly[(R)-3-hydroxybutanoate], $-(CHMeCH_2COO)_n-$, is an inexpensive starting material to which a recently-introduced amination procedure (di-tert-butyl azodicarboxylate) has been applied.[86] This leads to D-allothreonine and L-threonine with only moderate diastereoselectivity, but conformational restriction (conversion of the starting material into a dioxanone analogue) raises this to 99%. Not too distantly related, and capable of very high diastereoselectivity, are asymmetric Ugi[87a] and Strecker[87b] syntheses, employing 2,3,4,6-tetra-O-pivaloyl-β-D-galactopyranosylamine as chiral auxiliary (Scheme 5). The former route can yield enantiomerically pure D-amino acids. So does the Strecker route when the Lewis acid is $ZnCl_2$ in propan-2-ol, or $SnCl_4$ in THF, but the bias is towards L-amino acids using $ZnCl_2$ in $CHCl_3$.[87]

Photolysis of Cr-carbene complexes (11) containing a chiral, optically-active amino-alcohol moiety, gives lactones in high yields and with high diastereoselectivity (Scheme 6).[88] Hydrolysis of the products gives D-amino acids with return of the chiral auxiliary.

Ammonolysis of α-keto-acids in the presence of β-cyclodextrin-functionalized pyridoxamines mimics the *in vivo* transaminase-catalyzed process with moderate success.[89]

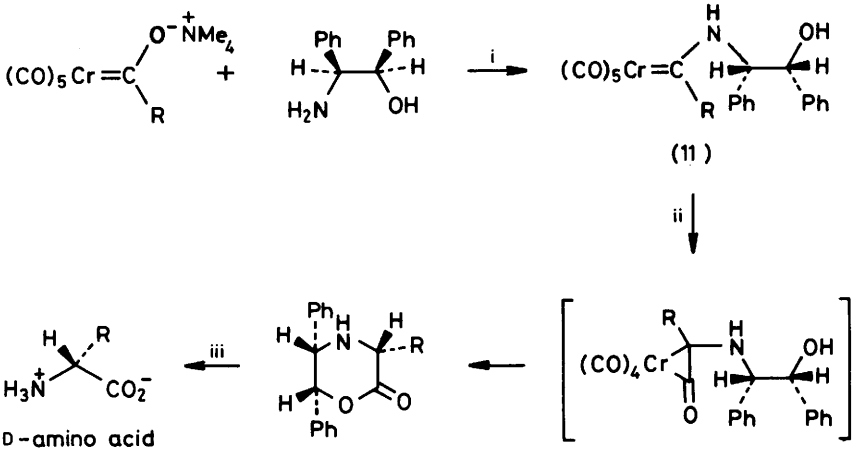
Reagents: i, R^1 = Br; aza – isobutyronitrile, toluene, 85 °C; ii, $CH_2{=}CR^2CH_2SnR_3$

Scheme 4



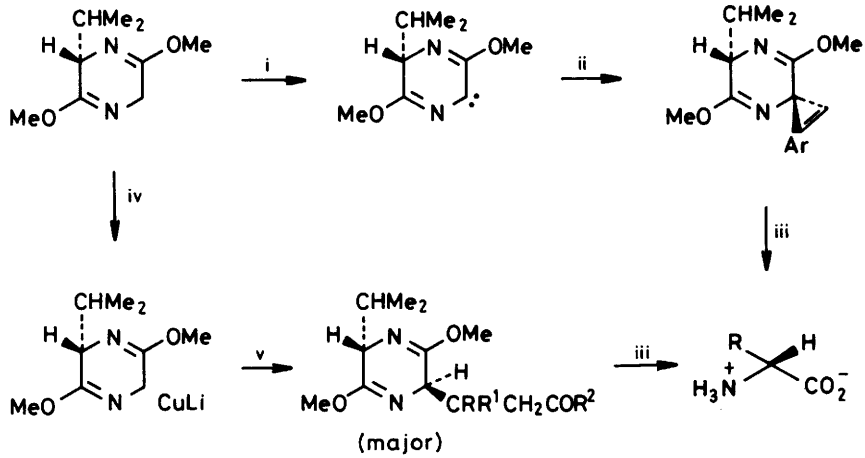
Reagents: i, R^2NC, R^1CHO, $ZnCl_2$, HCO_2H; ii, HCl–MeOH; iii, 6M–HCl, 80 °C, then Amberlite IR 200; iv, R^1CHO; v, Me_3SiCN, $ZnCl_2$(or $SnCl_4$/THF)

Scheme 5



Reagents: i, AcBr, then MeOH, argon; ii; 450 W lamp; iii H_2-$PdCl_2$

Scheme 6



Reagents : i, BuLi, $PhSO_3N_3$; ii, ArC≡CH ; iii, hydrolysis (H_3O^+); iv, BuLi, CuBr, Me_2S; v, $RR^1C{=}CHCOR^2$

Scheme 7

Synthesis of (S)-(-)-α-methylDOPA through an asymmetric Strecker route, and of (R)-(-)-α-methylserine and of D-serine by the 'bis-lactim ether' method, have been reviewed.[60] The latter method, amounting to the asymmetric alkylation of an enolized glycine moiety, has featured in every one of the last ten or more Volumes of this Specialist Periodical Report. It is featured again this year, in a showcase of papers from the research group that invented it.[61-64] Two of these reports are selected for display here, since they are both representative of the method and interesting (in different ways) in mechanistic terms (Scheme 7). In one project, (R)-N-Boc-amino-2-arylcyclopropene-1-carboxylates are formed through an asymmetric cycloaddition of a carbene to an alkyne;[62] in the other,[64] a bis-lactim ether-derived cuprate is added to αβ-unsaturated carbonyl compounds.

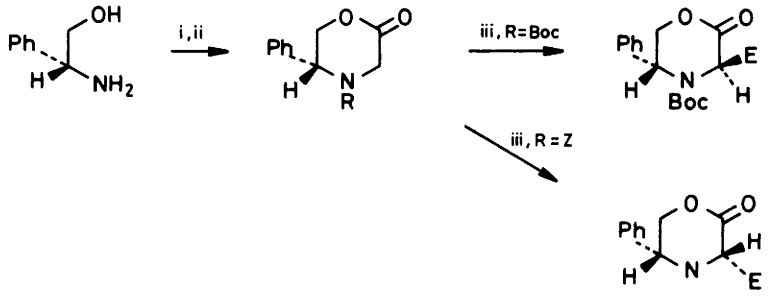
Further examples of diastereoselective substitution of glycine derivatives, the area in which most effort is being directed for asymmetric α-amino acid synthesis, have been reported. A paper that appeared at the end of 1988[65] describes a new chiral glycine enolate synthon derived from (R)-2-phenyl-2-aminoethanol ("D-2-phenylglycinol") for asymmetric syntheses (Scheme 8). This approach is modelled on an earlier classic study[66] which has been extended further (Scheme 9).[67,68] The chiral auxiliary used for this route (12) is commercially available, and can be recycled after recovery at the end of the synthesis.

There are puzzling aspects as far as diastereoselectivity is concerned, in these studies. In the 'asymmetric nucleophilic glycine' approach, the N-protecting group determines the bias as shown in Scheme 8; solvent and counterion, X^-, are also crucial.[68] Similar results in the 'asymmetric electrophilic glycine' approach[67] (Scheme 9) indicates that either retention or inversion accompanies substitution of the halogen atom, the anti-relationship resulting from substitution with inversion, being associated with easily crystallised products.

Alkynylstannanes $Bu_3SnC{\equiv}CR'$ have been used with the N-benzyloxycarbonyl analogue of (12) in an extension of the route in Scheme 9,[69] the resulting α-alkynyl-glycine derivatives being susceptible to hydrogenation/hydrogenolysis (H_2/Pd) to give γ-substituted D-butyrines.

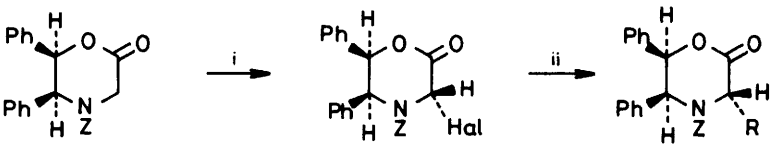
The basis of these routes is not new-found, but the natural conservatism that has made some workers reluctant to take up some of the earlier methods will be overcome by the thoroughness of studies such as these, and there should be much to report on them in future Volumes of this Report. A thoroughly-studied route with a longer history is based on the alkylation of chiral imidazolidinones (e.g. 13), and further results have been reported,[70] for the asymmetric synthesis of α-alkylated ornithines, lysines, and tryptophans.

An analogous approach using the N-acylated α-hydroxyglycine (14)[71] is flawed by the requirement of an indirect cleavage method for the acyl group at the end of the synthesis if racemization is to be avoided. The use of Steglich's α-bromo-N-Boc-glycine tert-butyl ester approach [but using the corresponding (-)-



Reagents: i, $BrCH_2CO_2Ph$; ii, RX; iii, EX

Scheme 8



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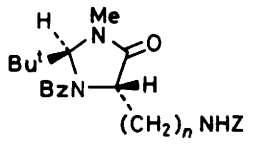
Reagents: i, NBS or Bu^tOCl; ii, R-M, $ZnCl_2$

Scheme 9



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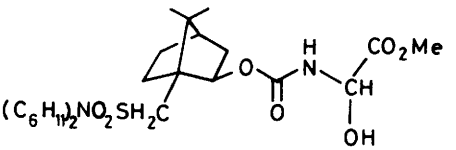

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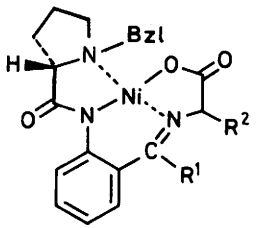

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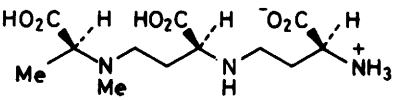


(16)

phenylmenthyl ester], represents another chiral electrophilic glycine equivalent, and has been developed further (photolysis of N-bromosuccinimide in CCl_4 for introduction of the bromine atom; substitution by Grignard reagents).[72]

Alkylation of the sodium enolate of the imine formed between glycine tert-butyl ester and (R)-camphor has been found to give low stereoselectivity, but other chiral ketones were no better from this point of view.[73] The enantiomeric excess, never more than 62%, secured in the allylation of the anion of the achiral imine $Ph_2C{=}NCH_2CO_2Me$ in the presence of a Pd(dibenzylidene acetate)-chiral diphosphine depends on the proportion of chiral ligand in the complex.[74] The analogous benzylation of ethyl N-benzylideneglycinate gives D-phenylalanine in 89.9% optical yield when conducted under phase transfer conditions in the presence of (-)-N-benzylcinchonidinium chloride.[75] The asymmetric aldol condensation between alkyl α-isocyanoalkanoates and paraformaldehyde in the presence of ferrocenyl - chiral phosphine - gold complexes to give α-alkyl serines has been studied.[76]

A substantial effort has been put into evaluating the use of the chiral nickel complexes (15), composed of an N-benzyl-L-prolinanilide moiety "overlapping" an N-arylidene-glycine[77-79] or -alanine[78-80] moiety. Alkylation by αβ-unsaturated aldehydes leads, after sodium cyanoborohydride reduction, to L-proline and its derivatives.[77] An alkyl chloride in DMF in the presence of solid NaOH leads to (2S,4S)-2,4-di-aminoglutaric acid (and the corresponding di-aminosuccinic acid)[79] and various α-methyl-α-amino acids, enantioselectivities up to 80% being achieved.[78]

A different Russian group is continuing a substantial study of $PdCl_2$ - (S)-(-)-phenylethylamine-catalyzed hydrogenolytic aminolysis of 4-arylidene-oxazol-5(4H)-ones ("azlactones").[81,82] One of these studies[81] was successfully directed towards the synthesis of (S)-p-fluorophenylalanine.

The remaining area, largely irrelevant as a practical asymmetric synthesis of α-amino acids but of continuing interest for mechanistic insights that it can offer, is the homogeneous-catalyzed hydrogenation of 2-aminoalken-2-oic acid derivatives. Rhodium(I) - chiral phosphine complexes catalyze the hydrogenation of α-acetamidocinnamic acid[83,84] with added triethylamine sharply reducing optical yields.[83] Less than 27% enantiomeric excesses were observed in an analogous study employing $[Ir(cod)(PhCN)(L)]^+ClO_4^-$ as catalysts, where L is a chiral phosphine ligand.[85]

The hydrogenation (H_2/Pd-C) of oximes and Schiff bases of amides formed between pyruvic acid and a chiral amine gives, in the best case [(R)-α-ethylbenzylamine], a 70% enantiomeric excess of L-alanine.[86]

4.3 Synthesis of Protein Amino Acids and Other Naturally-Occurring α-Amino Acids. Several examples of the synthesis of protein amino acids have been given already in this Chapter, and this Section has featured non-protein amino acids almost exclusively in recent years. A title such as this demands mention of the vast

literature on biotechnological production of protein amino acids, but as usual, only representative papers are cited here [readers are reminded of the easy access to this topic through Section 16 of Chemical Abstracts (Biotechnology and Industrial Fermentations)].

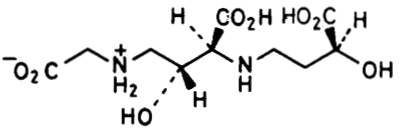
Reviews[87-93] have appeared, of large scale production of L-tryptophan,[87,88] L-phenylalanine,[89] and of natural α-amino acids generally.[90-93] By large scale in one of these reviews is meant 200 tons y^{-1} (of methionine, phenylalanine, or valine,[91] produced by acylase 'resolution' of N-acetyl-DL-amino acid esters).

The continuing importance of enzyme-catalyzed production of L-amino acids from DL-hydantoins,[88,92,94,95] and from keto-acids,[93,96,97,99] and more emphasis on unusual amino acids,[90] production of D-amino acids,[90,94,98,99] and reactor design,[91,100] is reflected in these reviews, and is also noticeable in the primary literature citations given. Particular commercial importance associated with L-DOPA,[96] glutamic acid,[101] arginine,[102] alanine,[103] and aspartic acid,[103] and several other protein amino acids, is reflected in these papers, but work on less common amino acids such as D- and L-pipecolic acid (the latter converted into L-aminoadipic acid)[98] is notable.

Just as the preceding citations are a small fraction of the total literature, research on amino acid biosynthesis pathways cannot be more than hinted at here. The first stage in conversion of crotonobetaine into L-carnitine by enterobacteria is hydroxylation of the double bond.[104] Further representative citations in this topic area can be found later in this Chapter (Section 6.2).

Reduction of methyl hydroximino-cyanoacetate HON=C(CN)CO_2Me at a lead or carbon cathode gives the amine, which by acetylation and further reduction gives the protected viomycin constituent, 2,3-diaminopropionic acid.[105] Other syntheses of relatively simple natural (non-protein) amino acids include L-discadenine [three steps and a tenfold yield improvement[106]] from L-homoserine lactone,[107,108] 3,4-seconicotianamine (16)[109] from N-trifluoroacetyl-L-aspartic β-semialdehyde ethyl ester and N-methyl-L-alanine. The interesting iron-chelating amino acid 2'-*epi*-distichonic acid A (17)[110] has been synthesized through a similar reductive coupling procedure involving (S)-$EtO_2CCH(OAc)CH_2CHO$, $NaBH_3CN$, and $NH_2CH(CO_2Bu^t)$-$CH(OAc)CH_2NBocCH_2CO_2Bu^t$ (for a further example of the same process, shown in Scheme 1, see Ref.24).

