1
Amino-acids

BY P. M. HARDY

The general format and topics covered in this chapter remain as in previous volumes. The purely biological effects of amino-acids or their simple derivatives have only been reported where there is some definite chemical interest involved, and biosynthetic studies as such are not included. Synthetic and degradative work is only discussed where novel methods or intermediates are involved. Amino-acid derivatives useful for peptide synthesis are dealt with in Chapter 3. Crystal structures are listed but not described. Reactions of protein- or peptide-bound amino-acid residues are only dealt with if they are also of interest in connection with free amino-acids or their simple derivatives.

1 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—As is customary in this section, only papers dealing with amino-acids which are rarely encountered or which are of particular current interest are mentioned.

The idea has been put forward that some compounds, such as the non-protein amino-acids, may be synthesized by many more plants, or even by all of them, than has hitherto been recognized. However, they may be formed in amounts that fall below the threshold concentration that can be recognized in plant extracts by routine analytical procedures.[1] An attempt has been made to test this concept by studying the nitrogenous fraction produced by the large-scale processing of sugar beet. Many non-protein amino-acids and derivatives are in fact present in this mixture in very small quantities, too small to have been recognized after a small-scale extraction. These include L-azetidine-2-carboxylic acid, ε-*N*-acetyl-lysine, γ-L-glutamyl-γ-aminobutyric acid, and γ-*N*-acetyl-L-α,γ-diaminobutyric acid; two new natural amino-acids from this source are reported below (p. 3). Fowden concludes that the particular genetic complexes necessary for the formation of individual secondary products may be more widely distributed in plants than often thought. Different patterns of product accumulation may reflect differences in the degree to which genes are 'switched on'.[1] ε-*N*-Acetyl-lysine is a new plant product, but further studies on its appearance in histones are reported.[2, 3]

[1] L. Fowden, *Phytochemistry*, 1972, **11**, 2271.
[2] W. F. Marzluff, D. M. Miller, and K. S. McCarty, *Arch. Biochem. Biophys.*, 1972, **152**, 472.
[3] W. F. Marzluff and K. S. McCarty, *Biochemistry*, 1972, **11**, 2677.

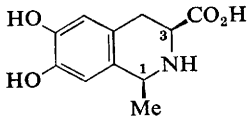
$N^{G}N'^{G}$-Dimethylarginine and $N^{G}N^{G}$-dimethylarginine have been identified in the basic A1 protein from bovine myelin. These residues produce methylamine and dimethylamine, respectively, on alkaline hydrolysis of the protein; these amines were identified by a g.l.c.–m.s. method as dimethylamine is not detected on amino-acid analysis.[4] Dityrosine, the fluorescent *oo'*-biphenol analogue of tyrosine, has now been isolated from bovine ligamentum nuchae;[5] it also occurs in uterine protein.[6] It can be simply synthesized by the action of horseradish peroxidase and hydrogen peroxide on tyrosine.[7] A survey of the protein of medulla cells from hair or quill of a number of mammalian species has been made, and the occurrence of the ε-(γ-glutamyl)lysine cross-link seems to be a general phenomenon. This cross-link contributes to the insolubility of these proteins.[8]

The antibiotic edeine D has been found to contain a residue of β-phenyl-β-alanine. This replaces a residue of β-tyrosine in the close analogue edeine A.[9] Participation of an ε-amino-group in a peptide bond in racemomycin-C has been confirmed by the isolation of ε-*N*-(L-β-lysyl)-L-β-lysine from a partial acid hydrolysate. The possibility of transpeptidation occurring during hydrolysis was ruled out by N-derivatization experiments.[10] The fungus *Boletus satanas* has been shown to contain γ-hydroxynorvaline. G.l.c. analysis of the *N*-[(*S*)-α-methoxypropionyl]-lactones derived from two isomers separated by ion-exchange chromatography showed one lactone to be the pure (2*S*, 4*R*)-isomer and the other to be partly racemized, (2*S*, 4*S*) : (2*R*, 4*R*) = 3 : 2.[11]

New Natural Free Amino-acids.—The antimetabolite rhizobotoxine, first isolated in 1965 from the root nodules produced by *Rhizobium japonicum* in the soybean *Glycine max* (L.) Merr.,[12] has now been identified as the enol-ether 2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-but-3-enoic acid, $CH_2OH \cdot CH(NH_2)CH_2 \cdot O \cdot CH{:}CH \cdot CH(NH_2)CO_2H$.[13] Its dihydro-derivative *O*-(2-amino-3-hydroxypropyl)homoserine is also produced by the same bacterium.[14] This is the first instance of an alkyl-ether derivative of homoserine being found in the absence of added alcohol.

[4] S. W. Brostoff, A. Rosegay, and W. J. A. Vandenheuvel, *Arch. Biochem. Biophys.*, 1972, **148**, 156.
[5] F. W. Keeley and F. S. LaBella, *Biochim. Biophys. Acta*, 1972, **263**, 52.
[6] J. W. Downie, F. S. LaBella, and M. West, *Biochim. Biophys. Acta*, 1972, **263**, 604.
[7] A. J. Gross and I. W. Sizer, *J. Biol. Chem.*, 1959, **234**, 1611.
[8] M. W. J. Harding and G. E. Rogers, *Biochim. Biophys. Acta*, 1972, **257**, 37.
[9] M. Wojciechowska, J. Ciarkowski, H. Chmara, and E. Borowski, *Experientia*, 1972, **28**, 1423.
[10] H. Tamiyama, Y. Sawada, K. Miyazeki, S. Tanaka, F. Miyoshi, and K. Hiraoka, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 1432.
[11] P. Matsinger, Ch. Catalformo, and C. H. Eugster, *Helv. Chim. Acta*, 1972, **55**, 1478.
[12] L. D. Owens and D. A. Wright, *Plant Physiol.*, 1965, **40**, 927.
[13] L. D. Owens, J. F. Thompson, R. G. Pitcher, and T. Williams, *J.C.S. Chem. Comm.*, 1972, 714.
[14] L. D. Owens, J. F. Thompson, and P. V. Fennessey, *J.C.S. Chem. Comm.*, 1972, 715.