Synthesis of alicyclic α-amino acids continue to engender ingenuity of planning (and also the application of routine methods). Previously-reported synthetic studies have been completed by establishment of (1S,2S)-stereochemistry for norcoronamic acid (1-amino-2-methylcyclopropane-1-carboxylic acid).[111] αβ-Dehydroglutamic acid has been subjected to standard cyclopropane synthesis (photolysis of the stable dihydropyrazole formed with diazomethane) to give "2,3-methanoglutamic acid".[112] Ring-opening of 1-arenesulphonyl bicyclobutanes (18 ; R' = CO_2H) provides 1-amino-cyclobutane-1-carboxylic acids, including both *cis*- and *trans*-



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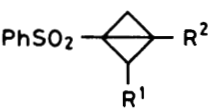

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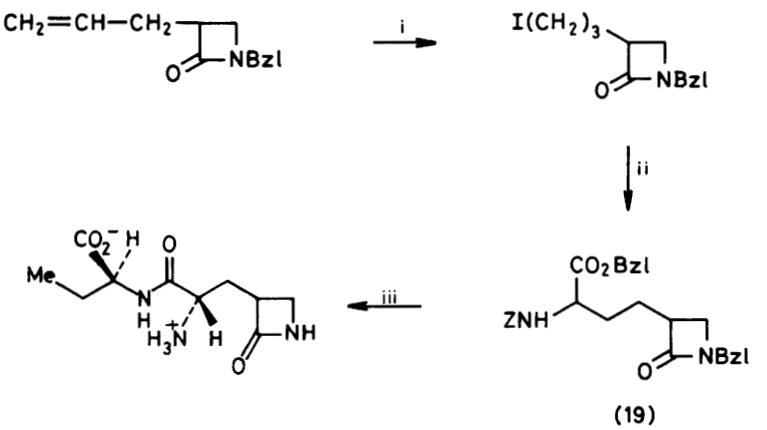
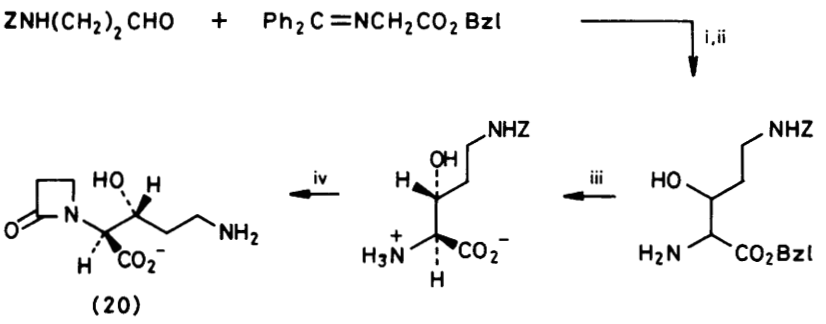


(19)

Reagents : i, dicyclohexylborane, then Chloramine T, NaI ; ii, Z - Gly - OBzl dianion; iii, routine elaboration

Scheme 10



(20)

Reagents : i, $(Me_3Si)_2NLi$; ii, 2M HCl - ether, then $NaHCO_3$; iii, *E. coli* acylase on *N*-$PhCH_2CO$ - derivative ; iv, established methods

Scheme 11

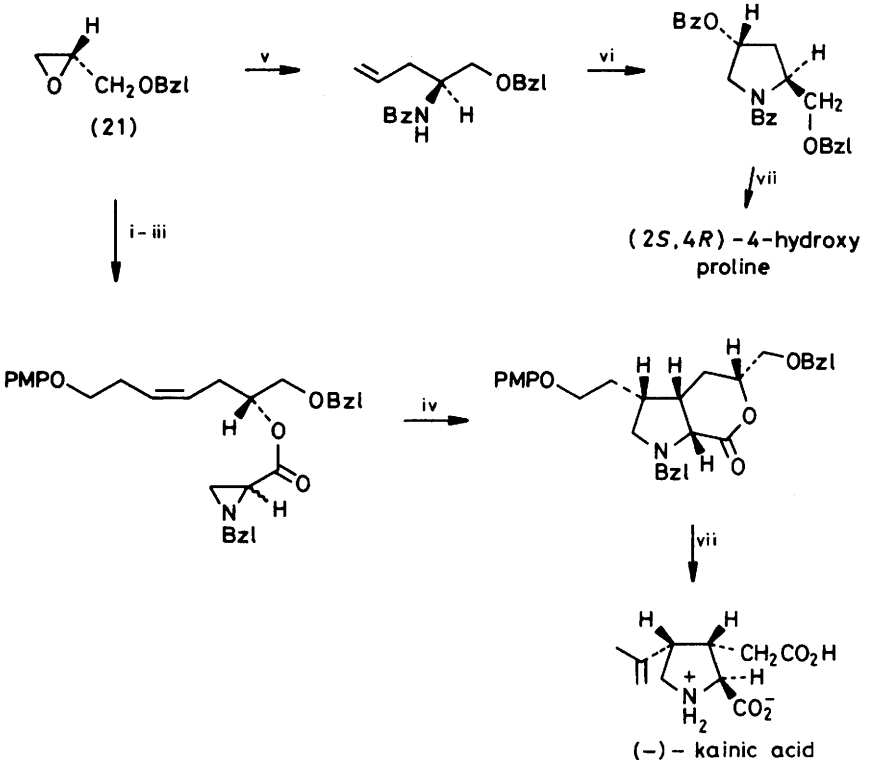
2,4-methanoglutamic acids.[113] A thorough coverage of the 1-aminocyclobutane-1-carboxylic acids from the legume Atelia herbert smithii, from the synthetic point of view,[113,114] has been extended with total syntheses of 2,4-methanoglutamic acid, 2,4-methanoproline, and cis-1-amino-3-(hydroxymethyl)-cyclobutane-1-carboxylic acid.[114] The last-mentioned compound was identical with one of the seed components [an example of the role of total synthesis in structure determination of trace constituents of plants], though the same structure for this constituent from the same plant, has been arrived at by another group (Vol.20, p.3).

This study[114] includes a synthesis of 2,4-methano-pyroglutamic acid, a possible further component of the seeds of this species. Smaller-ring lactams desoxytabtoxinine β-lactam (19)[115] and (2S,3R)-proclavaminic acid (20)[116] have been synthesized by the routes shown in Schemes 10 and 11, respectively. The synthesis of (20) involved construction of the azetidinone ring on L-β-hydroxylysine, and led to the assigned stereochemistry.

Proline derivatives provide targets for novel cyclization methods, especially since some of these compounds show extraordinary physiological properties (e.g. acromelic acids as potent neuroexcitatory agents from the poisonous mushroom, Clitocybe acromelalga). (2S,4R)-4-Hydroxyproline emerges from the iodine-mediated cyclization of 2-aminoalk-4-enols (Scheme 12),[117] and the same starting material, (S)-2-(benzyloxymethyl)oxirane (21), is used in a concise enantioselective route to (-)-kainic acid (Scheme 12; cf.Vol.20, pp.13,15).[118] A further route, an intramolecular Diels-Alder reaction with novel diastereoselectivity, has been applied for the same purpose (Scheme 13).[119] Syntheses of acromelic acids A and B (22 ; cf. Vol.20, p.15) from (-)-kainic acid, have been fully described.[120] The cobalt-mediated route established earlier (Vol.20,p.17) for proline ring closure can be used (Scheme 14) to provide acromelic acids without the restrictive requirement for (-)-kainic acid as starting material.[121]

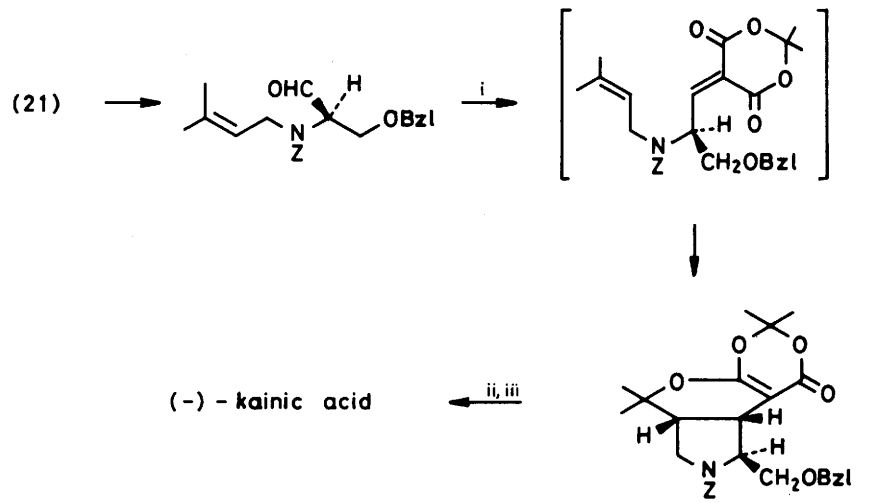
(2S,3S,5S)-3-Hydroxy-5-methyl-2-pyrrolidine-1-carboxylic acid (23 ; a component of actinomycin Z1), and its C-3 epimer, have been synthesized, starting with cycloaddition of MeC≡NO to a protected L-vinylglycine (Scheme 15).[122] The acidic proline derivative (24) from the marine red alga Schizymenia dubyi has been synthesized[123] from Z-L-pyroglutamic acid tert-butyl ester, the novel route (Scheme 16) being constructed using established synthetic operations. A similar starting point is used in a new synthesis of (-)-bulgecine (Scheme 17).[124]

Hydroxylated acyclic α-amino acids support an increasing proportion of the literature under the heading for this Section, even after relegating discussion of β-hydroxyglutamic acid to the later Section 4.16 'Synthesis of β- and Other ω-Amino Acids' because of its eligibility to be documented there, as well as here, coupled with its structural relationship with the statines discussed there. A conventional asymmetric glycine alkylation approach, in which N-(pyruvylideneglycyl)-D-phenylalaninato-copper(II) is condensed with imidazole-4-



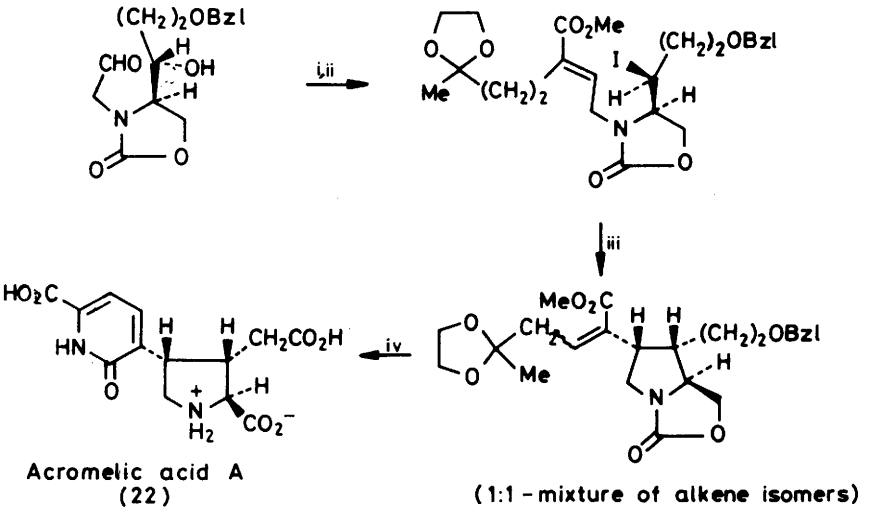
Reagents: i, MeLi, PMPO $CH_2CH{=}CH_2$; ii, H_2—Pd; iii, 2,3-dibromopropionyl chloride - Et_3N, then $BzlNH_2$; iv, 310°, xylene at high dilution, sealed tube; v, several steps including NaH - DMSO, H_2 - Pd, DEAD - phthalimide - Ph_3P; vi, I_2; vii, several routine manipulations

Scheme 12



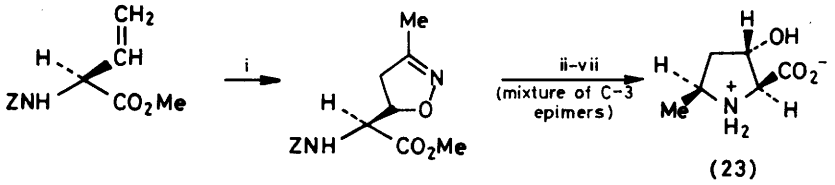
Reagents: i Meldrum's acid + $(CH_2NH_3)_2$ $2OAc^-$; ii, aq. dioxan; iii, TBDMSCl, then $NaBH_4$, Ac_2O, Bu^n_4NF, Jones' oxidation

Scheme 13



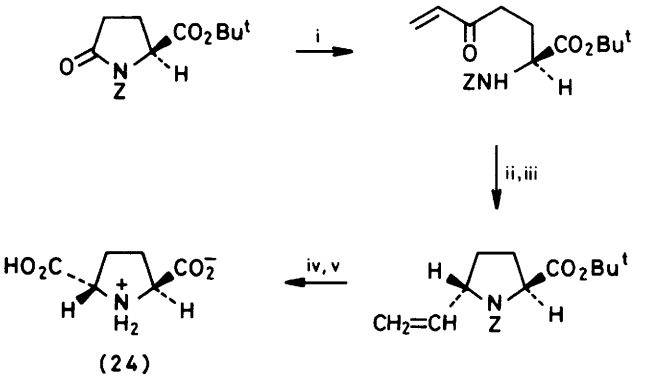
Reagents: i, $MeCO(CH_2)_2C(=PPh_3)CO_2Me$ protected as dioxalane; ii, $(CF_3SO_2)_2O$, py, NaI; iii, Co(I); iv, construction of pyridone ring (for precedent, see vol. 20, p.15)

Scheme 14



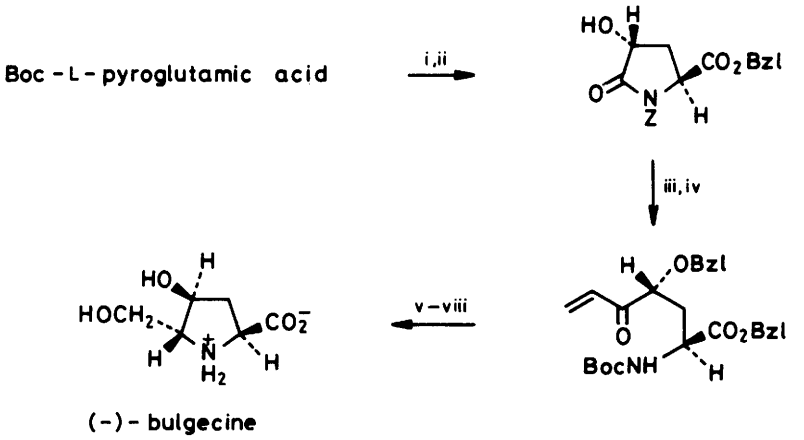
Reagents : i, $MeC\equiv\overset{+}{N}-\overset{-}{O}$; ii, separate diastereoisomers (silica gel); iii, Raney Ni, $B(OH)_3$, MeOH ; iv, H_2–Pd ; v, $NaBH_3CN$; vi, $Ba(OH)_2$; vii, ion-exchange separation

Scheme 15



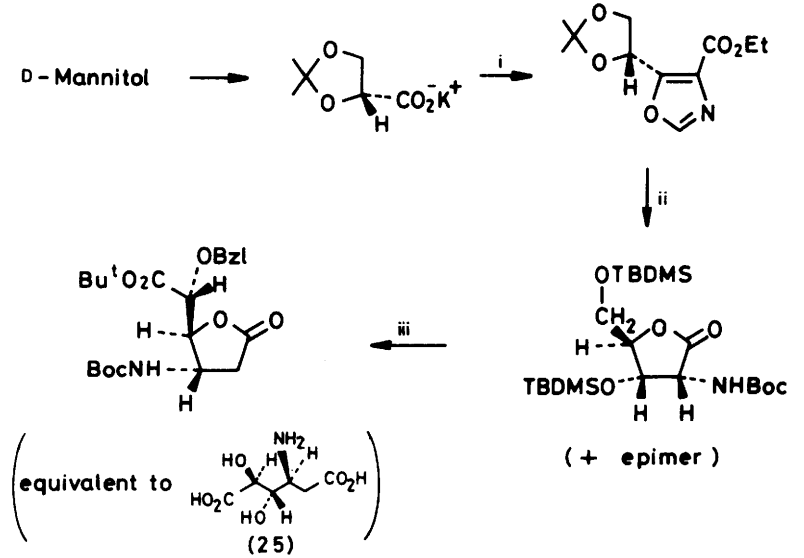
Reagents ; i, CH_2=CHMgX ; ii, $NaBH_4$, $CeCl_4$; iii, MsCl, Et_3N ; iv, O_3/–78°, MeOH ; v, TFA

Scheme 16



Reagents : i, $LiN(SiMe_3)_2$; ii, *N*-tosyl-3-phenyloxaziridine ; iii, BzOH, DEAD, Ph_3P ; iv, $CH_2{=}CHMgBr$; v, $NaBH_4$; vi, MSCl ; vii, O_3 ; viii, 1M NaOH

Scheme 17



Reagents: i, $CNCH_2CO_2Et$; ii, $MeSO_3H$, then $(Boc)_2O$, then H_2-Rh catalyst, TBDMSCl ; iii, CrO_3-py, then DIBAL, $Ph_3PCHOMe$, $Hg(OAc)_2$, NIS

Scheme 18

carbaldehyde, offers an efficient route to β-hydroxy-L-histidine, a component of the bleomycins.[125] The other β-hydroxylated α-amino acids covered this year present fearsome stereochemical challenges; these have been well confronted, syntheses of the hydroxy-amino acid moiety of AI-77-B, a gastroprotective substance from Bacillus pumilus AI-77 (25) being notable (Scheme 18).[126]

A general asymmetric synthesis for β-hydroxy-α-N-methylamino acids[127] involving efficient Sharpless epoxidation of an appropriately located side-chain double bond (but depending on the presence of a γ-CH_2 group) has been developed as a result of the ongoing interest in routes to 'MeBmt', the non-protein α-amino acid (4R)-4-[(E)-2-butenyl]-4-NN-dimethyl-L-threonine of cyclosporin. This approach is shown in Scheme 19; an alternative stereospecific synthesis[128] of this compound is shown in Scheme 20, involving the chiral auxiliary approach to generate the correct stereochemistry at the threonine side-chain moiety, and depending on this centre for all subsequent chirality (although inversion at C-2 was needed).

The syn relationship between α-amino- and β-hydroxy-groups is completely secure in a route (Scheme 21) providing D-threonine, γ-hydroxy-L-threonine, and 5-O-carbamoylpolyoxamic acid;[129] the latter compound has been synthesized from D-serine (Scheme 22), which gives a 6:1 bias in favour of the required diastereoisomer (26) in the initial stage.[130]

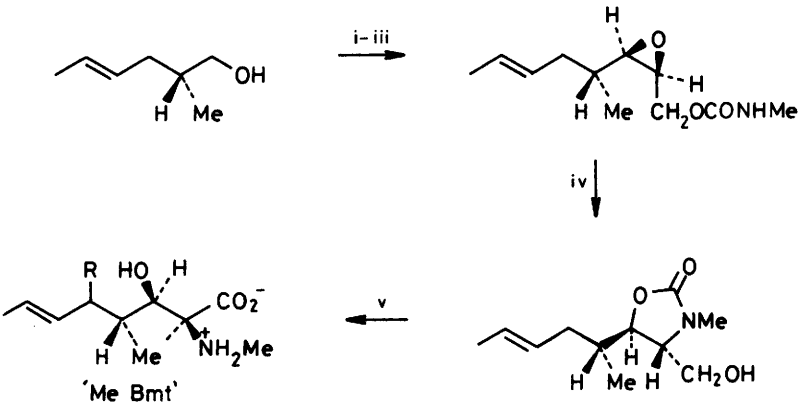
The less-common γ-hydroxy-α-amino acids are represented by the N-terminal residue of nikkomycin B, for which a synthesis has been accomplished that depends on intramolecular Diels-Alder addition of a glyoxylic acid N-benzoylimine (27 in Scheme 23) to introduce the required stereochemistry.[131]

Modifications of earlier routes (Vol.20, p.21) have led to improved syntheses of racemic ibotenic acids (28).[132,133]

4.4 α-Alkyl Analogues of Protein Amino Acids.- General methodology (some of which has been referred to in earlier sections) for the synthesis of these homologues includes the Schmidt reaction (rarely used for the purpose) with ketones ($R^1COCR^2R^3CO_2Et \rightarrow H_2NCR^2R^3CO_2Et$),[134] and alkylation of imines.[135] Michael addition of imines $RCH{=}NCHR^1CO_2R^2$ to $CH_2{=}CHR^3$ employs solid K_2CO_3 and MeOH[42] to give α-substituted α-amino acids; with aldehydes as electrophile, the same substrate gives corresponding serine homologues.[135]

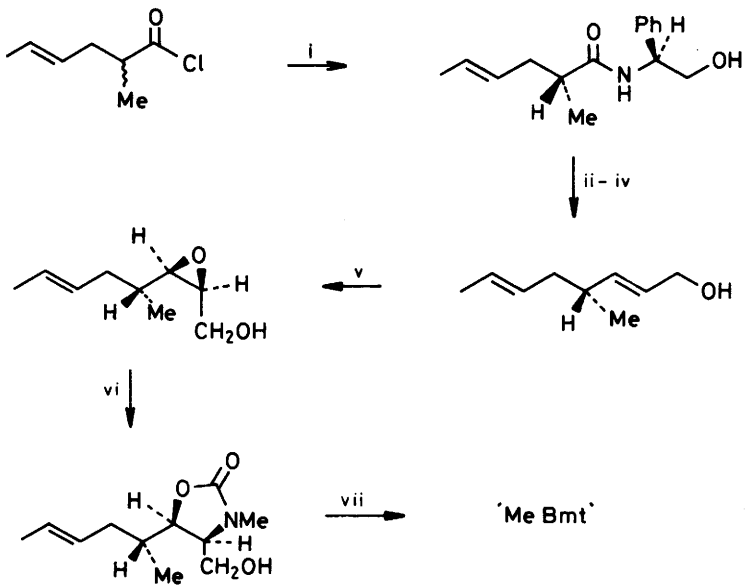
Variations of this latter process have been reported, such as the 'bis-lactim ether' route (Scheme 7) providing both enantiomers of 4-methylcycloserine.[136] Use of 4-trifluoromethyloxazoles for the synthesis (Scheme 24) of 2-substituted 3,3,3-trifluoroalanines[137] has been described.

Three standard methods leading to α-alkyltryptophans have been reported.[138] The direct approach, exemplified by the synthesis of α-methyl-L-methionine from L-methionine via the derived 1,3-oxazolidin-5-one (13 ; O in place of NMe) can be



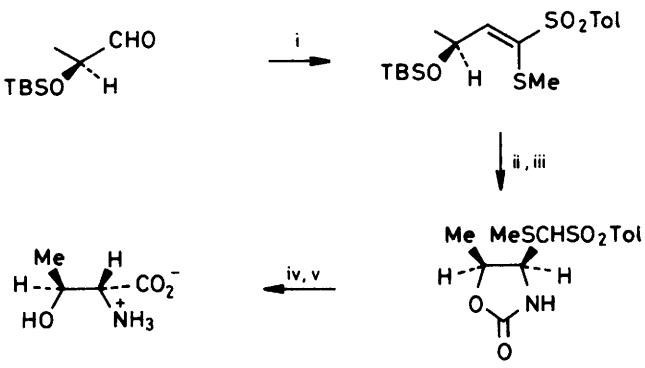
Reagents : i, DMSO-$(COCl)_2$; ii, $(MeO)_2P(O)CH_2CO_2Me$, DIBAL, Bu^tOOH, $Ti(OPr^i)_4$; iii, MeNCO; iv, NaH; v, Jones' reagent and routine elaboration

Scheme 19



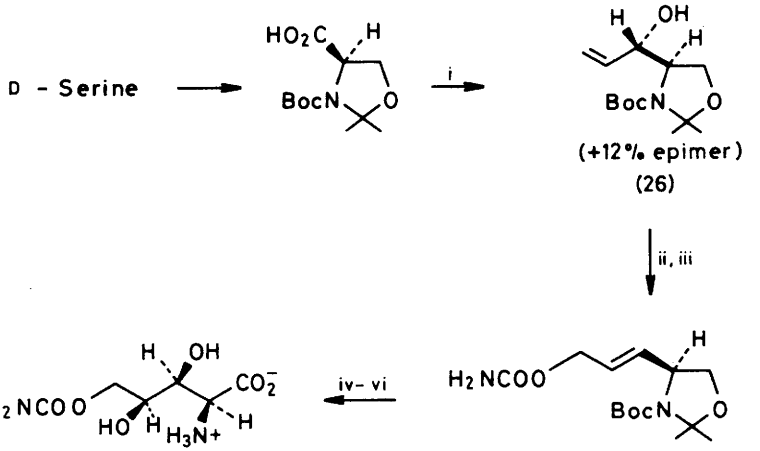
Reagents : i, L-Phenylalaninol; ii, $3N-H_2SO_4$; iii, $LiAlH_4$; iv, $(COCl)_2$-DMSO; $Ph_3P{=}CHCO_2Et$, DIBAL; v, $Ti(OPr^i)_4$, (-)-DIPT, TBMP; vi, MeNCO and NaH; vii, *cf.* Scheme 19

Scheme 20



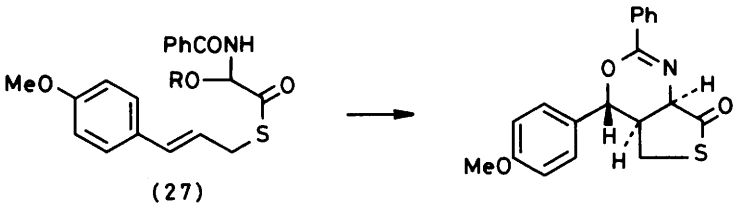
Reagents : i, $MeSCH_2SO_2Tol$, BuLi ; ii, 1M HCl then CCl_3CONCO ; iii, K_2CO_3-MeOH ; iv, MCPBA, then TFAA ; v, COSMe $\longrightarrow CO_2H$

Scheme 21



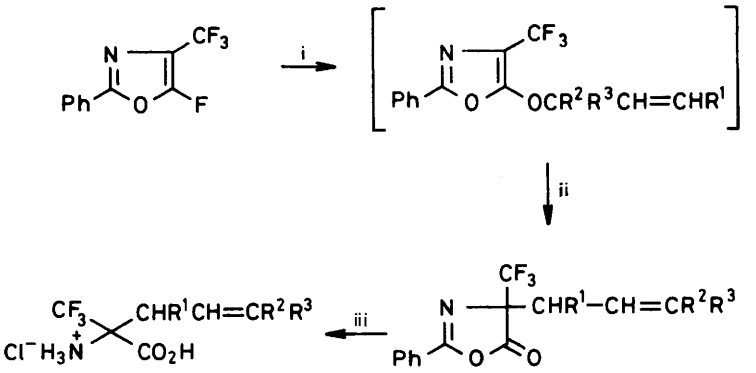
Reagents : i, $CH_2{=}CHMgBr$; ii, separate, p-NO_2-C_6H_4OCOCl, then NH_3 ; iii, $PdCl_2$ (allylic rearrangement) ; iv, TsOH, MeOH, then $KMnO_4$; v, *N*-bromo-urea (oxidation) ; vi, aq. HCl

Scheme 22



(27)

Scheme 23



Reagents: i, $R^1CH{=}CHCR^2R^3OH$; ii, spontaneous hetero-Cope rearrangement; iii, boiling conc. HCl

Scheme 24

(28)

operated on a moderately large scale (45 g L-methionine gave 10 g of its α-methyl homologue).[139]

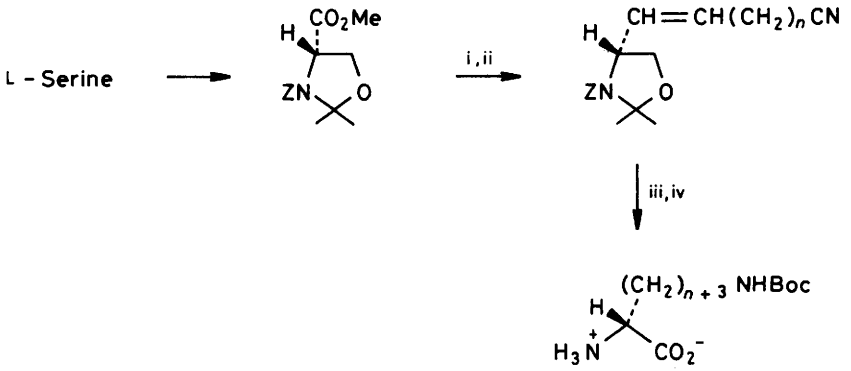
4.5 Other Aliphatic and Saturated Heterocyclic α-Amino Acids. - This section collects syntheses of aliphatic amino acids that, it so happens, are not known in natural sources. However, most of the methods are complementary to, or identical with, those in the preceding Sections of this Chapter.

Routine elaboration of the N-protected oxazolidine from L-serine (Scheme 25) provides a general enantioselective synthesis of D-α,ω-diamino-alkanoic acids,[140] but less routine is the cycloaddition of an ynamine to the oxazolone (29) leading to 3-substituted asparagines (Scheme 26).[141] All the other reports deal with cyclic structures, including enantioselective syntheses of 1-aminocyclopropane-1-carboxylic acids from the (-)-phenylglycinol-derived synthon (30)[142,143] (Scheme 27),[142] and of α-(carboxycyclopropyl)glycines from (S)-2-aminobut-3-enol (Scheme 28).[144] The fortunate capture of the halogen atom in a preparation of a 4-methyleneoxazolone and its retention during cyclopropanation with diazomethane (cf. also Ref. 112) provided a means of synthesis of 2,3-methanohomoserine and 2,3-methanomethionione.[145] Stereospecific synthesis of cis- and trans-1-amino-3-(hydroxymethyl)cyclobutane-1-carboxylic acids has been developed based on the easy availability of the bridged lactone (31).[146] All stereoisomers of 1-aminocyclopentane-1,3-dicarboxylic acid,[147,148] and of 1-aminocyclohexane-1,3-dicarboxylic acid,[148] have been prepared from corresponding 3-oxo-compounds. These, like several of the other cycloalkane-based amino acids mentioned above, attract interest as conformationally-restricted analogues of neurotransmitter amino acids; while cis-1R,3R-compounds mimic N-methyl-D-aspartic acid activity, the others are (-)-α-kainic acid-like in their effects.[147]

Gabriel-type synthesis of 3-phenylaziridine-2-carboxylates by amination of (E)-cinnamates is not stereospecific,[149] contradicting earlier assertions.[150] Ring expansion of these imino acids occurs (with retention of configuration) when they are treated with 2-chloroethanol and 2-chloroethanethiol, giving 4-oxa- and 4-thia-pipecolic acids respectively.[151] 4-Oxopipecolic acid is accessible through cyclization of tert-butyl 2-Boc-amino-4-oxo-hex-5-enoate.[152]

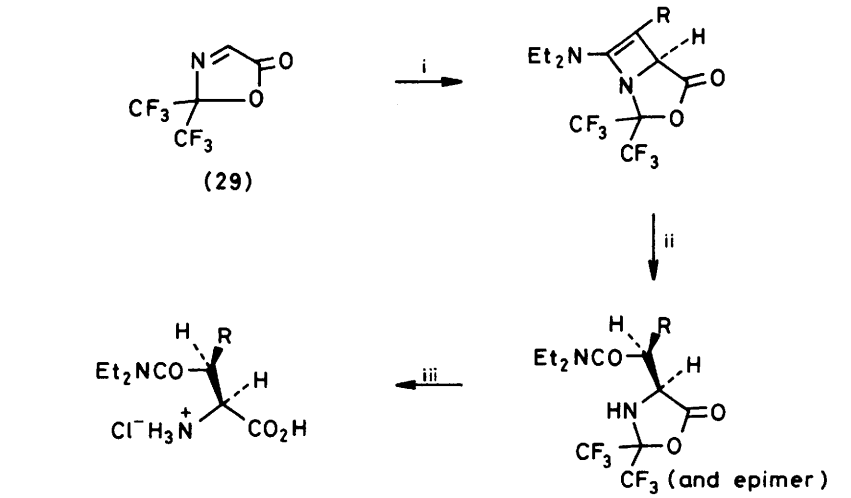
All stereoisomers of 3-ethylproline have been obtained through Michael addition of pent-2-enal or ethyl pent-2-enoate to an N-protected 2-aminomalonate, followed by routine elaboration, and resolution using (+)-dibenzyltartaric acid.[153]

Intramolecular Michael addition (32 → 33) giving 3-carboxymethylproline[154] is a simple exercise following the pioneering routes that had to be overcome for the much more demanding problems of stereospecific synthesis of the kainic acids described in preceding Volumes of this Report. The cis:trans ratio of the product (30:70) was amenable to equilibration (NaOEt/refluxing benzene) to 5:95.



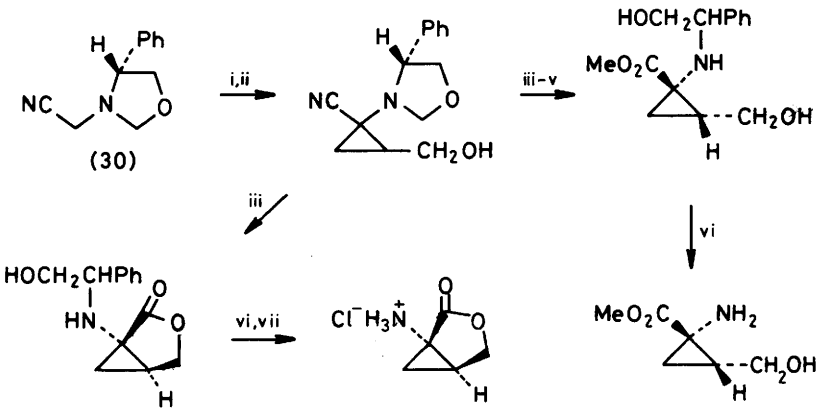
Reagents: i, DIBAL; ii, $Ph_3P{=}CH(CH_2)_nCN$; iii, $NaBH_4$ then $(Boc)_2O$; iv, TsOH, Jones' reagent, H_2–Pd

Scheme 25



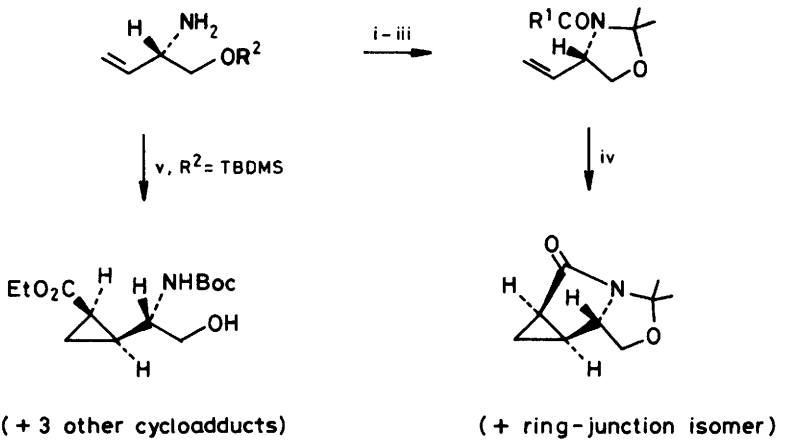
Reagents: i, $Et_2NC{\equiv}CR$; ii, 0·05M HCl; iii, conc. HCl

Scheme 26



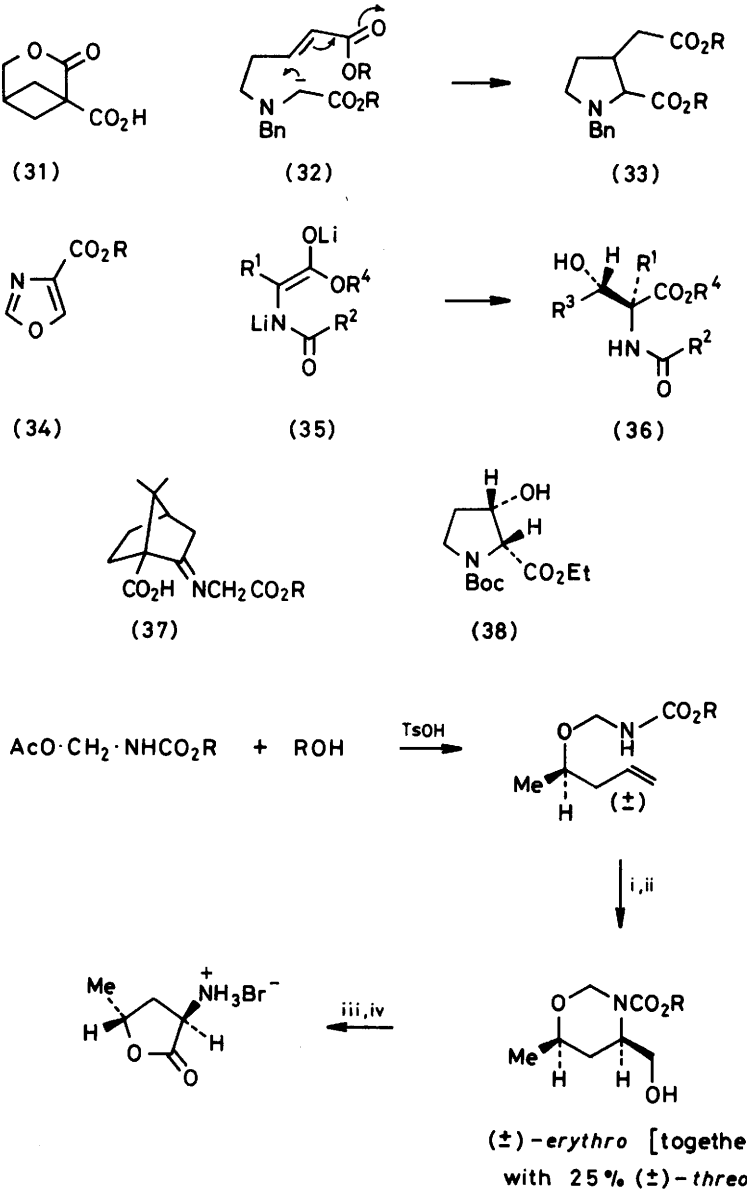
Reagents: i, LDA – HMPA; ii, epibromhydrin; iii, chromatographic separation; iv, aq. NaOH; v, $SOCl_2$ – MeOH; vi, H_2 – Pd, vii, HCl – EtOH