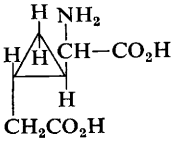
Four new amino-acids have been characterized from members of the Leguminosae; their structures, however, differ widely. A (1*S*, 3*S*) isoquinoline derivative (1) occurs in a variety of the velvet bean. It can readily



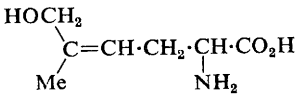
(1)

be synthesized from L-dopa and acetaldehyde in a largely stereoselective condensation.[15] 4,5-Dihydroxy-L-pipecolic acid has been isolated from *Calliandra haematocephala* Hassk. and synthesized by *cis*-hydroxylation of natural 4,5-dehydropipecolic acid with osmium tetroxide, but its stereochemistry is not yet fully known.[16] An amino-acid previously synthesized as an analogue of phenylalanine, *p*-aminophenylalanine, has now turned up in the form of its L-isomer in *Vigna vexillata*,[17] and *S*-(2-hydroxy-2-carboxyethanethiomethyl)-L-cysteine, $HO_2C \cdot CH(OH) \cdot CH_2S \cdot CH_2 \cdot SCH_2CH(NH_2)$-$CO_2H$, has been reported to occur in *Acacia georginae* seed.[18] The latter can be prepared from L-L-djenkolic acid by treatment with a limited quantity of sodium nitrite in acetic acid ($NH_2 \rightarrow OH$), but racemization during this substitution is almost complete.[18]

As far as other plants are concerned, Fowden and his colleagues have added a new cyclopropane amino-acid (2) and a new unsaturated amino-acid (3) to their series of such compounds isolated from the Sapidaceae



(2)



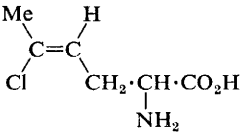
(3)

and Hippocastanaceae,[19] and the seeds of the Kentucky coffee tree have yielded two stereoisomers of β-hydroxy-γ-methylglutamic acid and other non-protein amino-acids as yet unidentified.[20] The sugar beet, *Beta vulgaris*, has been found to contain two new amino-acid derivatives, ε-*N*-acetyl-*allo*-δ-hydroxy-L-lysine and γ-*N*-lactyl-L-αγ-diaminobutyric

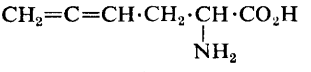
[15] M. E. Daxenbichler, R. Kleiman, D. Weisleder, C. M. Van Etten, and K. D. Carlson, *Tetrahedron Letters*, 1972, 1801.
[16] M. Marlier, G. A. Dardenne, and J. Casimir, *Phytochemistry*, 1972, **11**, 2597.
[17] G. A. Dardenne, M. Marlier, and J. Casimir, *Phytochemistry*, 1972, **11**, 2567.
[18] K. Ito and L. Fowden, *Phytochemistry*, 1972, **11**, 2541.
[19] L. Fowden, C. M. Macgibbon, F. A. Mellon, and R. C. Sheppard, *Phytochemistry*, 1972, **11**, 1105.
[20] G. A. Dardenne, J. Casimir, E. A. Bell, and J. R. Nulu, *Phytochemistry*, 1972, **11**, 787.

acid.[1] Details of the isolation of 4-methylene-DL-proline from *Eriobotrya japonica* have now been published.[21]

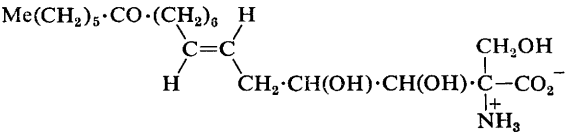
Four unsaturated amino-acids from fungi have been reported. The fruit bodies of *Tricholomopsis rutilans* contain L-2-amino-4-methyl-5-hexenoic acid,[22] and a New Guinea fungus, as yet only tentatively identified, yields L-2-aminohex-4-ynoic acid.[23] More exotic is the occurrence of *trans*-2-amino-5-chlorohexenoic acid (4) in *Amanita solitaria*, a mushroom with a chlorine-like odour. Although this compound is readily obtainable from the allenic amino-acid (5) known to occur in this fungus, it is probably



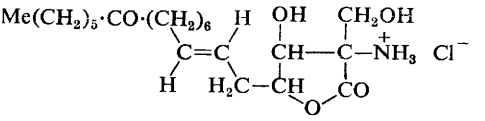
(4)



(5)



(6)

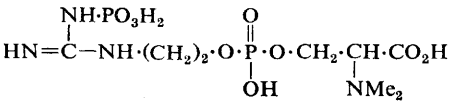


(7)

a true metabolite and not an artefact as addition to (5) requires treatment with hot 1M-HCl.[24] A strain of thermophilic fungi has been shown to produce the rather complex amino-acid thermozymocidin (6), an anti-fungal material. Methanolic hydrogen chloride converts thermozymocidin into the α-amino-γ-lactone hydrochloride (7). Although the stereochemistry has not been fully elucidated, it seems that the adjacent hydroxymethyl and secondary hydroxy-groups in the lactone have the *trans* configuration as ketal or acetal derivatives cannot be prepared.[25]

A new guanidino-compound, L-thalassemine, has been isolated from the body-wall muscle of the echuiroid worm *Thalassema neptuni*. This amino-acid, guanidoethylphospho-*O*-(α-*NN*-dimethyl)serine, occurs

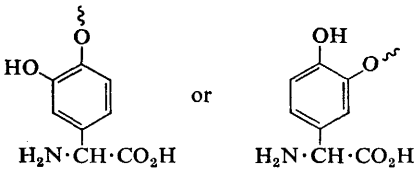
[21] D. P. Gray and L. Fowden, *Phytochemistry*, 1972, **11**, 745.
[22] R. Rudzats, E. Gellart, and B. Halpern, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 290.
[23] S.-I. Hatanaka, Y. Niimura, and K. Taniguchi, *Phytochemistry*, 1972, **11**, 3327.
[24] W. S. Chilton and G. Tsou, *Phytochemistry*, 1972, **11**, 2853.
[25] F. Aragozzini, P. L. Manachini, R. Cravieri, B. Rindone, and C. Scholastico, *Tetrahedron*, 1972, **28**, 5493.



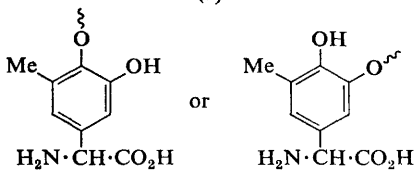
(8)

together with its N^1-phosphoryl-derivative (8); the occurrence of *NN*-dimethylserine either free or combined has not been previously noted.[26]

New Amino-acids from Hydrolysates.—Acid fission of the antibacterial compound ristocetin A yields a number of unusual amino-acids. One of these contains a diphenyl ether grouping with hydroxyl and $-CH(NH_2)-CO_2H$ units on one ring and methyl and $-CH(NH_2)CO_2H$ units on the other.[27, 28] The n.m.r. coupling pattern and nuclear Overhauser experiments favour the substitution patterns (9) and (10) for the two ring systems.[27]



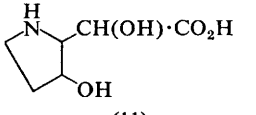
(9)



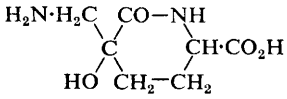
(10)