Scheme 27



Reagents: i *N*-Boc-Gly-*O*Su, Et_3N; ii, acetone, $(MeO)_2CMe_2$;
iii, $R^1 = BocNHCH_2 \longrightarrow CHN_2$; iv, $Pd(OAc)_2$; v, Boc_2O, then N_2CHCO_2Et, $Pd(OAc)_2$

Scheme 28



Reagents: i, $Hg(NO_3)_2$, MeCN; ii, KBr, O_2, $NaBH_4$; iii, Jones' reagent; iv, HBr–AcOH

Scheme 29

4.6 Prebiotic Synthesis of Amino Acids.- Some familiar science can be detected among recent papers reporting attempts to simulate the contents and characteristics of the primordial environment. High energy discharge experiments continue with u.v. picosecond laser pulsing at 266 nm through ammonium acrylate solution to give α- and β-alanines,[155] and electric discharge through H_2S, CH_4, N_2, NH_3, and H_2O to give methionine, thirteen protein amino acids, and one non-protein amino acid.[156]

Experiments with aqueous solutions of cyanides[157,158] reveal that much milder treatment leads to amino acids when these solutions overlay some common minerals. 2.2M-Aqueous KCN, after 26 days at 70° over sepiolite or zeolites, contains aspartic and glutamic acids, glycine, serine, threonine, and α- and β-alanines,[157] while a similar solution containing the ethyliminium cation over crystalline kaolinite, develops α-aminopropionitrile, hydrolysis giving α-alanine enriched in either L- or D-enantiomer, depending on the chirality at the adsorption site and the orientation of the cation at this site.[158] Clearly, this last-mentioned aspect is of extraordinary importance and will require confirmation.

The basis of a review[159] is that H_2S has special importance in prebiotic chemistry. A further example of the sort of armchair reasoning that has been regularly published on this topic discusses the fact that 16 of the protein amino acids possess a β-CH_2 group, and a further three possess a β-methine group;[160] it is proposed that this uniformly-sized "spacer group" has arisen through a form of prebiotic selection since properties of peptides and proteins are tailored to a purpose (here, the reasoning becomes more vague!) that is fulfilled by this structural feature.

4.7 Halogenoalkyl Amino Acids.- Noting some that some papers relevant to this section can be found in the preceding sections, since they also have relevance there, a conventional approach has been described for the synthesis of (2R,3S)- and (2R,3R)-3-bromobutyrines from appropriate threonines and from L-threo-3-methyl aspartic acid.[161]

4.8 Hydroxyalkyl Amino Acids.- A review has appeared, covering 4-alkoxycarbonyl-oxazoles (34) for use in synthesis as latent β-hydroxy-α-amino acids.[162]

Dilithiated N-acylamino acid esters give threo- and erythro-αβ-disubstituted serines through addition to aldehydes (35 → 36).[163] A similarly conventional approach has been used, but with the Schiff base (37) based on (+)-ketopinic acid as chiral auxiliary, for the synthesis of diastereoisomers of β-phenylserine.[164]

A new general method (see also Ref. 204) for the stereoselective synthesis of threo- or erythro-1,3-amino alcohols from a single precursor, based on intramolecular amidomercuration, has been used for synthesis of both

diastereoisomers of γ-hydroxy-DL-norvaline[165] (from 4-penten-2-ol; Scheme 29).

Continuation of studies (Vol. 20, p.15) employing monosaccharide lactones for stereoselective synthesis, is described in reports on (2R,3S,4R)-3,4-dihydroxyproline[166] and its enantiomer.[167] Protected (2R,3S)-3-Hydroxyproline (38) is obtained in better than 99% optical purity by yeast-mediated reduction of the corresponding 3-oxo-proline.[168]

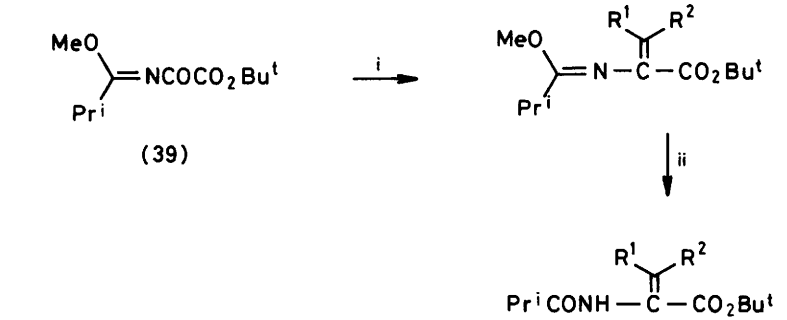
4.9 Aminoalkyl Amino Acids.- While noting that earlier sections have claimed mention of reports that could have been located here, one out-of-the-ordinary study, of the analogue of S-adenosyl-L-methionine in which the sulphur atom is replaced by the tertiary amine function (N≡ in place of $^{+}$S≡), is noteworthy.[169]

4.10 Amino Acids with Unsaturated Side Chains.- αβ-Unsaturated α-amino acids have been reviewed.[170] Standard routes based on azlactones (see also Refs. 81,82,185) include an unusual application[171] of DMF-imines RN=CHNMe$_2$ (R = various 6-membered diaza-heterocycles) for the preparation of corresponding β-RNH-substituted-methylene-α-amino acids. The first report of Wittig-Horner olefination of the cationic amino acid synthon, ethyl N-carbethoxyoxamate (39 in Scheme 30) has appeared,[172] as well as a conventional Wittig approach employing N-acyl α-(diethoxyphosphonyl)glycine ethyl esters.[173]

Interesting new routes to these 'dehydro-amino acids' involve N-carboxyanhydride formation from a 2-azido-3-substituted alkanoic acid, $COCl_2$, and $NaReO_4$,[174] and preparation of trifluoroacetyl N-methyl derivatives (Scheme 31).[175]

Some routes to DL-vinylglycine give disappointing overall yields, far lower than the 52.2% for the base-catalyzed condensation of diethyl 2-acetamidomalonate with phenyl vinyl sulphoxide in DMSO at elevated temperatures.[176] Alkylation of acetamidomalonates with tert-butyl propargylate gives '*trans*-βγ-dehydro-glutamates', from which, by routine elaboration, correspondingly unsaturated L-ornithine and L-arginine have been prepared.[177] General routes to these βγ-unsaturated amino acids have been reviewed;[178] this article essentially covers reductive elimination of 5-chloromethyloxazolines catalyzed by Vitamin B_{12} or cobalt esters for the purpose, also the highly stereoselective amidoalkylation of (E)- or (Z)-alkenylsilanes with *in situ*-generated acyliminium cations.[178]

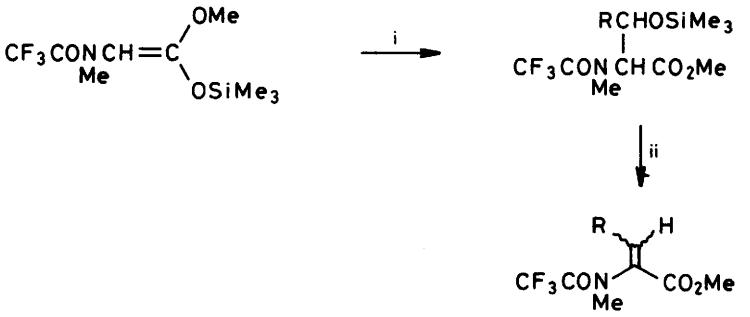
4.11 Aromatic and Heteroaromatic Amino Acids.- Some conventional studies, involving conversion of protein amino acids with aromatic or heteroaromatic groups in their side chains, into homologues through substitution reactions, are featured every year in this section (and also in the later Section 6.3). Oxygenation of L-phenylalanine, to give DOPA (and other hydroxylated phenylalanines, total yield ca. 50%) can be effected under Udenfriend conditions (Fe^{2+} - O_2 with electrochemical reduction of Fe^{3+} formed in the process).[179] Both 2- and 3-



(39)

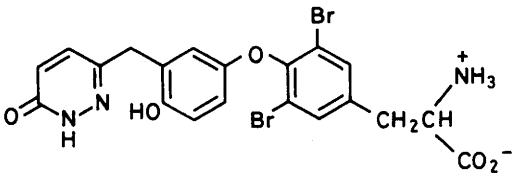
Reagents : i, $Ph_3P{=}CR^1R^2$; ii, aq. HI

Scheme 30



Reagents: i, RCHO, $ZnBr_2$; ii, 100–110° with CF_3SO_3H or $(MeSO_2)_2O$ - DMAP

Scheme 31



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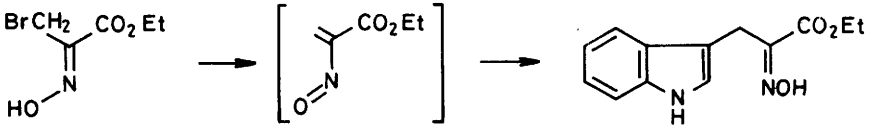


(41)

monofluorotyrosines are formed through direct (F_2) fluorination of O,N-diacetyl L-tyrosine methyl ester, the short time being the crucial feature recommending the route for use in synthesis of isotopically-labelled analogues.[180]

Synthesis of (40 ; alias SK & F L-94901, a novel thyromimetic and, from the point of view of this Section, a mixed aromatic - heteroaromatic system) has been described (see also Ref.225).[181]

Most of the 'heteroaromatic interest' focuses on tryptophan analogues. 8-Aza-tryptophan has been prepared through the bislactim ether approach, usually used (cf. Section 4.2) for asymmetric synthesis but unaccountably used in this study for the preparation of the racemate.[182] Oximes (41) formed through cyclo-addition of indoles with α-nitroso-acrylates can be reduced (Zn/AcOH) to 2-substituted tryptophans.[183] Nitration of N-trifluoroacetyltryptophan methyl ester with HNO_3 gives 60% 6-nitro- and 6.7% 2-nitrotryptophans.[184]

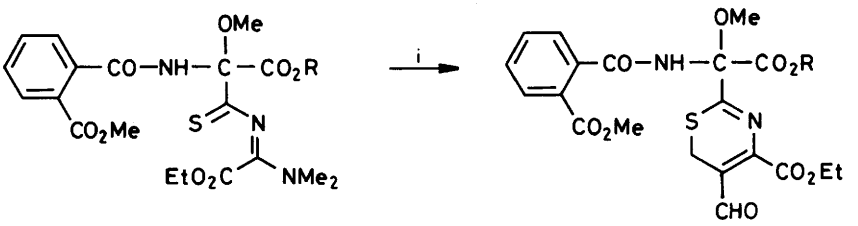
τ-N-Alkylhistidines have been prepared through the azlactone route (condensation of 1-alkyl-4-formyl-1H-imidazoles with hippuric acid and acetic anhydride).[185] Interest in the thiazine ring (present in peptide-based cephalosporins) has been of long standing, and an approach based on earlier routes (Scheme 32) for the synthesis of amino acids containing this side-chain moiety has been reported.[186]

4.12 N-Hydroxy Amino Acids.- Oxidation of Schiff bases with m-chloroperbenzoic acid and opening of the resulting oxaziridines with hydroxylamine is a serviceable route to these derivatives.[187]

4.13 Amino Acids containing Sulphur or Selenium.- Sulphenyl chlorides RSCl add to acrylic esters to give α-chloro-β-alkylthioalkanoates as major regioisomers, from which, by conventional azidolysis but unconventional reduction of the resulting azides (with H_2S-pyridine or H_2S-Re_2S_7), correspondingly substituted cysteines are obtained.[188] A similar addition approach underlies the formation of thiazoline-4-carboxylic acids from α-chloro-acrylic acid and N-methythiobenzamide,[189] N-formyl-β-alkylthio-α-amino acid esters from 'αβ-dehydroamino acids' and alkanethiols,[190] and ring-opening of cis- and trans-2-phenylaziridine-3-carboxylic acid (-)-menthyl esters with H_2S to give β-mercaptophenylalanine enantiomers.[143]

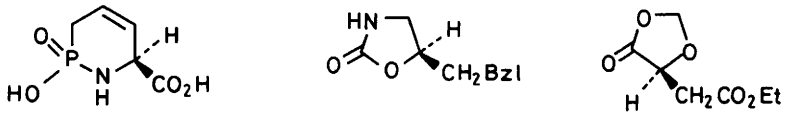
(S)-β-2- and 3-Selenienylalanines have been prepared by two standard asymmetric synthesis methods, Rh-chiral phosphine-catalyzed hydrogenation or the use of the bis-lactim ether method.[191] The enzymic resolution approach continues to show its breadth of application, with use of O-acetylhomoserine sulphydrylase for the preparation of L-selenocysteine and L-selenohomocysteine from Na_2Se_2 and acetyl derivatives of serine and homoserine.[192] The former of these has been used for the preparation of the new selenium-containing amino acid L-selenodjenkolic acid.

4.14 Phosphorus-containing Amino Acids.- Methyl α-methoxyhippurate and the N-

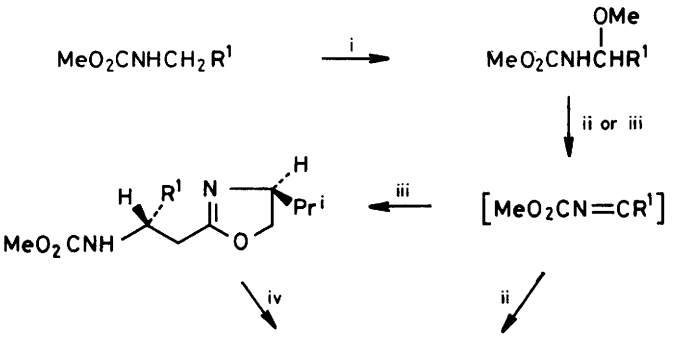


Reagents : i, CH_2=CHCHO, Amberlyst 15

Scheme 32



(42) (43) (44)



Reagents : i, e^-, MeOH ; ii, $MeCO_2R^2$, LDA, THF, - 70 °C ; iii, (*S*) - 4 - isopropyl - 2 - methyloxazoline, LDA, THF, - 70 °C; iv, 1M-HCl, then HCl - MeOH

Scheme 33

benzyloxycarbonyl analogue yield the corresponding α-phosphonylglycines, and these have been converted into phosphites with Lewis acids.[193]

Diethyl 2-(p-chloromethylbenzyl)-2-acetamidomalonate has been shown to be susceptible to Arbuzov reaction with triethyl phosphite, as well as to other halogen-substitution processes (e.g. → $CH_2SO_3^-Na^+$).[194] The plumbemicin A and B (N-1409) component (42) has been synthesised by Ugi 4-component condensation and other general methods using the aldehyde OCHCH=CHP(O)(OEt)OR and straightforward elaboration of the resulting protected 3,4-didehydro-5-phosphono-norvaline.[195]

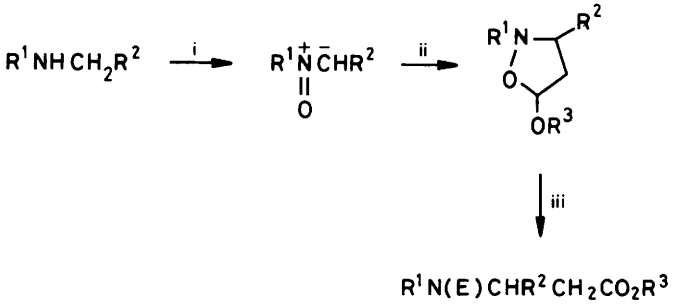
4.15 Amino Acids Synthesized for the First Time.- No reader searching for newly-synthesized amino acids should concentrate only on this Section, since several such compounds for which other points of interest are discernible, are mentioned elsewhere in this Chapter. New di-and trichlorophenylalanines,[38] ring-substituted phenylalanines and tryptophans,[196] and thyroxines,[41] have been reported.