One of the co-occurring amino-acids seems to be identical except that it lacks the methyl group.[27, 28] The structure of the depsipeptide detoxin-D has been elucidated. It contains the novel pyrrolidine amino-acid (11); the o.r.d. spectrum of the *N*-valyl lactone derivative of (11) formed upon alkaline hydrolysis of detoxin-D shows the ring substituents to be *cis*.[29] A hitherto unidentified amino-acid produced by acid hydrolysis of chlorosis-inducing toxins from the plant pathogen *Pseudomonas* has now been characterized as 3-aminomethyl-6-carboxy-3-hydroxy-2-piperidone (12).[30]

[26] N. van Thoai, Y. Robin, and Y. Guillon, *Biochemistry*, 1972, **21**, 3890.
[27] J. R. Fehlner, R. E. J. Hutchinson, D. S. Tarbell, and J. R. Schenk, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2420.
[28] N. N. Lomakina, M. S. Yurina, V. N. Sheinker, and K. F. Turchin, *Antibiotki Moscow*, 1972, **17**, 488.
[29] K. Kakinuma, N. Otake, and H. Yonemara, *Tetrahedron Letters*, 1972, **69**, 2420.
[30] P. A. Taylor, H. K. Schnoes, and R. D. Durbin, *Biochim. Biophys. Acta*, 1972, **286**, 107.



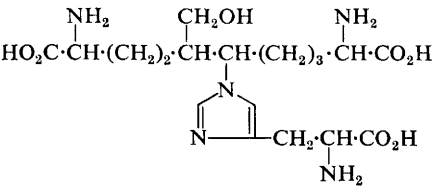
(11)



(12)

Hydrolysis of the antitubercular peptides tuberactinomycins A and N gives *threo*-γ-hydroxy-β-lysine if hydrochloric acid is used, but the *erythro*-isomer if sulphuric acid is used. It is therefore not clear which stereoisomer exists in the original peptide.[30a]

Although chlorination of the aromatic ring of tyrosine is known to occur under some conditions during acid hydrolysis with hydrochloric acid, 3-chlorotyrosine has not previously been reported to occur naturally. Three reports have appeared in 1972 of its isolation, from the scleroprotein of the whelk *Buccinum undatum*,[31] from locust cuticular protein,[32] and in the cuticle of *Limulus polyphemus*.[33] In the latter 3,5-dichlorotyrosine was also present. The conditions of isolation in all three cases were thought to be such as to make chlorination during protein degradation unlikely. Two papers concerning new collagen cross-links have been published. Isolation of 2,10-diamino-5-hydroxymethyl-6-(N^{τ}-histidyl)-undecandioic acid (13), termed aldol-histidine, from borohydride-reduced cow-skin



(13)

insoluble collagen provides the first evidence for a histidine-containing cross-link. Its biosynthesis probably involves a Michael addition of the histidine imidazole to the known collagen component 2,10-diamino-5-formyl-5-undecendioic acid; the product on reduction would yield (13).[34] After reduction of calf insoluble collagen with NaB^3H_4, a labelled degradation product has been identified as *N*-(δ-hydroxynorleucino)-2,3,4,5,6-pentahydroxyhexylamine. This no doubt arises by the reduction of the Schiff base formed by interaction of the carbonyl group of a hexose sugar and the ε-amino-group of δ-hydroxylysine. The structure of the amino-acid was established by mass spectral comparison with N^{ε}-galactosyl-

[30a] T. Wakamiya, T. Shiba, and T. Kaneka, *Bull. Chem. Soc. Japan*, 1972, **45**, 3668.
[31] S. Hunt, *F.E.B.S. Letters*, 1972, **24**, 109.
[32] S. O. Anderson, *Acta Chem. Scand.*, 1972, **26**, 3097.
[33] S. Welinder, *Biochim. Biophys. Acta*, 1972, **279**, 491.
[34] R. B. Fairweather, M. L. Tanzer, and P. M. Gallop, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 1311.

hydroxylysine, but the specific hexose configuration remains to be determined.[35]

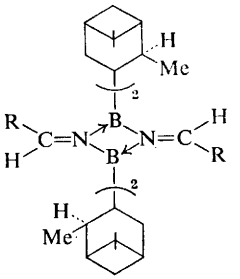
2 The Chemical Synthesis and Resolution of Amino-acids

General Methods of Synthesis.—The preparation of α-amino-acids from α-keto-acids continues to be explored. A detailed study of the temperature dependence of the hydrogenolytic asymmetric transamination of ethyl pyruvate by several optically active amines has now appeared. Amines of the *R* configuration produce (*R*)-alanine (D-alanine) at lower temperatures, but as the reaction temperature increases (*S*)-alanine is formed. Using (*R*)-α-methylbenzylamine, for example, configurational inversion occurs between 10 and 20 °C. The highest optical purity recorded for the alanine was 68%.[36] Reduction of α-keto-acids by (*R*)-(+)-α-phenethylamineborane or its enantiomer gives better yields of α-amino-acids than does the use of sodium cyanoborohydride, but the optical purity of the products is low. This is ascribed to the relatively large distance between the asymmetric carbon of the reducing agent and the developing tetrahedral carbon of the product in the transition state.[37] The imine obtained from *R*- or *S*-α-methylbenzylamine and ethyl glyoxylate reacts with enneacarbonyldi-iron to form two diastereoisomeric complexes of the structure $Fe(CO)_4$-(PhCHMeN:CH·CO_2Et) which can be readily separated. Treatment of these with halogeno-compounds such as benzyl bromide or ethyl bromoacetate followed by catalytic hydrogenation leads to phenylalanine and aspartic acid, respectively. Optical purities of 77 and 78% were obtained. Treatment of the iron complex with acetyl chloride gives some alanine as well as threonine; the intermediate acyl complex apparently undergoes partial decarbonylation.[38]

Di-isopinocamphenylborane has been used as the chiral reagent in the asymmetric synthesis of α-amino-acids from nitriles. The imino-borane intermediate [i.r. shows it to be a mixture of the monomer and dimer (14)] is treated with acetone cyanohydrin as a source of HCN, and subsequent cleavage from the boron with methanol liberates the α-aminonitrile ready for hydrolysis. Convenience is claimed as an advantage of this method. Valine prepared from 2-methylpropionitrile in this way had an optical purity of 12.4%.[39]

Two papers concerning the use of dehydroamino-acid derivatives as α-amino-acid precursors have appeared. Reduction of α-acylamino-acrylic acids using catalysts prepared from Rh(hexa-1,5-diene)Cl_2 and *o*-anisylmethylcyclohexylphosphine (*i.e.* a phosphine in which the chirality

[35] M. L. Tanzer, R. B. Fairweather, and P. M. Gallop, *Arch. Biochem. Biophys.*, 1972, **151**, 137.
[36] K. Harada and T. Yoshida, *J. Org. Chem.*, 1972, **37**, 4366.
[37] R. F. Borch and S. R. Levitan, *J. Org. Chem.*, 1972, **37**, 2347.
[38] J. Y. Chenard, D. Commereuc, and Y. Chauvin, *J.C.S. Chem. Comm.*, 1972, 750.
[39] U. E. Diner, M. Worsley, J. R. Lown, and J. Forsythe, *Tetrahedron Letters*, 1972, 3145.