4.16 β- and Higher Amino Acids.- A new β-amino acid synthesis[197] based on nucleophilic addition of enolate anions of alkyl acetates to N-methoxycarbonyl imines is displayed in Scheme 33. Use of chiral optically-active 2-methyl-oxazolines instead of alkyl acetates leads to 72-90% enantiomeric excesses. A high yield route (Scheme 34) is based on alkylation of amine oxides with vinyl ethers.[198] Hydrocyanation of phthalimido-alkynes catalyzed by nickel powder leads to mixtures of β- and γ-amino acids.[199]

Particular examples of synthesis targets include (S)-isoserine $NH_2CH_2CH(OH)CO_2H$, for which three independent studies claim the first enantioselective synthesis. (S)-Malic acid, via (43) formed with $(PhO)_2P(O)N_3$ and Et_3N in refluxing benzene,[200] or via Curtius rearrangement of (44),[201] and (-)-8-phenylmenthyl glyoxylate monohydrate,[202] are the chosen starting materials. In the latter study,[202] KF-promoted addition to $MeNO_2$ gives only 50% yield after work-up, but the product is optically-pure. Enantiomers of cucurbitine (45 in Scheme 35) and the near relative, (-)-3-amino-tetrahydrothiophen-3-carboxylic acid (45 ; S in place of NH), have been synthesized through a 1,3-dipolar cycloaddition sequence followed by pig liver esterase-catalyzed hydrolysis.[203]

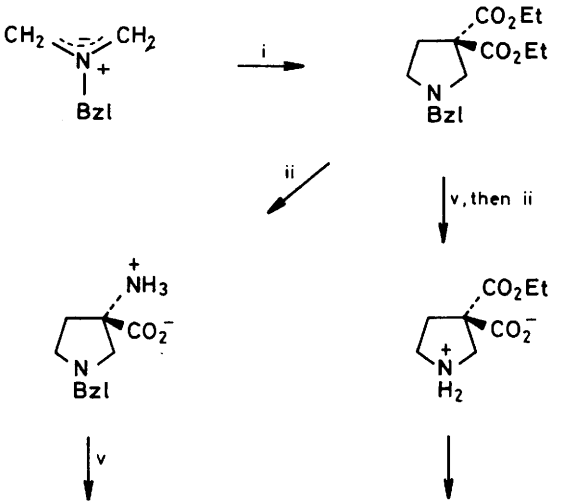
Stereoselective synthesis of racemic threo-γ-hydroxy-β-lysine lactone[204] from $PhtN(CH_2)_2CH(OCH_2NHCOOC_6H_{11})CH=CHCH_2OBzl$ provides a further example of cyclization by the amidomercuration method (see Scheme 29; ref.165).

γ-Amino acids are represented by simple examples (46)[205] and (R)-carnitine (Scheme 36)[206] as far as stereospecific synthesis is concerned, but pre-eminently by the β-hydroxy-γ-amino acid, statine and its analogues, for which several new syntheses[207-213] have been reported. A further route[207] to (3R,4S)-N-Boc-statine starting with N-Boc-L-leucinal employs the boron enolate of (47), related to the chiral synthon that has been used in an analogous synthesis of (3S,4S)-(+)-statine



Reagents: i, H_2O_2; ii, $CH_2{=}CHOR^3$; iii, E-X, then base

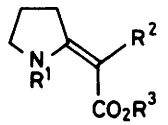
Scheme 34



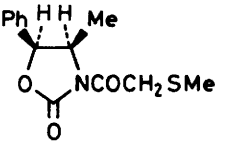
(-) - cucurbitine (45) (+) - cucurbitine

Reagents: i, $CH_2{=}C(CO_2Et)_2$; ii, pig liver esterase; iii, $ClCO_2Et$; iv, NaN_3; v, Pd-C/H_2

Scheme 35



(46; R^2 = –NC → –NHAc)



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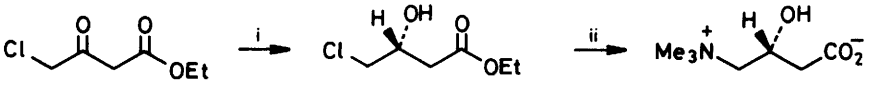
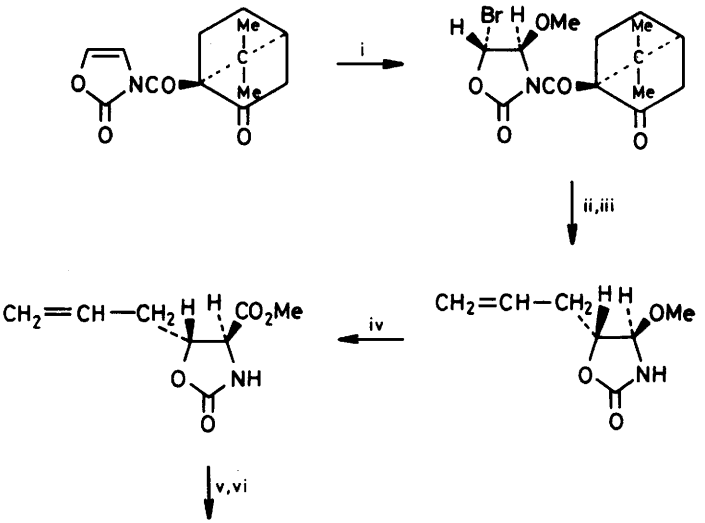


Reagents: i, (*S*)- BINAP - Ru (II), H_2, 100 atm; ii, Me_3N

Scheme 36



(2*S*, 3*R*) - 3 - hydroxyglutamic acid

Reagents: i, Br_2 - $MeC(OMe)_3$ - TMSOTf; ii, CH_2=$CHCH_2SnBu_3$; iii, Bu_2CuLi; iv, TMSCN, then MeOH; v, $KMnO_4$ + $NaIO_4$; vi, established methodology

Scheme 37

(Scheme 37),[208] and applied also to the synthesis of (2S,3R)-3-hydroxyglutamic acid.[208] Addition of the chiral acetate enolate $CH_2=C(OLi)CHPhCPh_2OLi$,[209] and of anions of malonate half-esters,[210] to α-(Boc-amino)aldehydes, and condensation of malonic esters with N-protected amino acids[211,212] followed by reduction (including yeast-catalyzed reduction[211]), provide alternative routes to statines. A nine-step route to (+)-statine starts with (R)-2,3-O-isopropylideneglyceraldehyde.[213]

An extended example of the demands of syntheses of the 1,2-amino-hydroxy grouping on an alkanoic acid is provided by galantinamic acid (7 in Scheme 38), which together with seven 6R-diastereoisomers, has been synthesized starting from D-or L-lysine.[214]

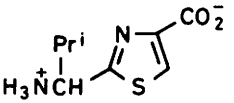
Less demanding methodology leads to 5-amino-4-oxopentanoic acid (4-oxo-pentanoic acid → 5-bromo; Gabriel synthesis)[215] and to the condensed dipeptide (48) from natural sources, which could be classified as an ω-amino acid derivative [Z-Val-C(=NH)OEt + cysteine ethyl ester].[216]

4.17 Labelled Amino Acids.- Most of the labelling patterns represented for amino acids in preceding Volumes of this Report are featured again this year, with ever-increasing medical interest in short-lived ^{11}C and ^{18}F isotopomers. Coverage in this Section is in order of ascending atomic mass.

Large-scale synthesis of (R)- and (S)-[1-^{2}H]glycine from [1-^{2}H]furfural or 4-methoxybenzaldehyde[1-^{2}H] can be achieved through condensation, respectively, with (+)- or (-)-B-isopinocamphenyl-9-borabicyclo[3,3,1]nonane followed by amination and oxidation of the initial aldehyde substituent to CO_2H using O_3 or RuO_4.[217] The same chiral auxiliary has been used in a route to (2S,3S)-phenylalanine-[3-^{2}H].[218] (R)- and (S)-α-methyl-[3,3,2-$^2H_3$]alanines have been prepared by the bis-lactim ether method.[219] (2S,4R)- and (2S,4S)-[4-^{2}H]homoserine lactone hydrochlorides and (2S,3R)-[3-^{2}H]-, (2S,3S)-[2,3-$^2H_2$]-, and (2S,3R,4R)-[3,4-$^2H_2$]-analogues, have been synthesized from corresponding aspartates, some of these being prepared through long-established routes from fumarates.[220] The last-mentioned labelled homoserine has been secured [stereochemistry (2RS,3S,4S)] by alkylation of (S,S)-[2,3-$^2H_2$]-oxirane by the dianion of hippuric acid.[221] Standard routes have been applied to the synthesis of per-^{2}H-DL-leucine from acetone-$^2H_6$.[222] [3,3-$^2H_2$]- and [2,3,3,3-$^2H_4$]-β-Alanines have been prepared from $NCCH_2CO_2Et$ through treatment of the $LiAl^2H_4$ reduction product by oxidation or refluxing ^2HCl, respectively.[223]

[3-^{3}H]-Phenylalanines are accessible through subjecting N-Boc phenylalanines to Pd-catalyzed exchange with $^3H_2$.[224] A labelled version of the novel thyromimetic, SK & F L-94901 (40) with ^{3}H in both ortho-positions, and β-^{14}C in the tyrosine moiety, has been reported.[225]

[3-^{11}C]-DL-Alanine, α-aminoisobutyric acid, norvaline, norleucine and phenylalanine have been synthesized by phase-transfer alkylation of N-(diphenylmethylene)glycine tert-butyl ester, and L-enantiomers of alanine and



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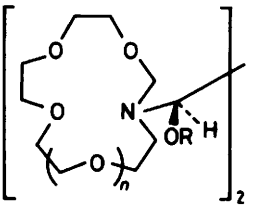

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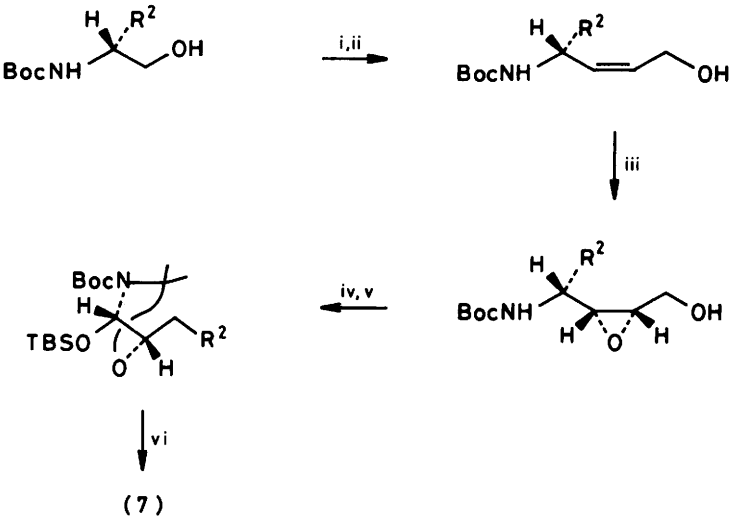
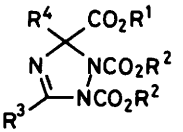


Reagents : i, SO_3-py, DMSO, then $(CF_3CH_2O)_2P(O)CH_2CO_2Me$; ii, DIBAL; iii, MCPBA; iv, $LiAlH_4$; v, TBMSCl; vi, OsO_4

Scheme 38



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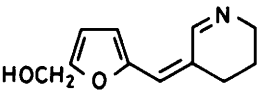


(51)

phenylalanine have been obtained from their labelled racemates using D-amino acid oxidase.[226] ^{11}C-Labelled L-valine, and L-enantiomers of those amino acids in this list that are not substrates for this enzyme, were synthesized by asymmetric alkylation of [(+)-2-hydroxypinanyl-3-idene]glycine tert-butyl ester, in 80-82% enantiomeric excesses; but the 50-55 minutes' overall reaction time meant that radiochemical yields were only 9-25%.[227] L- and D-[Methyl-^{14}C]methionines can be prepared by methylation of S-benzylhomocysteines[228] (and correspondingly labelled selenomethionines[229]), and from L-homocysteine thiolactone.[230] These routes start with $^{11}CO_2$, and the use of this and of ^{11}CO have been reviewed.[231]

L-[2-^{13}C]Serine formation from [2-^{13}C]glycine and HCHO, catalyzed by serine hydroxymethylase, can be followed by ^{13}C-n.m.r.[232] N-Tosyl-[2-^{13}C]glycine has been used for the synthesis of 2-amino-1,5-dihydro-1-[N-methyl-^{13}C]-4H-imidazol-4-one-[5-^{13}C], alias creatinine-$^{13}C_2$.[233] [2-^{14}C]-α-Aminoisobutyric acid and -2-amino-2-methylbutanoic acid, intriguingly described in the abstract as "potential tumour-seeking agents", have been synthesized by modified Bucherer reaction.[234]

S-Adenosyl L-[1-^{14}C]homocysteine can be prepared from L-methionine-[1-^{14}C] via S-benzylation followed by reaction with 5'-O-tosyladenosine.[235]

Isotopically-enriched indoles condensed with L-serine catalyzed by E.coli tryptophan synthetase provide [1-^{15}N]-, [indole C-2-^{13}C]-, or [indole C-3-^{13}C]-labelled L-tryptophans.[236] Microbial syntheses employing $(^{15}NH_4)_2SO_4$ have provided ^{15}N-labelled leucine and isoleucine, and their [^{15}N,3-^{13}C]-analogues.[237]

Direct aromatic substitution of phenylalanine and tyrosine by AcO^{18}F gives 2-[^{18}F]- and 3-[^{18}F]- analogues, respectively,[238] the use of $^{18}F_2$ for the purpose being less efficient.[239] A conventional synthesis route from p-nitrobenzaldehyde that involves $^{18}F^-$ displacement of the nitro-group and resolution by chiral chromatography, has been used for the synthesis of L-4-[^{18}F]-phenylalanine (cf.Ref.539).[240] 4- and 6-[^{18}F]-5-Hydroxytryptophans have been prepared by $^{18}F_2$ substitution.[241]

Aromatic bromination provides radiobromine-labelled 6-bromo-m-tyrosine.[242]

4.18 Resolution of Amino Acids.- Enzymic resolution continues to develop into new and perhaps unexpected variants of well-established applications, which have been reviewed.[243] Thus, α-nitro-α-methylalkanoate esters can be kinetically resolved using α-chymotrypsin[48] as a component of a general amino acid synthesis, and the same enzyme yields methyl L-phenylalaninate through catalyzing the asymmetric transesterification of n-propyl DL-phenylalaninate in MeOH.[244] The more conventional approach is represented in yeast-catalyzed asymmetric hydrolysis of ethyl N-acetyl-DL-fluorophenylalaninates,[245] Aspergillus niger lipase with Z-amino acid 2-chloroethyl esters,[246] and Mycobacterium neoaurum cells acting on α-alkyl-α-amino acid amides.[247] The principle has been adapted for assessing the optical purity of amino acids using L- or D-amino acid oxidase-coated electrodes.[248]

Diastereoisomer-forming derivatization also continues to serve the need for enantiomerically-pure α-amino acids, illustrated by (S)-2-chloropropionylation of α-alkyl-α-amino acids.[247] Further development of the use of chiral binaphthyl hosts that show high chiral recognition for phenylglycinates is reported,[249] and conventional uses of resolving agents [(+)-1-phenylethanesulphonic acid and DL-*p*-hydroxyglycine;[250] (+)-tartaric acid and *N*-salicylylidene-DL-thiazolidine-4-carboxylic acid[251]] have the added virtue that the L-enantiomer that remains in solution in these cases is epimerized. Diastereoisomeric salt formation with (+)-dibenzyltartaric acid has been used for the resolution of *cis*- and *trans*-3-ethyl prolines.[153]

Preferential crystallization of one enantiomer from supersaturated solutions is attracting a wider circle of users [DL-valine hydrochloride;[252] DL-thiazolidine-4-carboxylic acid;[253] *N*-acetyl DL-α-aminoisobutyric acid and DL-norvaline ammonium and alkylammonium salts[254]], with an extraordinary result[255] for [1-^{14}C]-DL-leucine, resolved by seeding with unlabelled D- or L-leucine.

Chromatographic resolution (discussed further in Section 7.5) is also developing rapidly, with promising results for new chiral polymers[256] and detailed information on use of the chiral medium formed by bonding L-phenylalanine to polyacrylamide (Biogel P4) through Mannich condensation with formaldehyde, loaded with copper(II) ions, for ligand exchange resolution of DL-[1-^{3}H]-amino acids.[257]

Explanations for prebiotic resolution of amino acids developed in the recent literature deal with amplification of small local enantiomeric excesses (the Frank model; see Vol.20, p.34) through the slightly different rate constants shown by enantiomers in a a given reaction,[258] and with the slightly different energy of one kaolinite enantiomer compared with the other, resulting in preferential adsorption and consequent activation of one enantiomer of a DL-amino acid (see also Ref.158).[259] The underlying 'weak interaction' principle that assigns microscopically-different energies to each of a pair of enantiomers underlies these reports, and the general topic has been exhaustively reviewed.[260]

5 Physical and Stereochemical Studies of Amino Acids

5.1 X-Ray Crystal Analysis.- This Section does not, as a rule, need an introductory paragraph of general information, but it seems an appropriate way of drawing attention to a review of the crystallization behaviour of amino acids.[261]

Crystal structures of familiar amino acids and simple derivatives are featured in the recent literature: glycine cyclo-tetraphosphate,[262] DL-alanine, β-alanine, and sarcosine,[263] L-histidinium dihydrogen phosphate,[264] DL-lysine monohydrochloride monohydrate and D-lysine monohydrochloride dihydrate,[265] L-α-aminoadipic acid,[266] N^{α}-carbamoyl-L-asparagine,[267] N^{α}-Z-L-arginine hemihydrate,[268] the α-methylDOPA derivative 3,4-dimethoxy-α-methyl-DL-phenylalanine

sesquihydrate,[269] N$^{\alpha}$-pivaloyl-L-proline N-methylamide,[270] 1-amino-cyclopentane-1-carboxylic acid derivatives,[271] and amino acid constituents of the ristomycins.[272]

5.2 Nuclear Magnetic Resonance Spectrometry.- The continual raising of the level of sophistication of n.m.r. instruments seems to occur in annual increments, as reflected in this section Volume by Volume. Solid state studies, illustrated this year[273] by ^{2}H-n.m.r. of polycrystalline L-[3,3-$^{2}H_2$]methionine, can be expected to become ever more prominent. These data have been interpreted in terms of side-chain conformational changes as a function of temperature over the range -35 to 106°.[273]

400 MHz ^{1}H-n.m.r. spectra have been published for 38 amino acids in $^{2}H_2O$ at p^{2}H values 2, 7 and 12 (a total of 114 spectra).[274] ^{1}H-N.m.r. data for N-trityl-(2S,3R)-[3-^{2}H]- and -(2S,3S)-[2,3-$^{2}H_2$]homoserine lactone have been reconsidered, explaining a previous mis-assignment of C-4 proton resonances through unique shielding and deshielding effects of the trityl group.[275] Further ^{1}H-n.m.r. studies of a more routine nature deal with N-acetylvaline, norvaline, and α-aminoisobutyric acid methylamides[276] and (3R,4S)- and (3S,4S)-statines.[277] The conformational information agrees well with that inferred from molecular orbital calculations in the former case, while the ^{1}H-n.m.r. of statines, backed up with i.r. and X-ray data, give insights into conformational mobility and the propensity of these γ-amino acids towards self-association. Shift reagent - ^{1}H-n.m.r. estimation of enantiomeric purity of N-acetylamino acids has been elevated to an accurate operation.[278] Prototropic equilibria HO·····N ⟨====⟩ O^{-}·····HN^{+} for N-(1H-2-oxopyrimidin-4-yl)amino acids in DMSO have been quantified through a combined ^{1}H-n.m.r. - FT-i.r. study.[279] A detailed ^{1}H-^{13}C-n.m.r. investigation of ethyl N-(β-benzoylethyl)-β-alaninate has been reported.[280]

Solid-state ^{17}O-n.m.r. of a single crystal of ^{17}O-enriched glycine reveals five ^{17}O transitions, each comprising two lines, each caused by a dipolar interaction between a ^{17}O atom and nearest protons in the unit cell.[281]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- Fundamental studies of vibrational c.d. (v.c.d.; differential absorption of left- and right-circularly polarized light in the infrared wavelength region) continue for alanine, observing the general topography of the spectra with much still to be understood.[282] N-Boc- and Z-amino acids have been studied by ^{13}C-n.m.r., i.r., and v.c.d. in chloroform or DMSO;[283] these show a strong v.c.d. couplet in the carbonyl stretch wavelength region while ^{13}C-n.m.r. spectra indicate that the amide configuration is predominantly cis at room temperature.

A folded conformation is indicated by intense c.d. for DMSO solutions of the Markownikov adducts formed between N-acetyl-L-cysteine and the exo-vinyl group of bilirubin-IXα.[284] This study, and a use of c.d. to investigate binding of 6-

nitro-L-tryptophan to human serum albumin,[285] are representative of major applications of the technique over many years.

5.4 Mass Spectrometry of Amino Acids.- Like the n.m.r. field, considerable changes in instrumentation are taking place in m.s. Multiphoton ionization m.s. of amino acids and derived N-phenylthiohydantoins have been reported, including the observation of the molecular ion of L-arginine for the first time.[286]

FAB M.s. of 24 amino acids, noting metastable ions and collisional activation spectra of $[M + H]^+$ and $[M - H]^-$ ions,[287] illustrate substantial progress in the operation of this m.s. variant, which is sufficiently mild in molecular structural terms to study easily decarboxylated species such as 5-substituted proline 4,4-dicarboxylic acids formed between γ-carboxyglutamic acid and aldehydes,[288] yet can generate 'precursor ions', e.g. [Leu + metal atom + glycerol]$^+$ and [Leu + metal atom]$^+$ through ionization of amino acids and alkali metal halides in a glycerol matrix.[289]

^{2}H-Labelling has been resorted to, to aid identification of ions formed from amino acids by high-energy collisional activated fragmentation.[290] These spectra show characteristic fragmentation patterns, promising to assist m.s. identification of those β-branched amino acids that are difficult to distinguish from isomeric and β-unbranched amino acids by classical m.s. methods.

Desorption of valine through bombardment with fast alkane ions $(C_nH_m)^+$ has been achieved,[291] offering a secondary molecular ion approach to the difficult problem of obtaining m.s. of underivatized amino acids. The sputtering yield, of valine negative molecular ions per incident carbon atom in these bombarding alkane ions, increases with increasing numbers of carbon atoms.[292]

5.5 Other Spectroscopic Studies.- A non-routine, but preliminary, study of amino acids by near-i.r. reflectance spectrometry (1100-2500 nm) has been reported.[293]

5.6 Other Physical Studies.- Reports of complex formation involving amino acids are often the prelude to novel chromatographic separations, and the preferential binding of one enantiomer of an amino acid ester by chiral porphyrin - Rh(III)Cl complexes may be exploitable.[294] The binding of amino acids to benzo crown ethers occurs between the amine group and the ether oxygen atom;[295] 1:1- and 1:2-complexes of the crown ether (49) with L- or D-valine methyl ester hydrochloride or with NH_4Cl are probably of the sandwich type;[296] no enantioselectivity is shown.

Thermal data have been collected,[297-300] including a novel method for determining the optical purity of an amino acid based on differential scanning calorimetric measurement of enthalpy of solid-solid phase transitions,[297] and a use of the same instrument to quantify the interaction of amino acids with phospholipids.[298]

Apparent molal volumes,[301] partial molar heat capacities and volumes,[302] and viscosity coefficients, heats of solution and surface activity measurements[303] are representative of a vast number of routine papers.

Results based on somewhat similar laboratory methods, but with a wider significance, have been reported for polarities of amino acids; side chain distribution coefficients between vapour and cyclohexane, 1-octanol, and neutral water are closely related to 'inside-outside' distribution of side-chains seen in globular proteins.[304] The acidities in DMSO of a series of Schiff bases (six of the type $Ph_2C{=}NCHRCO_2Et$, five $ArCH{=}NCHRCO_2Et$) has special relevance to practical procedures for optimizing mono-alkylation at the expense of di-alkylation (or vice versa).[305]

Sooner or later, improved understanding of fundamental thermodynamic and other physical properties will feed in to the understanding of biological transport processes of amino acids, e.g. L-alanine transport in rabbit kidney luminal membrane vesicles,[306] and 1-aminocyclohexanecarboxylic acid transport across the blood-brain barrier.[307]

5.7 Molecular Orbital Calculations.- Calculations have been outlined for charge densities and dipole moments of amino acids[308] and N-acetylaspartyl methylamide ion and similar systems,[309] including GABA, GABA imine, and aminoxyacetic acid.[310] The influence of hydration on rotation barriers of glycine has been computed.[311]

The validity of an m.o. approach (AM1) has been tested with calculations of equilibrium structures of the non-ionic tautomer of representative amino acids.[312]

6 Chemical Studies

6.1 Racemization.- Racemization rates in aqueous acidic media are very slow with some well-known exceptions among natural amino acids,[313] and even slower in normal environmental conditions, thus permitting age estimations for relatively young fossils based on degree of racemization of amino acids extracted from them. The criticisms have been eloquently stated, and reviewed in these pages, as far as free amino acids are concerned, and recent criticisms[314] have been extended to include protein-bound amino acids. Fragmentation of a protein into dioxopiperazines has been established to occur rapidly enough, so that it must be a major degradation pathway to free amino acids;[315] however, the general validity of age estimation based on racemization survives the criticisms because the dioxopiperazines are not unduly prone to racemization.[315]

The use of L-isoleucine epimerization measurements has been described from the geologist's viewpoint.[316]

6.2 General Reactions of Amino Acids.- This Section collects papers describing

current interests in reactions of amino or carboxy groups separately, or reactions involving the NH_2---CO_2H moiety as a whole.

Classic α-amino acid reactions, such as ninhydrin oxidation and other colour-forming processes, and Maillard condensations and Amadori rearrangements with carbohydrates, seem to gain a new lease of life on an annual basis. Ninhydrin conversion into CO_2, of amino acids separated by preparative g.l.c. from tissue hydrolysates, allows $^{13}C:^{12}C$ ratios to be determined.[317] Ten O-acylated benzene derivatives have been compared with ninhydrin for their colour-forming reactions with amino acids.[318] α-Alkyl-α-amino acids produce a very low colour yield with ninhydrin, and low relative fluorescence yields with the o-phthaldialdehyde (OPA) - 2-mercaptoethanol reagent, due to incomplete derivatization.[319] The use of OPA with simple thiols is reliable for amino acid quantitative analysis in experienced hands, when exact protocols are followed that take account of the decay of the fluorophore, and NN-dimethyl-2-mercaptoethylammonium chloride is advocated to yield an iso-indole showing more stable ε values.[320]

Diethyl azodicarboxylate and Ph_3P accomplish oxidation of amino acid esters to α-keto-esters.[321] N-Acylamino acid esters yield adducts (50) with this system,[321] but give α-methoxy derivatives through anodic oxidation in methanol.[322]

Hypochlorous acid oxidation of amino acids is not what it might be surmised to be; chloramine originating in this reaction involves the nitrogen atom of the amino acid, even if NH_4^+ salts are present, and it ends up as cyanogen chloride (conversion yields 3-4% for valine, leucine, isoleucine, 11.2% for serine, 13.7% for threonine).[323] Aqueous chlorine degradation of amino acids <u>via</u> N-chlorination and subsequent decomposition is featured in other papers;[324,325] in one of these studies,[324] ClO_2 was surprisingly found to be unreactive towards most of the familiar amino acids.

Careful studies (see also Ref.398) of radicals formed from aliphatic α-amino acid derivatives continue to explore the factors discriminating between various sites for deprotonation by SO_2Cl_2 or by N-bromosuccinimide,[326] which lead to the α-carbon radical (surprisingly, more stable than the tertiary β-carbon radical in the case of valine). At the other end of the spectrum of amino acid chemistry, but closely related to some of the foregoing papers in mechanistic terms, is α-carbanion formation by proton abstraction by a nucleophilic site of D-amino acid oxidase coupled with electron transfer from the amino-N of the substrate to the oxidized flavin cofactor.[327] Routine (repetitive, even) mechanistic studies of amino acid oxidation employing familiar inorganic oxidants [manganese-based;[328-330] chromium(VI) - methionine;[331] silver(I) and cerium(IV)[332]] continue to be reported. One citation (use of N-bromobenzamide to form aldehydes from amino acids[333]) is representative of many such studies employing organic oxidants.

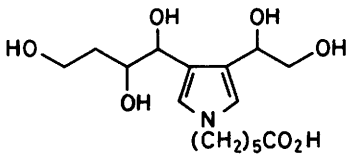
Heterocyclic compounds formed by reaction at both amino and carboxy groups of the amino acid include N-carboxyanhydrides formed using crystalline (but very

expensive) 'triphosgene' [bis(trichloromethyl)carbonate],[334] and corresponding derivatives of αβ-dehydroamino acids.[335] Boroxazolidinones are formed between amino acids and borinic and boronic esters.[336] Conversion of β-amino acids into β-lactams employing diphenylphosphinic chloride[337] or tris(*N*-2-oxazolinonyl)phosphine oxide[338] offers improved methodology. β-Amino acids are formed by stereospecific ring-opening of aziridinecarboxylic acids by thiols.[339] Reclosure of the resulting α-arenethio-β-amino acids using Ph_3P and bis(pyrid-2-yl)disulphide gives β-lactams.[339]

Betaines $Me_3N^+(CH_2)_nCO_2^-$ are effective phase-transfer catalysts for reactions involving dichlorocarbene.[340]

Reports of reactions at the amino group of amino acids include some of interest in their own right, as well as the interest in products formed in this way, which include novel *N*-protecting groups and improved routes to known derivatives. The extraordinary range of products from the Maillard reaction (more than one hundred volatile compounds - furans, pyrroles, pyrazines, etc - from the condensation of valine with D-glucose[341]) is only now being appreciated, the result of improved analytical separation procedures in organic chemistry. ^{13}C-N.m.r. identification of four products (α-, β-pyranose and -furanose forms of D-fructosylglycine)from a D-glucose - glycine reaction mixture (initial Schiff base formation followed by Amadori rearrangement) has been reported.[342] The early phase (130°), developed phase (130-150°), and final phase (>150°, leading to insoluble polymers) in the 1 : 1 - D-glucose : DL-phenylalanine Maillard reaction have been defined.[343] More control of the process is possible, with (*E*)-5-(3,4,5,6-tetrahydropyrid-3-ylidenemethyl)-2-hydroxymethylfuran (51) shown (by *X*-ray analysis) to be formed from D-glucose and L-lysine,[344] 1-alkyl-2-formyl-3,4-diglycosylpyrroles (52) and (53) from glucose or xylose with 6-aminohexanoic acid in the presence of sodium sulphite (which inhibits the formation of brown fluorescent melanoidins in the later stages of the Maillard reaction,[345] and xylose - lysine condensation yielding the 3-(pyrrol-2-yl)cyclopentenone (54)[346] and a second crop of products [(3-N^{ε}-lysino)lactic acid and D-glyceric acid; the first set was established to be N^{ε}-carboxymethyl-lysine and D-erythronic acid] identified among products from oxidative cleavage of the Amadori rearrangement product, N^{α}-formyl-N^{ε}-fructolysine.[347] At a simpler level as far as products are concerned, malondialdehyde reacts with amino acids to give highly fluorescent 1,4-dihydropyridines (55) *via* Michael addition of alkylidene-malondialdehydes with enaminals.[348] Unidentified strongly-coloured green products formed between ethyl caffeate with tyrosine, phenylalanine, and histidine (weaker colour depth was seen with aliphatic amino acids; no colour with proline) may have some connection with the preceding processes.[349] The reaction may have some diagnostic usefulness since it is more sensitive than the biuret reaction.

Attention continues to be given to reactions of the NH_2 group that preserve



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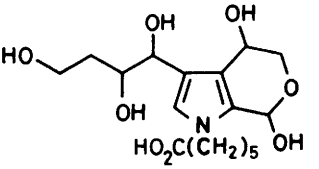

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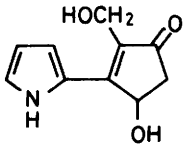

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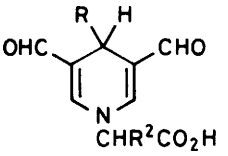

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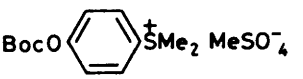

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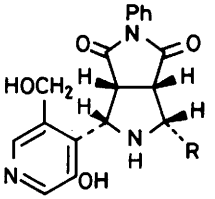

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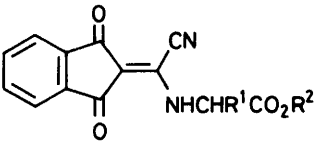

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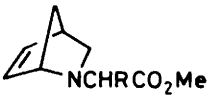

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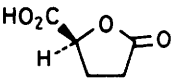

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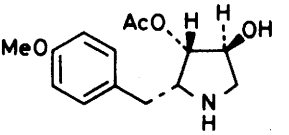

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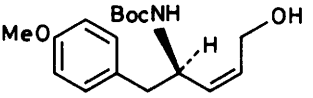

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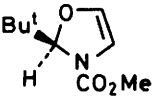

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other structural features intact. Some of the recent papers under this heading deal with reactions broadly applicable to amines in general, for example, that N-nitrosation of sarcosine and proline, is subject to nucleophilic catalysis by thioureas,[350] and that these imino acids react with 4-methyl-*o*-benzoquinone to give 4-methylcatechol and N-(5-methyl-*o*-benzoquinon-4-yl)imino acids in a type of redox process.[351] Degradation of labelled amino acids by hydroxylamine O-sulphonic acid in aqueous EtOH has been used for formation of ^{14}C-labelled acids.[352]

Many other papers are specifically concerned with N-protection strategies for amino acids, including N-α-thiasuccinoylation starting with a polymeric xanthate PEG-$OCS_2CH_2CONH_2$,[353] tritylation of N,O-di(trimethylsilyl)amino acids using trityl chloride in refluxing $CHCl_3$,[354] preparation of Boc-amino acids using the water-soluble reagent (56; see also ref.402);[355] introduction of two Boc groups at N,[356] and formation of N-Boc-N-Z-amino acids.[356] Replacement of Z by Boc under neutral conditions involves either hydrogenolysis (Pd-C) in the presence of $(Boc)_2O$,[356] or $Et_3SiH/Pd(OAc)_2/(Boc)_2O$.[357] N-Arenesulphonyl groups can be removed from α-amino acids carrying hydroxyalkyl side-chains by electrolysis using phenol as proton source;[358] the earlier-reported alkaline hydrolysis regime[359] was tried, without success.[358] Results of preliminary screening in a search for micro-organisms capable of stereospecific cleavage of N-methoxycarbonyl-DL-amino acids, have been reported.[360]

Further novel N-protection strategies include N-(3-cyano-4,6-dimethylpyridyl)-sulphenylation,[361] N-[2-*p*-biphenylyl)-2-propyloxycarbonyl]ation,[362] the latter group cleavable under mild conditions (CH_2Cl_2 - 0.5% TFA), and N-[bis(4-nitrophenyl)-ethoxycarbonyl]ation (base-labile).[363] N-Acetyl-N-(benzoyloxy)lation of amino acid esters employs acetyl chloride and benzoyl peroxide.[364]

The volume of work reported by Grigg's group, on amino acid-derived imines, can only be described as substantial, with several full papers appearing in the year under review. α-Amino acids and αα-disubstituted amino acids react with pyridoxal and N-phenylmaleimide to give two series of cycloadducts, one (57) from azomethine ylides from decarboxylated pyridoxal imines, the other (58) from azomethine ylides formed by 1,2-prototropy.[365] These results are relevant to the mechanism of Strecker degradation and will also assist progress in establishing the mode of action of decarboxylases. Further results, based on cycloadditions to maleimides, deal with kinetics[366] and anionic cycloaddition to imines (59) formed between amino acid esters and (1,3-dioxo-indan-2-ylidene)malononitrile.[367]

The Schiff base formed *in situ* between an amino acid ester and formaldehyde can be trapped by *aza*-Diels-Alder addition to cyclopentadiene;[368] an extraordinary sensitivity to amino acid structure is indicated in the distribution of isomers in this reaction (60:61 = 93:7 for L-isoleucine, but 20:80 for D-phenylglycine).