(14)

is on the phosphorus itself) gave products of up to 90% optical purity.[40] Reductive hydrolysis of unsaturated azlactones with Raney nickel in alcoholic ammonia under 2—3 atmospheres of hydrogen gives acylamino-acid amides and hence α-amino-acids in yields often better than those obtained from such azlactones by other methods.[41]

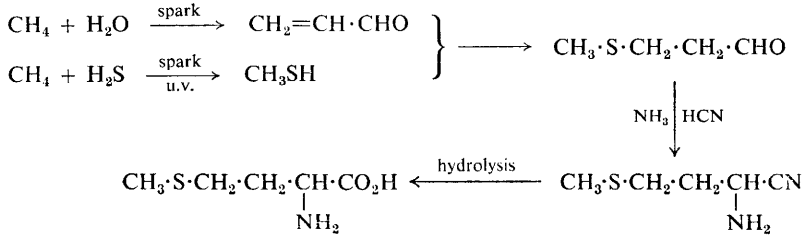
An admirably brief communication reports a new general method of α-amino-acid synthesis through amination of α-lithiated carboxylic acid salts. Phenylacetic acid, for example, on successive treatment with lithium isopropylamine and *O*-methylhydroxylamine gave, after work-up, a 55.5% yield of α-phenylglycine. This is the first report of the conversion of carboxylic acids into α-amino-acids by a one-stage procedure.[42] A way in which the optical purity of intermediates in the asymmetric synthesis of α-amino-acids can be established has been put forward,[43] and the preparation of α-amino-acids by the C-alkylation of glycine as its bis-(*N*-salicylidene glycinato)cobaltate(III) complex by acrylonitrile, methyl acrylate, and acetaldehyde has been described.[44]

Synthesis under Simulated Prebiotic Conditions.—It has been pointed out that reliance on *Rf* values or column chromatographic elution positions alone for the identification of amino-acids produced under simulated prebiotic conditions is insufficient for unambiguous identification. Examination of products from the action of an electric discharge on a mixture of methane, nitrogen, water, and traces of ammonia by g.l.c.–m.s. has shown that a peak at the isoleucine position on the amino-acid analyser is in fact α-hydroxy-γ-aminobutyric acid. The spectrum of products also indicates that no selective synthesis of the branched-chain amino-acids present in proteins occurs.[45] All the non-protein amino-acids found in the Murchison

[40] W. S. Knowles, M. J. Sabacky, and B. D. Vineyard, *J.C.S. Chem. Comm.*, 1972, 10.
[41] A. Badshak, N. H. Khan, and A. R. Kidwai, *J. Org. Chem.*, 1972, **37**, 2916.
[42] S. Yamada, T. Oguri, and T. Shioiri, *J.C.S. Chem. Comm.*, 1972, 623.
[43] J. C. Fiaud and A. Horeau, *Tetrahedron Letters*, 1972, 2565.
[44] Yu. N. Belokon, N. I. Kuznetsova, R. M. Murtazin, and M. M. Dolgaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2772.
[45] J. E. Van-Trump and S. L. Miller, *Science*, 1972, **178**, 859.

meteorite are produced in this electric discharge reaction, and the pattern of relative abundances is on the whole quite similar.[46]

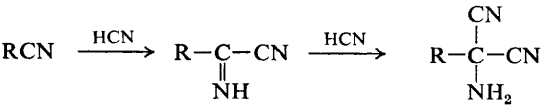
The hitherto neglected prebiotic synthesis of the sulphur amino-acids has now received attention. Addition of hydrogen sulphide to the gas mixture detailed in the preceding paragraph leads to the formation of methionine; a synthetic scheme has been proposed (Scheme 1). The presence



Scheme 1

of acrolein in the products was established, and this is thought to be a key intermediate in the formation of glutamic acid, homocysteine, α,γ-diaminobutyric acid, and α-hydroxy-γ-aminobutyric acid as well as methionine.[47]

The prebiotic synthesis of amino-acids by the attack of cyanide ion on nitriles (Scheme 2) does not appear to be feasible under the dilute conditions



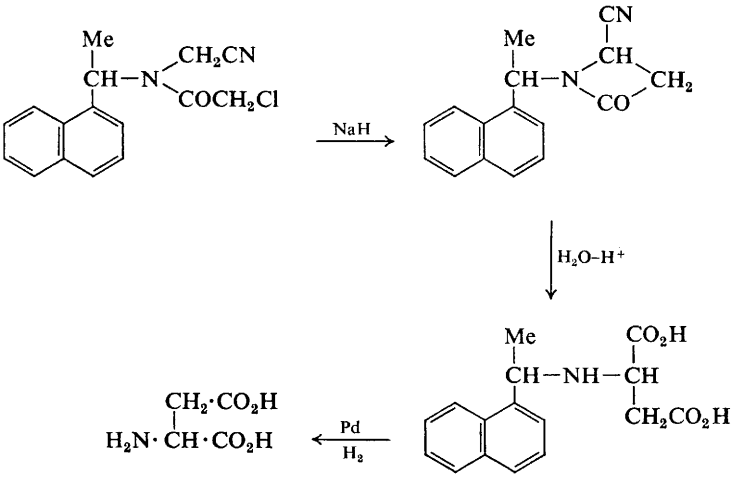
Scheme 2

considered reasonable except in the cases of aspartic and glutamic acids. Only in these instances does the inductive effect of the side-chain group R result in a rapid enough addition.[48] Morphological and compositional resemblances between material occurring in precambrian rock from South Africa and the products of simulated prebiotic syntheses have been noted.[49]

Two papers concerning the study of prehistoric amino-acids have appeared. The racemization of amino-acids near neutral pH has been considered and related to organic geochemistry,[50] and the dating of fossil bones using the extent of racemization of isoleucine has been proposed.[51]

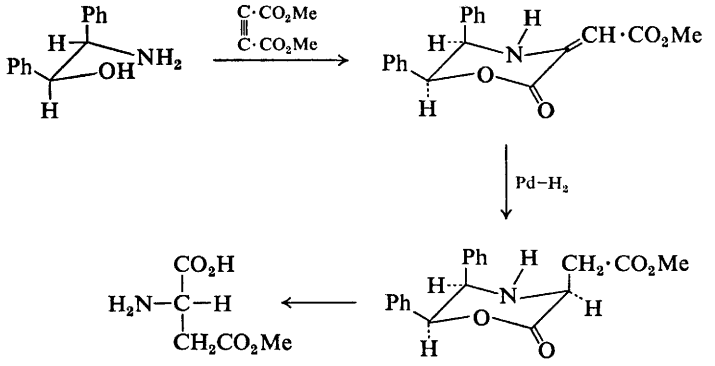
[46] D. Ring, Y. Wolman, N. Friedmann, and S. L. Miller, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 765.
[47] Y. Wolman, W. J. Haverland, and S. L. Miller, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 809.
[48] Y. Wolman and S. L. Miller, *Tetrahedron Letters*, 1972, 1199.
[49] C. Simonescu, F. Denes, and E. Schreiber, *Compt. rend.*, 1972, **275**, *D*, 703.
[50] J. L. Bada, *J. Amer. Chem. Soc.*, 1972, **94**, 1371.
[51] J. L. Bada, *Earth Planet Sci. Letters*, 1972, **15**, 273.

Protein and other Naturally Occurring Amino-acids.—The preparation of aspartic acid through β-lactams derived from *N*-alkyl-*N*-chloracetamidoacetonitriles has been studied in detail. Derivatives of (*R*)(+)-α-(1-naphthyl)ethylamine (Scheme 3) gave aspartic acid of the highest (54—67%)



Scheme 3

optical purity in quite high yield. Equilibration studies indicated that asymmetric induction is occurring during ring-closure rather than by epimerization of the α-carbon atom.[52] Full details of a stereospecific synthesis of aspartic acid first reported in 1968 have now appeared (Scheme 4). In this method L-*erythro*-1,2-diphenylethanolamine is used to form an

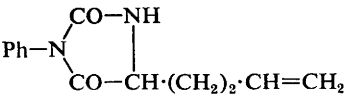


Scheme 4

[52] T. Okawara and K. Harada, *J. Org. Chem.*, 1972, **37**, 3286.

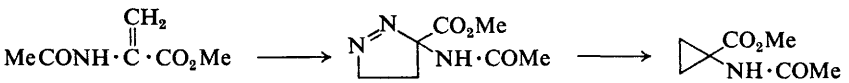
oxazinone which is hydrogenated and hydrolysed to give a 98% yield of L-aspartic acid.[53] DL-Glutamic acid has been prepared in 20% yield by a reaction sequence involving the addition of copper salicylideneglycinate to methyl acrylate in the presence of base. The corresponding Schiff base with acetaldehyde gave 4% of glutamic acid in a similar reaction.[54]

A convenient synthetic route to the reduction products of several collagen cross-links and cross-link precursors has been developed. The key intermediate 5-(but-3-enyl)-3-phenylhydantoin (15) can be used as a source of



(15)

hydroxynorleucine, 5,6-dihydroxynorleucine, hydroxylysinonorleucine, and hydroxylysinohydroxynorleucine.[55] A new synthesis of 1-aminocyclopropane-1-carboxylic acid from methyl α-acetamidoacrylate involves the addition of diazomethane to give a pyrazoline which loses nitrogen on pyrolysis (Scheme 5).[56] A novel method of preparing L-(−)-α-methyl-3,4-



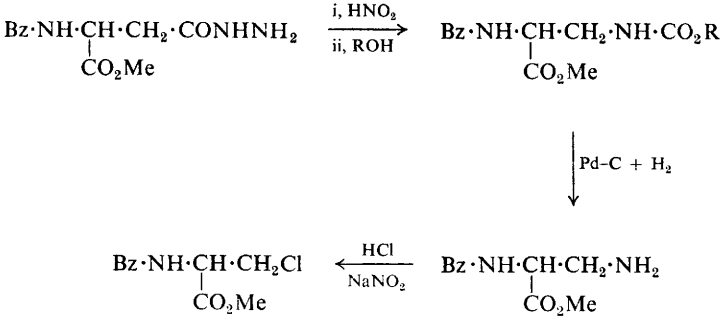
Scheme 5

dimethoxyphenylalanine by an Ugi reaction involving L-(−)-α-methylbenzylamine has been developed. The resulting diastereoisomeric L(or D)-α-methylbenzyl-DL-amino-acids can be easily separated.[57]

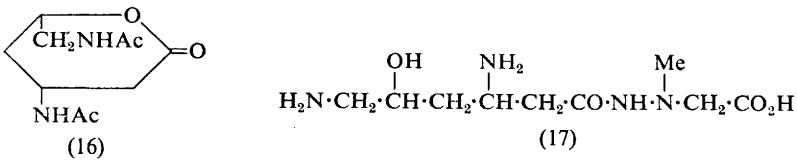
***C*-Alkyl and Substituted *C*-Alkyl Amino-acids.**—The preparation of 27 *C*-alkyl amino-acids, 14 of which are novel, has been reported; four of these are methionine analogues. Five of these materials are toxic to *Escherischia coli*.[58] L-β-Chloroalanine has been prepared from L-aspartic acid through its β-hydrazide (Scheme 6).[59]

Amino-acids with Aliphatic Hydroxy-groups in the Side-chain.—The synthesis of both enantiomers of δ-hydroxy-β-lysine from D-galacturonic acid and 3-amino-3-deoxy-D-glucose *via* the lactone of 3,6-diacetamido-5-hydroxyhexanoic acid (16) has led to the synthesis of negamycin (17) and its optical

[53] J. P. Vigneron, H. Kagan, and A. Horeau, *Bull. Soc. chim. France*, 1972, 3836.
[54] Yu. N. Belokon, V. M. Belokon, N. I. Kuznetsova, and M. M. Dolgaya, *Bull. Acad. Sci. U.S.S.R.*, 1972, 1288.
[55] N. R. Davis and A. J. Bailey, *Biochem. J.*, 1972, **129**, 91.
[56] I. Bregovec and T. Jakovcic, *Monatsh.*, 1972, **103**, 288.
[57] K. Freter, M. Gotz, and K. Grozinger, *J. Medicin. Chem.*, 1972, **15**, 1072.
[58] C. J. Abshire and G. Planet, *J. Medicin. Chem.*, 1972, **15**, 226.
[59] K. Okumura, T. Iwasaki, T. Okawara, and K. Matsumoto, *Bull. Inst. Chem. Res. Kyoto Univ.*, 1972, **50**, 209.