Papain-catalyzed esterification of Z-DL-amino acids[369,370] and other N-protected amino acids[370] give corresponding N-protected-L-amino acid esters, while variation

of the reaction medium allows serine protease-catalyzed esterification of N-acyl amino acid 2-chloroethyl esters to be directed either to D- or L-ester.[371] DMAP-Catalyzed formation of tert-butyl esters of N-protected amino acids can be achieved using either tert-BuOH - DCCI[372] or $(Boc)_2O$ - pyridine.[373] Valid recipes for N-Boc-L-O-ethyltyrosine pentafluorophenyl, pentachlorophenyl, and N-hydroxysuccinimidyl esters have been published,[374] and a careful assessment of the optimum specification for active esters accompanies the description of the preparation and use of representative 2,3,5,6-tetrafluorophenyl esters.[375] Full details of the highly reactive tetrahydrothiophen 1,1-dioxide-based active esters (TDO-esters)[376] and base-labile bis(4-nitrophenyl)ethyl esters[363] have been published.

Pd(0)-Catalyzed rearrangement of allyl esters of amino acid Schiff bases yields isomeric α-allyl-α-amino acid derivatives.[377]

Friedel-Crafts acylation using N-methoxycarbonyl-L-aspartic α-acid chloride is the first step in a synthesis of enantiomerically-pure β-amino-γ-aryl-γ-butyrolactones.[378] Similar acylation of primary organometallic reagents gives α-Fmoc-aminoalkyl ketones, elaboration of which gives enantiomerically-pure α-substituted alkanoic acids.[379]

Anodic α-substitution of N-protected amino acids leading essentially to solvent incorporation has been mentioned a number of times in preceding sections. The curious fact that this 'non-Kolbe' behaviour is the opposite of that of analogous hydroxy acid derivatives, has been pointed out.[380]

A full account is available[381] of N-Boc amino acid symmetrical anhydrides, some of which have already been characterized, but reports are scattered through the literature of the last ten years or so. Fmoc-Amino acid mixed anhydrides have been used to acylate cyclopentadienyl iron carbonyls, $Cp(CO)_2FeNa$ to give Fmoc-NHCHRCOFe$(CO)_2$Cp.[382]

A mechanistic study shows hydrolysis of 4-(N-methylalanyl)morpholine at 50° in aqueous HCHO to proceed *via* the 5-oxazolidinone, implicating N-hydroxymethylation at an initial stage.[383] Much is being made, in terms of potential rewards, of the chances of improving the slender chiral discrimination seen in the hydrolysis of racemic esters in the presence of D- or L-amino acid derivatives, and the converse equivalent process. Many papers cited in recent Volumes are supplemented this year by accounts of stereoselective hydrolysis of amino acid *p*-nitrophenyl esters[384-387] in the presence of L-histidine derivatives[384-387] covalently linked to poly(ethyleneimine)[384] or contained in surfactant co-aggregates formed by cholesterol-containing amphiphiles.[386] The last-mentioned system[386] and an analogous chiral copper(II)-chelating micelles[387] show the highest enantioselectivity yet reported for such processes. The (S)-1-benzyl-2-[(2-hydroxyethylamino)methyl]pyrrolidine - copper(II) complex shows modest chiral discrimination in catalyzing the hydrolysis of DL-valine methyl ester.[388]

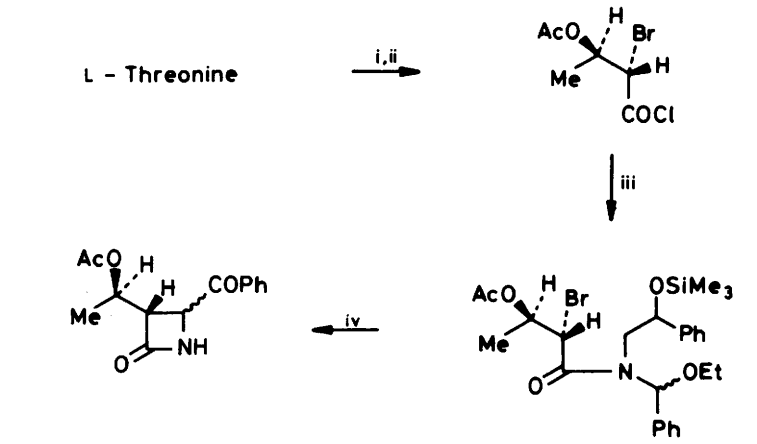
6.3 Specific Reactions of Amino Acids.- These are reactions associated specifically with amino acid side chains, though also involving the NH_2---CO_2H moiety in some cases.

There is an ever-increasing number of applications of natural amino acids in asymmetric synthesis of natural products, though these often employ straightforward methodology until a point has been reached in a synthetic scheme where the structural link with the amino acids has been extinguished. Thus, no attempt at complete coverage is offered in this Section; representative syntheses incorporating less routine reactions of amino acids include 2,3-deoxy-D-ribose from L-glutamic acid *via* (62),[389] (-)-anisomycin (63) from D-tyrosine *via* (64),[390] (+)- and (-)-*N*-methylpseudoconhydrine by anodic α-methoxylation of protected lysine enantiomers followed by replacement of the methoxy group using allylTMS/$TiCl_4$[391] and diastereoisomers of the near relative, 5-hydroxypipecolic acid, from L-glutamic acid.[392]

L-Threonine is the starting point for (3*R*,4*R*)-3-[1-(*R*)-hydroxyethyl)]-4-(benzyloxy)azetidin-2-one (Scheme 39), revealing a use of the novel phenyl alkoxymethyl *N*-protecting group.[393] The uses in asymmetric synthesis, of enantiomers of *N*-methoxycarbonyl 2-tert-butyloxazoline (65 ; from L-serine) and its 4-methyl homologue (from threonine) have been surveyed.[394] Methyl L-pyroglutamate has been employed in a route to (5*S*)-2-[(3',4'-methylenedioxy)phenylethyl]-5-(α-hydroxybenzyl)pyrrolidines as potential hypotensive agents.[395] Oxidative decarboxylation is a useful synthetic operation at or near the end of asymmetric syntheses such as these, and iodosobenzene is capable of effecting the conversion of cyclic imino acids (proline, pyroglutamic and pipecolic acids) into lactams under neutral conditions.[396]

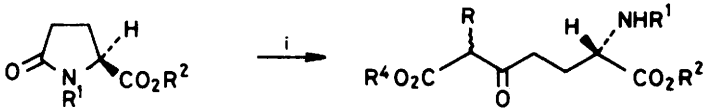
Conversions of easily-available enantiomers of amino acids continue to provide alternative access to other amino acids that are only available with difficulty. The preparation of β-aryl-α-alkyl amino acids from different stereoisomers of *N*-phthaloylthreonine methyl ester β-*O*-methanesulphonate and arenes proceeds without racemization except for C-3 in allothreonines (where it is extensive).[397] Full details of mechanistic studies that have been featured in this section in recent years describe the formation of β-chloro- and (to a lesser extent) γ-chloro-valine through reactions of SO_2Cl_2 with *N*-benzoylvaline methyl ester (cf. Ref.326).[398]

Adaptation of the L-lysine side-chain in suitably-protected derivatives gives corresponding amides through Ru_2O_4 oxidation[399,400] (L-2,4-diaminobutyric acid and L-ornithine behave similarly[399]). N^{α}-Benzyloxycarbonyl lysine is converted into (2*S*)-amino-6-hydroxyhexanoic acid through treatment with aqueous sodium nitroprusside at pH 9.5, thence into N^{α}-*Z*-L-aminoadipic acid through RuO_2-$NaIO_4$ oxidation.[401] N^{ϵ}-Benzyloxycarbonyl lysine has been prepared, to demonstrate the use of the novel water-soluble acylating agent (56; *Z* in place of Boc; cf. *Ref.* 355).[402]



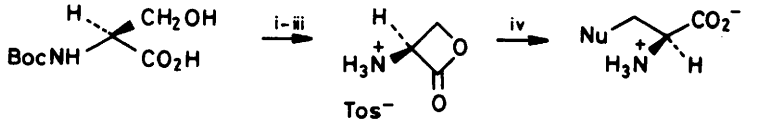
Reagents: i, Ac_2O; ii, oxalyl chloride; iii, $PhCH{=}NCH_2CHPhOSiMe_3$; iv, K_2CO_3, MeOH (some reagent details lacking in ref. 393)

Scheme 39



Reagents: i, (*E*)-$R^3CH{=}C(OLi)CO_2R^4$

Scheme 40



Reagents: i, Ph_3P, DEAD, −78 °C; ii, TFA; iii, TsOH; iv, Nu − X

Scheme 41

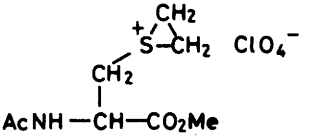
Cleavage of N-alkoxycarbonyl-L-pyroglutamates occurs through mono-addition of lithium enolates, to give (2S)-amino-5-oxoalkanoic acids, useful as starting materials for routes to carbapenams (Scheme 40).[403] α-t-Butyl-γ-methyl-N-trityl-L-glutamate reveals itself as capable of acting as a 'γ-amino acid anion equivalent' through conversion into the γ-ester enolate through use of lithium isopropyl cyclohexylamide; the anion adds stereospecifically to electrophiles (aldehydes in this study).[404]

Uses of serine enantiomers for the stereospecific synthesis of other amino acids are perhaps more varied and extensive than those of other protein amino acids. A further application stems from the first preparation of 3-amino-2-oxetanone as its stable toluene-p-sulphonate salt, through Mitsunobu cyclization of N-Boc-L-serine followed by deprotection with TFA (Scheme 41); nucleophilic ring-opening to give β-substituted L-alanines has been explored.[405]

Photo-exchange between N^{ε}-acetyllysine and cytosine (or 5-methylcytosine) occurs at pH 7.5 to give 2-acetylamino-6-(1'-cytosinyl)hexanoic acid (or the 5'-methyl-1'-cytosinyl analogue).[406] A study of the condensation of arginine with phenyl and substituted-phenyl glyoxals[407] is distantly related in the sense that it illustrates mechanistic interest in amino acid reactions of potential biochemical significance. Other papers concerning arginine deal with protection of the side-chain (for a review, see ref. 408), the N^{α}-9-anthracenesulphonyl group being removeable by mild reduction (dissolving metals).[409] A one-pot preparation of mono- and di-Z-histidines has been reported,[410] and studies of N^{τ}-trityl histidines and distribution (14%π, 70%τ) of the Boc group on side-chain nitrogen atoms through reacting $(Boc)_2O$ with N^{α}-Z-histidine methyl ester.[411]

Reactions of cysteine and its derivatives [rate constants for S-nitrosation,[412] and formation of N-acetylcysteic acid *via* N-acetyl-S-nitrosocysteine through use of excess $NaNO_2$ or NO;[413] synthesis of the episulphonium salt (66) from N-acetylcysteine *via* its S-(2-trifluoroacetoxyethyl) derivative;[414] and estimation of ratios of singly-charged cysteine tautomers in solution by u.v.spectrometry[415]] include important revisions of earlier work. Condensation of L-cysteine with aromatic aldehydes to give thiazolines is not stereoselective;[416] and cysteine reacts with 1,4-benzoquinone to give more complex adducts (67) than previously reported.[417]

Modifications of aromatic and heteroaromatic side-chains include chlorination of L-tyrosine [Cl_2-$(MeO)_2CMe_2$] to give the 3',5'-dichloro homologue.[418] Iodination is faster at higher pH, in marked contrast with peroxidase-catalyzed iodination, which operates at maximum rate at low pH.[419] Substitution in the phenolic moiety of tyrosine by laser-irradiated (308 nm) 5-bromouracil,[420] and analogous substitution of tryptophan and histidine, gives highly fluorescent products. Highly stereoselective *threo*-β-hydroxylation of Boc-tyrosine by $K_2S_2O_8$ with 2 equivalents of $CuSO_4$ (50° - 70° during 1.5-4h) is explained by the formation of a



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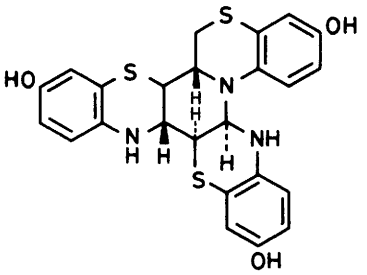

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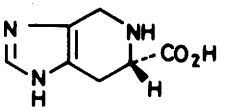

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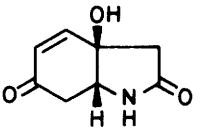

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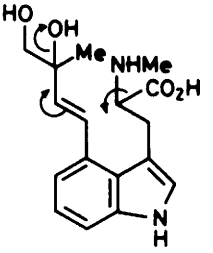

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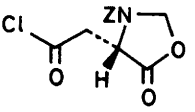

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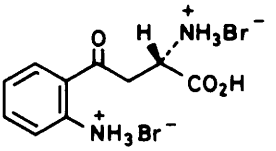

(72)

cyclic carbamate involving the degraded Boc group.[421] A similar strapping of the amino group to the side chain is seen in the condensation of L-histidine with trifluoroacetaldehyde ethyl hemiacetal to give two diastereoisomers (but with low bias, 68:32) of 4-(trifluoromethyl)-L-spinacine (68),[422] and in Rose Bengal-sensitized photo-oxidation of L-tyrosine to give (69) via the corresponding hydroperoxide, in 10-20% yields (proline was decarboxylated through the same treatment).[423] Cyclisation of (70), synthesised from 4-formylindole as a likely biosynthetic precursor to chanoclavine I, occurs under mild conditions.[424]

Pd(0)-Catalyzed cross-coupling of *p*-Boc-aminophenyl trimethylstannane with the L-aspartic acid derivative (71) using $Pd_2(DBA)_3$ gives the homophenylalanine derivative (72), which was converted through routine further steps into L-kynurenine.[425] Overall, this represents a convenient route that will allow access to analogues of this tryptophan degradation product. 5-Hydroxylation of N^{α}-trifluoroacetyltryptophan methyl ester can be achieved using H_2O_2 - SbF_5 - HF.[426]

Simpler structures are involved in reports of four different routes to α- and β-benzyl esters of N-benzylaspartic acid,[427] and reductive esterification of α-acetamido-acrylic acid [CH_2=C(NHAc)CO_2H → N-acetylalanine methyl ester] using H_2/MeOH catalyzed by $RhCl_3$ or Rh(I)chloro(1,5-hexadiene dimer).[428] Apple tissue is employed in the conversion of 2,3-dimethyl-1-aminocyclopropane-1-carboxylic acids into mixtures of cis- and trans-butenes (results are consistent with a stepwise enzymatic radical mechanism).[429] The dehalogenating enzyme present in Proteus mirabilis IFO 3849 can act upon L-2-amino-4-chloropent-4-enoic acid to degrade it to 2-ketopent-4-enoic acid.[430] Ovothiol C (alias $N^{\alpha}N^{\alpha}N^{\tau}$-trimethyl histidine-5'-thiol; Vol.20, p.3) acts as a H_2O_2 scavenger in sea urchin eggs, i.e. as a glutathione peroxidase substitute, preventing oxidative damage to the eggs at fertilization.[431]

Higher amino acids are represented by conversion of appropriate Boc-statine stereoisomers into (3S,4S)- and (3R,4S)-3-aminodeoxystatines (in Scheme 37; 3-OH replaced by NH_2) through subjecting the carboxy derivative [-CO_2H → -CONHOMe] to Mitsunobu treatment.[432]

6.4 Effects of Electromagnetic Radiation on Amino Acids.- Excitation effects of radiation, as opposed to radiation-induced chemical changes, provide this section with its raison d'être, and papers cited here apply, almost exclusively, to phenylalanine, tyrosine or (particularly) to tryptophan.

During sonolysis, a rapidly-growing field of study, radiation acts on the solvent rather than solute, to generate H and OH radicals in Ar-saturated neutral aqueous amino acid solutions; products are readily accounted for on this basis.[433] Single crystal pulse radiolysis of tryptophan yields a transient absorption at 450 nm.[434]

Developing techniques are also featured in u.v.-Raman excitation profiles (217 -

240 nm),[435] and circularly-polarized laser (266 nm) photolysis of aqueous DL-tyrosine,[436] the result of the latter treatment being faster destruction of the D-enantiomer. Although enantioselective photodegradation has been claimed before, it has also been disputed before; another result that is relevant to this general topic is the 15% more efficient formation of the intermolecular excimer involving N-acetyl-L-pyrenylalanine methyl ester, compared with its D-isomer, in a chiral medium [(+)-octan-2-ol and (+)-methyl 2-chloropropionate].[437]

Some protection against He-Ne laser degradation of L-tryptophan in solutions containing haematoporphyrin is observed, but rather less protection is offered by meso-tetra(p-sulphophenyl)porphin.[438] Fluorescence-quenching of tryptophan in 90% methanol, in the presence and absence of 18-crown-6,[439] laser-induced fluorescence following supersonic jet-induced solvation of tryptophan derivatives,[440] and problematical long-wavelength fluorescence of tryptophan and tyrosine solutions,[441] represent less-routine fluoresence studies. There is no longer a need to rationalize the long-wavelength fluorescence data since optical artifacts (second order diffraction) are responsible (rather than molecular excitation).[441]

7 Analytical Methods

7.1 General.- Routine amino acid analysis in the clinical laboratory has been reviewed.[442]

7.2 Gas-Liquid Chromatography.- Finer details are now being dealt with for g.l.c. analysis of amino acids, since the main principles of methodology are well established. One of these established principles is derivatization of amino acids in order to achieve adequate volatility, although pyrolysis-g.l.c. has its uses, e.g. in estimation of cysteine and methionine in proteins; complex mixtures are formed, of course, but estimates of these amino acids based on H_2S and MeSH peaks show 10-12% and 5-6% standard deviations, respectively.[443]

N-Trifluoroacetylamino acid n-butyl esters,[444] isobutyl esters,[445] and n-butylamides[446] continue to have their champions; in one of these papers, the simple change of solvent from CH_2Cl_2 to $CHCl_3$ for the acylation step is shown to be beneficial.[446] Equally widely used are N-heptafluorobutyroyl n-butyl esters,[447-449] these papers illustrating the analysis of amino acids in streptococcal peptidoglycan polysaccharide complexes,[449] and the equality of g.l.c. with ion-exchange analysis, for reliable quantitation of proline, threonine and serine in mixtures.[447] N-Pentafluoropropionyl α-alkyl-α-amino acid n-propyl esters and heptafluorobutyroyl analogues are formed somewhat incompletely, and low results are therefore obtained for these sterically-hindered α-amino acid analogues.[319] Continuing advances are being made on behalf of tert-butyldimethylsilyl derivatives,[21] including one-step derivatization of amino acids

by N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide.[450]

G.l.c.-m.s. studies are becoming more prevalent in the literature,[444,449-451] with equally good results, on the basis of selected-ion monitoring,[451] for analyte levels at the lowest limits applying to h.p.l.c. and ion-exchange methods.