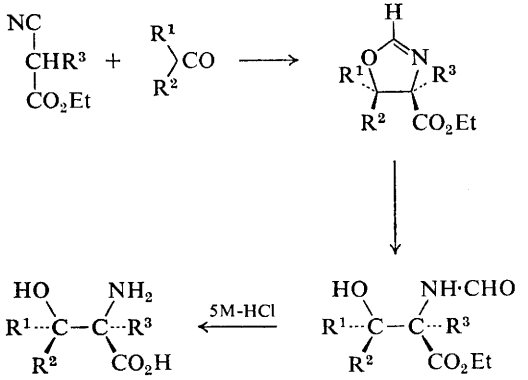


Scheme 6



(16) (17)

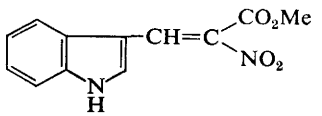
antipode; the latter has some biological activity.[60] Ethyl 2-oxazolinone-4-carboxylates have been found to form ethyl α-formylamino-β-hydroxy-acid esters almost quantitatively on warming with aqueous ethanolic triethylamine, although such alkaline hydrolysis is unusual for 2-oxazolinones. Since ethyl 2-oxazolinone-4-carboxylates are readily available from ethyl isocyanoalkanoates and carbonyl compounds in basic media, this affords a new synthetic route to β-hydroxy-α-amino-acids (Scheme 7).[61, 62]



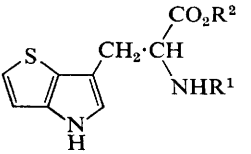
Scheme 7

[60] S. Shibahara, S. Kando, K. Maeda, H. Umezawa, and M. Ohno, *J. Amer. Chem. Soc.* 1972, **94**, 4353.
[61] D. Hoppe and U. Scholkopf, *Annalen*, 1972, **763**, 1.
[62] D. Hoppe and U. Scholkopf, *Angew. Chem. Internat. Edn.*, 1972, **11**, 432.

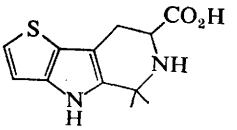
Aromatic and Heterocyclic Amino-acids.—L-Tryptophan has been enzymically prepared from indole, ammonia, and sodium pyruvate in the presence of tryptophanase in good yield; 5-hydroxytryptophan can be made if 5-hydroxyindole is used in place of indole.[63] An electrolytic synthesis of DL-tryptophan from (18) has been reported,[64] and an improved synthesis of indole-3-acetylaspartic acid *via* the *p*-nitrophenyl ester of indole-3-acetic acid has been described.[65] A thieno-[3,2-*b*]pyrrole analogue of tryptophan



(18)



(19)



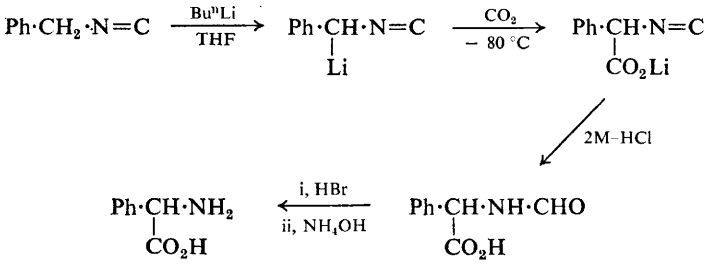
(20)

(19; $R^1 = R^2 = H$) has been prepared and found to be less stable than the indole compound. Hydrolytic deacylation of a precursor (19; R^1 = COMe; R^2 = Me) proved difficult; attempts to cleave with hydrazine led to the formation of (20) as well as the desired amino-acid. The *gem*-dimethyl group arose from the use of acetone to precipitate the product and consume excess hydrazine.[66]

Alkylation of the methyl ester of $N^{\alpha}N^{\pi}$-dibenzoyl-L-histidine with trimethyloxonium fluoroborate provides a convenient route to N^{τ}-methyl-L-histidine. Hydrolysis of the resulting ester affords 75% of the desired compound.[67] Pyrazole will add on to α-acetamidoacrylic acid in boiling acetic acid to give an excellent yield of the acyl derivative of β-(pyrazolyl-*N*)-DL-alanine; this is a convenient new synthesis for this amino-acid.[68] A new preparation of 3-(3,4-dihydroxyphenyl)-L-alanine involving oxidation of an *N*-acyl-L-tyrosine methyl ester with benzoyl peroxide in chloroform has been published.[69] 1,4-Cyclohexadiene-L-alanine hydrate, a naturally occurring inhibitor found in certain bacteria, has been found to undergo

[63] H. Nakazawa, H. Enei, S. Okumura, H. Yoshida, and H. Yamada, *F.E.B.S. Letters*, 1972, **25**, 43.
[64] E. V. Zaparazhets, I. A. Aurutskaya, and M. Y. Fioshin, *Electrokhimiya*, 1972, **8**, 1809.
[65] R. C. Mollan, D. M. X. Donnelly, and M. A. Harmey, *Phytochemistry*, 1972, **11**, 1485.
[66] A. J. Humphries, R. L. Keener, K. Yano, F. S. Skelton, and E. Freiter, *J. Org. Chem.*, 1972, **37**, 3626.
[67] H. C. Beyerman, L. Moat, and A. Van-Zou, *Rec. Trav. chim.*, 1972, **91**, 246.
[68] I. Murakoshi, S. Ohmiya, and J. Haginawa, *Chem. and Pharm. Bull.* (*Japan*), 1972, **20**, 409.
[69] H. Varbrüggen and K. Krolikiewicz, *Chem. Ber.*, 1972, **105**, 1168.

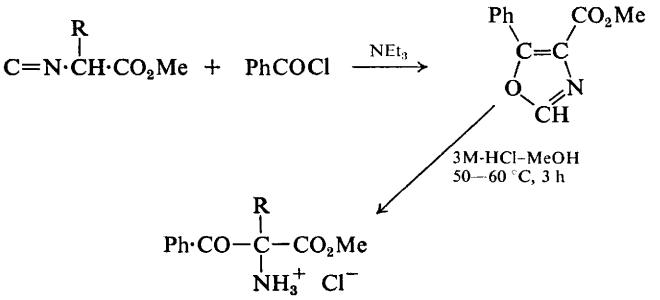
dehydrogenation in the solid state in the presence of oxygen to form L-phenylalanine. All attempts to desiccate the hydrate have led to this dehydrogenation; this and other evidence suggest that the water of crystallization may have a role in this reaction.[70] The carboxylation of α-lithiobenzyl isocyanide enables α-phenylglycine to be rapidly synthesized (Scheme 8),[71] and the formation of oxazoles from acid chlorides simply



Scheme 8

using triethylamine as the base (Scheme 9) provides a convenient route to *C*-aroyl amino-acids.[72]

Azetidine-3-carboxylic acid has been prepared for the first time. Its p*K* values are similar to those of proline, and an *X*-ray study shows the ring to be puckered by less than 1°. In the last step in its synthesis (hydrogenolysis of an *N*-benzhydryl group) use of Pd–C as catalyst effected no cleavage, but palladium hydroxide gave a quantitative yield of the desired material.[73] In the preparation of aziridine-2-carboxamides by the reduction of 2*H*-azirine-2-carboxamides, sodium borohydride is an effective agent but lithium aluminium hydride is not.[74] A new synthesis and resolution of

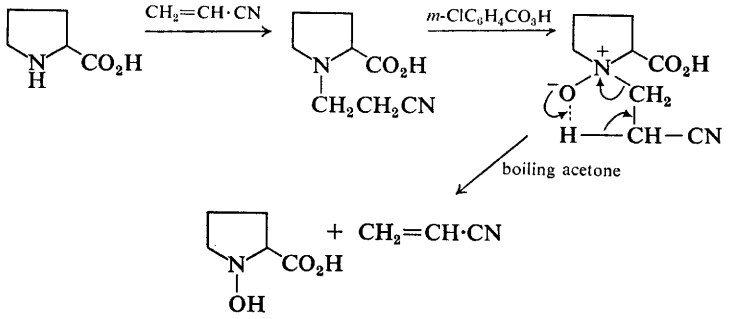


Scheme 9

[70] C. Ressler, *J. Org. Chem.*, 1972, **37**, 2933.
[71] W. Vallburg, J. Strating, M. G. Waldring, and H. Wynberg, *Synthetic Comm.*, 1972, **2**, 423.
[72] M. Suzuki, T. Iwasaki, K. Matsumoto, and K. Okumura, *Synthetic Comm.*, 1972, **2**, 237.
[73] A. G. Anderson and R. Lok, *J. Org. Chem.*, 1972, **37**, 3953.
[74] T. Nishiwaki and F. Fujiyama, *Synthetic Comm.*, 1972, **2**, 569.

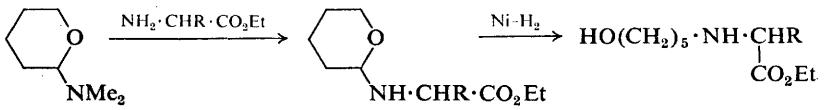
cis- and *trans*-5-methylproline has been reported,[75] and homologues of proline containing 12—15-membered rings have been prepared. These latter syntheses involve the base-catalysed rearrangement of α-halogeno-ω-aminolactams prepared from lactams derived from oximes by the Beckmann rearrangement.[76]

N-Substituted Amino-acids.—The first synthesis of N^{δ}-hydroxyornithine has been reported. The preparation involves the tosylation of *O*-benzylhydroxylamine, alkylation with 1,3-dibromopropane to give γ-(*N*-tosyl-*N*-benzyloxy)aminopropyl bromide, and then treatment with diethyl sodioacetamidomalonate as in a conventional amino-acid synthesis. N^{δ}-Tosyl-N^{δ}-benzyloxy-L-ornithine was prepared by a papain-catalysed resolution.[77] A reaction of general utility for the synthesis of cyclic *N*-hydroxyamino-acids and *N*-hydroxy-*N*-alkylamino-acids has been developed. The reaction sequence involves cyanoethylation, N-oxidation, and a Cope elimination of acrylonitrile (Scheme 10). 1-Hydroxyproline prepared by this method



Scheme 10

from L-proline has been shown to be optically pure.[78] The preparation of *N*-(ω-hydroxyamyl)amino-acids by hydrogenation in the presence of Raney nickel of *N*-(α-tetrahydropyranyl)amino-acids derived by the transamination of *NN*-dimethyl-α-aminotetrahydropyran has been described (Scheme 11).[79]



Scheme 11

[75] C. G. Overberger, K. H. David, and J. A. Moore, *Macromolecules*, 1972, **5**, 368.
[76] J. A. Elberling and H. T. Nagasawa, *J. Heterocyclic Chem.*, 1972, **9**, 411.
[77] Y. Isowa, T. Takashima, M. Ohmori, H. Kurita, M. Sato, and K. Mori, *Bull. Chem. Soc. Japan*, 1972, **45**, 1461.
[78] H. T. Nagasawa, J. G. Kohlhoff, P. S. Fraser, and A. A. Mikhail, *J. Medicin. Chem.*, 1972, **15**, 483.
[79] G. Glacet and J. Tesse, *Compt. rend.*, 1972, **275**, *C*, 147.

α-Amino-acids containing Sulphur.—The copper-catalysed alkaline autoxidation of homocysteine is a useful source of homocysteinesulphinic acid.[80]

A List of α-Amino-acids which have been Synthesized for the First Time.—

Compound	*Ref.*
2,6-Diamino-4-oxohexanoic acid	81
4,5-Dihydroxy-L-pipecolic acid	16
S-(2-Hydroxy-2-carboxyethanethiomethyl)-L-cysteine	18
2-Amino-3-(6-thieno[3,2-*b*]pyrrolyl)propionic acid	66
Azetidine-3-carboxylic acid	73
3,3′,5,5′-Tetramethyl-L-thyronine	82
δ-Hydroxy-β-lysine	83
L-2-Aminohex-4-ynoic acid	23
N^{δ}-Hydroxyornithine	77
N-[δ-(2-Amino-6-purinylthio]valerylglycine	84
N-[δ-(2-Amino-6-purinylthio]valeryl-DL-valine	84
N-[δ-(2-Amino-6-purinylthio]valeryl-L-leucine	84
N-[δ-(2-Amino-6-purinylthio]valeryl-L-aspartic acid	84
N-[δ-(2-Amino-6-purinylthio]valeryl-L-glutamic acid	84
4-Aminobut-2-ynoic acid	85
4-Morpholinobut-2-ynoic acid	85
4-Piperazinobut-2-ynoic acid	85
4-Piperidinobut-2-ynoic acid	85
4-Pyrrolidinobut-2-ynoic acid	85
α-(*S*-L-Cysteinyl)-β-(5-imidazolyl)propionic acid	86
L-{2-[(2-Amino-2-carboxyethyl)thio]ethyl}trimethylammonium bromide	87
L-α-Amino-γ-nitroguanidinobutyric acid	88
α-Amino-β-(1-imidazolyl)propionic acid	89
4-Nitrohistidine	89
2-Methyl-3-(2′,4′-di-iodo-5′-hydroxyphenyl)-DL-alanine	89
4-(3′-Amino-2′,4′,6′-tri-iodophenyl)-DL-isovaline	90
4-(3′-Acetamido-2′,4′,6′-tri-iodophenyl)-DL-isovaline	90
4-(3′-Hydroxy-2′,4′,6′-tri-iodophenyl)-DL-isovaline	90
Ethyl 4-oxo-1-phenylpyrrolidine-3-carboxylate	91
4-Anilino-1-phenylpyrrole-3-carboxylic acid	91
2-Amino-4-thiosulphobutyric acid	92
S-(3-Aminopropyl)homocysteine	93
9-{*S*-[4-(2-Amino)butyric acid]-5′-thiopentyl}adenine	93
S-(Cyclopentylmethyl)homocysteine	93