Derivatization also underpins the use of g.l.c. for standard approaches to the determination of enantiomer ratios, either based on separating N-trifluoroacetyl,[452] N-heptafluorobutyroyl,[12,455] or N-trimethylsilyl[453] amides[453] and esters[432,454] over chiral stationary phases (n-pentyl-or -acetyl-α- or β-cyclodextrins,[452] Chirasil-Val®[453-455]), or by separating enantiomer mixtures which have been derivatized so as to form diastereoisomer mixtures (e.g. by acylation with N-trifluoroacetyl-L-prolyl chloride[315]), over achiral stationary phases.

Experienced practitioners in g.l.c. of amino acids have reported differences between results obtained with wide bore glass capillary columns, in comparison with packed columns.[456]

7.3 Ion-Exchange Chromatography.- Useful modifications of the amino acid analyzer and adjustments to the classic chemistry involved, allow acidic amino acids such as phosphoserine, phosphothreonine, phosphotyrosine, cysteic and homocysteic acids, to be accommodated,[457] and allow easier methodology through use of a set of sodium citrate buffers.[458] The substantial series of one-man papers on amino acid analyzer techniques, started two or three years ago, is lengthening with assessments of buffer preparation[459] and integrator reliability.[460]

Difficulties experienced with ion-exchange analysis of α-alkyl-α-amino acids (as with other standard analytical methods for these sterically-hindered α-amino acid analogues) have been discussed.[319]

An accolade to the reliability of this classic method for amino acid analysis, has emerged as a recommendation that concentrations of solutions of reference standard proteins should be determined on the basis of the ion-exchange analysis of their hydrolysates, but estimates of protein concentration obtained in this way tend to be low.[461] Routine use of the amino acid analyzer has been reported for mixtures,[462] 3-hydroxyproline,[463] and diaminopimelic acid in proteins,[464] the latter di-amino acid being accessible after performic acid oxidation prior to hydrochloric acid hydrolysis of proteins containing it (methionine is analysed in such hydrolysates as its sulphone). The numerous protein precipitation techniques introduce sources of error when applied for the preparation of standard samples for hydrolysis and determination of cysteine content by ion exchange analysis, but h.p.l.c. of o-phthaldialdehyde-derivatized hydrolysates is satisfactory.[465]

7.4 Thin-Layer Chromatography.- Before arriving at h.p.l.c. (next Section), this Chapter navigates through ever-more-routine methods. All relevant aspects of t.l.c. are, surely, almost second nature, perhaps genetically-imprinted into

organic chemists and biochemists. However, there are those who continue to publish on the technique, covering t.l.c. analysis of amino acid N-phenylthiohydantoins (for a review, see ref. 466) on silica impregnated with simple metal salts.[467] The unlikely suggestion is made[467] that reported differences are explained by ion-pairing phenomena. Improved solvent systems have been proposed for t.l.c. of amino acids and their derivatives.[468] A modified spray regime has been proposed, claimed to reveal most amino acids on t.l.c. plates, involving successive treatment with fluorescein isothiocyanate and ninhydrin, with intermediate heating and air-drying and finally heating at 90° for 10 minutes.[469] This generates distinctive colours for 0.5 - 1 microgram amounts of amino acids; finally, observation in 280 nm u.v. light reveals certain amino acids that have not been revealed by the foregoing regime.

T.l.c. data in seven solvent systems are included with molecular weight and van der Waals volume data, in a scheme for parametrization applied to forty eight amino acids (see also Ref. 507).[470]

7.5 High Performance Liquid Chromatography.- Those operating h.p.l.c. analytical methods for amino acids, in preference to g.l.c. or ion-exchange, are being offered new options on a regular basis, such as supercritical liquids as mobile phase. For those with long-established roots in analysis of N-phenylthiohydantoins (PTHs), a paper describing rapid, efficient h.p.l.c. employing supercritical CO_2 will be a tempting introduction to the technique.[471] A timely review of electrochemical detection methods in h.p.l.c.[472] consolidates the experience of several years of increasing numbers of practitioners. Completing this paragraph of citations of work of general significance, an extraordinary observation that is perhaps obvious when thought through, has been published; a non-racemic mixture will chromatograph as two peaks in some cases on an achiral stationary phase, one being the enantiomer in excess and the other being the racemate.[473] It will be interesting to see whether re-evaluations of problematical h.p.l.c. traces will start to appear in the literature, embodying this intriguing principle.

As far as the reliable analysis of amino acids in physiological fluids is concerned, a warning has been published, that unless immediate deproteinization is carried out, levels of certain amino acids can increase. Thus, in a check on a routine h.p.l.c. analysis of glutamic acid and glutamine in serum,[474] it was found that the glutamic acid level had doubled, two hours after sampling.

The main preoccupation of this Section in recent years has been to report the search for the derivatization protocol shown to be 'best' for a particular purpose. Reagents come and go, but there is no doubting the pre-eminence of the o-phthaldialdehyde + alkanethiol approach (OPA), or that it is being caught, or even overtaken, by phenyl isothiocyanate (PITC) derivatization. A reasoned

comparison of these two methods[475] concludes that the PITC method has several advantages, in the stability of the products under the conditions of the reaction itself and under the analytical separation conditions, and in the fact that proline and hydroxyproline (and other imino acids) give PTC derivatives but imino acids do not react with OPA without processing. A comparison of the OPA method, not only with PITC but with dansylation, dabsylation and PTH formation, offers similar support for the PITC technique.[476] A notable feature of this study is the use of the same h.p.l.c. column for the comparison, tending to reduce the variables inherent in such a comparison. OPA Derivatization has been compared (very favourably) with a further standard method (but one which is used less, year by year), viz., fluoresceamine.[477]

There are several papers, among a large group of papers describing applications of the OPA method, that explore possible improvements. Efficient ion-pair separation of amino acids, and post-column reaction with OPA - EtSH, seems to offer a good compromise, minimising the time taken between derivatization and absorbance measurement.[478] Low 'colour yields' are achieved with (sterically-hindered) α-alkyl-α-amino acids in the OPA - 2-mercaptoethanol reaction, as with all other derivatization methods, but more drastic reaction conditions are in some ways self-defeating due to the easily-degraded fluorophore formed in the process.[319] Nowadays, the OPA technique is generally a matter of pre-column derivatization, and recent specific applications[479-485] include leucine and other branched aliphatic amino acids,[479] analysis of γ-aminobutyric acid, glutamic acid, and glycine using 5-amino-n-valeric acid as internal standard,[480] acidic amino acids including N- and O-phosphorylated compounds, lombricine and N-phosphoryl-lombricine.[481] A combination of the OPA - 3-mercaptopropionic acid method (for primary amino acids) and Fmoc chloride (for imino acids) has been carefully evaluated.[482] The sensitivity of post-column electrochemical detection of OPA - 2-mercaptoethanol derivatives has been stressed,[485] as for the OPA - t-BuSH reagent used for rat brain neurotransmitter amino acids,[486] and in recent developments, employing naphthalene-2,3-dicarboxaldehyde in place of OPA, and electrochemical detection, an extraordinary 36 attomole lower limit for the detection of asparagine has been claimed.[487]

PITC Derivatization of amino acids, giving N-phenylthiocarbamoylamino acids (PTC-amino acids) suitable for h.p.l.c. separation, has been reviewed[488,489] and a lower limit of 1 pmol,[490] or a rather higher figure,[491] established for it. The chemistry involved is identical with the Edman 'coupling' step for sequence analysis of peptides, which, applied to amino acids, will lead easily to PTH derivatives; this may be the reason for variable yields that have been shown to be the result of derivatization in the presence of salts.[492] Yields also depend on the way in which the vacuum drying stage is conducted.[492] Analysis of glutamic and aspartic acids as PTC-derivatives gives low yields, particularly in analysis

of protein hydrolysates that have been conducted rapidly (i.e. acid hydrolysis at 160° for short times).[493] These losses are, surprisingly, associated with the presence of materials extracted from glass surfaces.[493] An improved buffer for conducting the amino acid - PITC reaction is water:MeCN:pyridine:triethylamine = 35:30:25:10.[493] Comments at the start of this Section, quote practitioners' experience with the high stability of PTC-amino acids, and the method has been used confidently[494-499] for assays of branched chain amino acids (norleucine as standard),[494] assay of basic amino acids (N^{ε}-trimethyl-lysine and N^{ω}-mono- and dimethyl-arginines),[495] and assay of acidic amino acids [glutamic and aspartic acids;[493] γ-carboxy-glutamic acid;[496] and opines (alanopine, strombine, tauropine, β-alanopine)[497]]. An interesting application is the derivatization of amino acids cleaved from peptide C-termini by carboxypeptidase P to allow C-terminal identification.[499]

PTH Derivatives have been found to be unstable in various widely-used h.p.l.c. solvents; for example, PTHs of amino acids with aliphatic side chains (alanine and leucine) have half-lives of 15-20 h in mixtures containing tetrahydrofuran,[500] so it is likely that PTHs of protein amino acids with side-chain functional groups will be even more rapidly degraded. Thiazol-5-ones from aliphatic amino acids were shown earlier to undergo oxidative dimerization in solvents prone to peroxide formation (such as dioxan and tetrahydrofuran),[501] and it is conceivable that this PTH 'degradation' may be exploited as a pathway to more stable characteristic derivatives that are amenable to h.p.l.c. identification. The h.p.l.c - thermospray m.s. combination gives spectra showing strong $[M + H]^+$ ions for 19 PTHs.[502]

DNP-Amino acids are still favoured,[503,504] allowing identification at low picomole levels,[503] and establishing cellulose as a useful h.p.l.c. phase that tolerates iso-amyl alcohol - MeCN - aqueous buffers at various pH and ionic strengths.[504] H.p.l.c. of dansylamino acids[505-507] also offers sensitive analysis of canavanine and canaline,[505] and has been used as a test-bed for comparison of laser polarimetry, refractive index, and u.v. absorption as detection methods,[506] and as part of a multivariate characterisation process for amino acids (see also ref.470).[507]

Fluoresceamine has been applied to α-amino nitriles[508] as well as to amino acids,[477,508] and 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-F) continues to show its merits as a highly fluorescent tagging agent, for S-sulphocysteine[509] and for tryptophan.[510,511] The case of tryptophan is interesting, since its NBD-derivative lacks the intense green fluorescence shown by other NBD-amino acids,[511] but electrochemical oxidation converts it into fluorescent NBD-dioxindolylalanine.[510] The careful NBD-tryptophan study has established detection limits down to 10 fmole for NBD-amino acids.[510] Femtomole levels are also routine for Fmoc derivatives formed using Fmoc-Cl and using 1-aminoadamantane to remove excess reagent.[512] The

h.p.l.c. of dabsylamino acids has been developed further.[513]

Among numerous h.p.l.c. studies employing routinely some of these derivatization methods, or relying on the inherent absorption or fluorescence properties of the amino acids themselves in some cases (aromatic and heteroaromatic amino acids), the following references complete what is intended to be a representative review: glutamic acid released from perfused cerebellar slices;[514] cysteine and *N*-acetylcysteine;[515] 45 common cysteine-based mixed-disulphides;[516] *S*-adenosyl-L-methionine;[517] *O*-galactosyl-hydroxylysine;[518] 3-hydroxypyridinium crosslinking amino acids (pyridinoline and deoxypyridinoline);[519] phenylalanine[520,521] tyrosine[521] and its *o*- and *m*-isomers;[522] tryptophan;[523] and 3-methylhistidine.[524] The h.p.l.c. - FAB-m.s. combination has been explored for derivatized amino acids.[525]

H.p.l.c. flourishes as the method of choice for determining the enantiomeric composition of amino acids. The topic has been reviewed.[526] Analytical resolution is accomplished either by conversion of the sample into diastereoisomeric derivatives using a chiral derivatizing agent, or through using derivatives such as those portrayed above, in a chiral h.p.l.c. environment (chiral mobile phase or chiral stationary phase). While the latter approach is more widely used now, there are many convenient aspects associated with diastereoisomeric derivatives, since some derivatization techniques follow well established protocols; e.g. OPA with *N*-acetyl-L-cysteine as the thiol component, used for α-substituted glutamic acids[527] and for other acidic amino acids (aspartic, glutamic, and lombricine).[528] Other derivatization procedures have been used,[529,530] notably Marfey's reagent [*N*-5-(1-fluoro-2,4-dinitrophenyl)alaninamide];[529] in one of these applications[530] [to Baclofen; β-(*p*-fluoro- or -chloro-phenyl)-γ-aminobutyric acid] the greater distance apart of chiral centres in the derivative does not hinder the resolution. A novel application of established principles arises in the use of a post-column reactor packed with immobilized L-amino acid oxidase, with quantitation of products based on peroxyoxalate chemiluminescence.[531] Chiral stationary phases [2-carboxymethylamino-1,2-diphenylethanol[532] or an analogue (2-methyl in place of 2-phenyl),[533] (-)-*trans*-1,2-cyclohexanediamine,[534] *N*-(2-naphthoyl)-L-leucine,[535] or *N*-(3,5-dinitrobenzyl)-D-phenylglycine[535] bonded to silica, or analogous tris(3,5-dimethylphenyl carbamate)s of cellulose or amylose[536]] effect resolution, in some cases[532-534] through the ligand-exchange principle with underivatized amino acids in a copper(II)-containing mobile phase. One of these studies[535] requires *N*-acylamino acid butyl esters, another[536] is based on *N*-protected amino acids. Dansylamino acids[537,538] have been resolved by the ligand-exchange h.p.l.c. technique, and determination of the enantiomer composition of amino acids labelled with short-lived isotopes ([methyl-^{11}C]methionine,[230] [^{18}F]-5-fluoroDOPA[539]) has benefited from rapid chiral-h.p.l.c.

7.6 Other Analytical Methods.- Attomole levels of dansylamino acids (5 x 10^{-8} M for methionine ≡ 37 amole, and 5 x 10^{-7} M for aspartic acid ≡ 450 amole, are the amounts actually injected) can be assayed by capillary zone electrophoresis with thermo-optical absorbance detection.[540] Similar studies of OPA-derivatives have claimed attomole levels.[541] Levels at an astonishing 4 orders of magnitude smaller can be attained by derivatization by fluorescein isothiocyanate and laser-induced fluorescence quantitation; 9 x 10^{-21} M alanine (≡ 6000 molecules) are actually assayable.[542] Differences in fluorescence lifetimes of o-, m-, and p-tyrosines imply that analytical exploitation might be worthwhile as a topic of study.[543]

Extension of earlier studies of amino acid analysis based on isotachophoresis has been reported.[544]

Potentiometric titration of all common amino acids except tyrosine, cysteine, and cystine, is feasible using $HClO_4$ in AcOH or in 90% MeCN-AcOH.[545]

7.7 Assay of Specific Amino Acids.- The term 'specific' in this context conjures up the topic of enzymatic analysis with biosensor connotations, and, indeed, most of (but not, by any means, all) the relevant recent literature is enzyme-based. Electrodes carrying immobilized L-lysine decarboxylase[546] or L-glutamic decarboxylase[547] respond to the corresponding L-amino acids, while an electrode carrying immobilized *Proteus mirabilis* whole cells combined with an ammonia sensor has been advocated for L-asparagine analysis.[548] L-Glutamic acid synthetase is the crucial part of an ion-selective field effect transistor-type L-glutamate sensor.[549] Branched chain L-amino acids are amenable to analysis by a continuous flow bioluminescence method based on immobilized L-leucine dehydrogenase.[550]

A review has appeared of the analysis of glutamic acid in foods by enzymatic assays.[551] Assays of the respective L-amino acids based on NAD-dependent L-phenylalanine dehydrogenase[552] and L-arginine kinase,[553] and of S-adenosyl-L-methionine (DNA - cytosine methyltransferase) employing [methyl-^{3}H]-S-adenosyl-L-methionine,[554] have been published.

Other 'wet' methods, but based on classical chemical and instrumental techniques, involve molybdenum blue formation from cysteine and ammonium molybdate (concentration linear with absorbance at 780 nm),[555] cysteine-cystine ratios based on the inhibitory effect of Hg(II) salts on catalyzed photo-oxidation of thiosemicarbazones,[556] and exploitation of a 'potential jump' that occurs at the point of formation of the insoluble L-cysteine - copper(II) acetate 2:1-complex.[557] More sophisticated polarographic and cathode-stripping voltammetric methods applied to S-adenosylmethionine are claimed to operate successfully with very low levels of analyte.[558] Arginine levels in hair can be measured by catalytic polarography after $NiNO_3$ - borax treatment of hydrolysates.[559] Nopaline and octopine can be revealed on electrophoretograms by the Sakaguchi reagent.[560]

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