[80] P. Lucki and C. de Marco, *Analyt. Biochem.*, 1972, **45**, 236.
[81] R. C. Hider and D. I. John, *J.C.S. Perkin I*, 1972, 1825.
[82] P. Block and D. M. Coy, *J.C.S. Perkin I*, 1972, 633.
[83] S. Shibahara, S. Kondo, K. Maeda, H. Umezawa, and M. Ohno, *J. Amer. Chem. Soc.*, 1972, **94**, 4353.
[84] A. Černy, R. Yotva, and M. Semanský, *Coll. Czech. Chem. Comm.*, 1972, **37**, 2606.
[85] P. M. Beart and G. A. R. Johnston, *Austral. J. Chem.*, 1972, **25**, 1359.
[86] Y. A. Yankeelov and G. J. Jolley, *Biochemistry*, 1972, **11**, 159.
[87] H. A. Itano and E. A. Robinson, *J. Biol. Chem.*, 1972, **247**, 4819.
[88] J. W. Van Nispen and G. I. Tesser, *Synthetic Comm.*, 1972, **2**, 207.
[89] G. E. Trout, *J. Medicin. Chem.*, 1972, **15**, 1259.
[90] G. Shtacher and S. Dayagi, *J. Medicin. Chem.*, 1972, **15**, 1174.
[91] V. J. Bauer and S. R. Safir, *J. Medicin. Chem.*, 1972, **15**, 440.
[92] C. De Marco and P. Lucki, *Analyt. Biochem.*, 1972, **48**, 346.
[93] J. K. Coward and W. D. Sweet, *J. Medicin. Chem.*, 1972, **15**, 381.

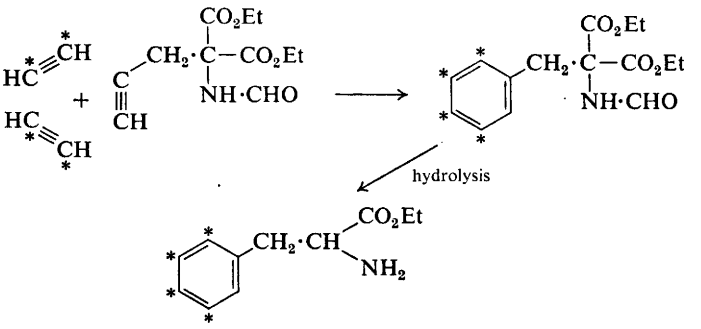
Compound	*Ref.*
5-Amino-4-chloro-6-[3-(homocysteinyl)-n-propyl]aminopyrimidine	93
L-3-{[*p*-(Hydroxymethyl)-αα-diphenylbenzyl]thio}alanine	94
L-3-{[αα-Bis-(α-hydroxy-*p*-tolyl)-benzyl]thio}alanine	94
L-3-{[(4-Biphenylyl)diphenylmethyl]thio}alanine	94
L-3-[(αα-Diphenylpiperonyl)thio]alanine	94
N-[Tris(hydroxymethyl)methyl]alanine	95
2-Ethylamino-DL-alanine	96
2-Propylamino-DL-alanine	96
2-Butylamino-DL-alanine	96
2-Amylamino-DL-alanine	96
2,2-Diethylamino-DL-alanine	96
2-(2′-Hydroxyethyl)amino-DL-alanine	96
5′-Guanosylhomocysteine	97
12—15-membered-ring homologues of proline	76
14 new *C*-alkyl amino-acids with aliphatic side-chains	58

Labelled Amino-acids.—A simple and general procedure for labelling α-amino-acids at their α-C atoms by deuterium (or tritium) by a catalytic exchange in $MeCO_2D$–D_2O containing DCl at 120 °C has been developed. That such a reaction is of utility demonstrates that there is a limitation to the conditions under which such labelled compounds can be used for tracer studies.[98] The preparations of deuterium- and tritium-labelled (3*S*)- and (3*R*)-phenylalanines [99] and (2*R*, 3*S*)-[3-$^3H_1$]- and (2*S*, 3*R*)-[3-$^3H_1$]-tyrosines [100] have been described. These materials have been used to investigate the stereochemical courses of elimations to αβ-unsaturated acids catalysed by L-phenylalanine ammonia-lyase [99] and enzymes from maize and potatoes.[100] The formation of L-αγ-diamino[4-^{3}H]butyric acid, L-[5-^{3}H]ornithine, and DL-[6-^{3}H]lysine by the catalytic tritiation of β-, γ-, and δ-cyano-compounds has been detailed,[101] and a systematic study of the catalytic microtritiation of αβ-unsaturated amino-acid derivatives has been made.[102] A re-evaluation of the loss of tritium labels from positions 3 and 5 of the aromatic ring of tyrosine on acid hydrolysis indicates that such exchange has been hitherto underestimated. Similar losses occur from the aromatic nucleus of tryptophan.[103]

DL-3-[2′,3′,4′,5′-$^{14}C_4$]Phenylalanine has been prepared by the co-cyclotrimerization of [$^{14}C_2$]acetylene and ethyl propargylformamidomalonate

[94] K. Y. Zee-Cheng and C. C. Cheng, *J. Medicin. Chem.*, 1972, **15**, 13.
[95] J. Galsonias, C. Frezou, and P. Vieles, *Compt. rend.*, 1972, **274**, *C*, 1392.
[96] R. S. Asquith and P. Carthew, *Tetrahedron*, 1972, **28**, 4769.
[97] J. A. Elberling and H. T. Nagasawa, *J. Heterocyclic Chem.*, 1972, **9**, 411.
[98] J. L. Garrett, B. Halpern, and R. S. Kenyon, *J.C.S. Chem. Comm.*, 1972, 135.
[99] R. M. Wightman, J. Staunton, A. R. Battersby, and K. R. Hanson, *J.C.S. Perkin I*, 1972, 2355.
[100] P. G. Strange, J. Staunton, M. R. Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Mavir, *J.C.S. Perkin I*, 1972, 2364.
[101] S. Thyagarajan, I. Mezö, I. Teplán, and J. Márton, *Acta Chim. Acad. Sci. Hung.*, 1972, **73**, 23.
[102] J. Márton and A. Kovács, *Acta Chim. Acad. Sci. Hung.*, 1972, **73**, 11.
[103] L. A. Holt and B. Milligan, *Photochem. and Photobiol.*, 1972, **16**, 511.

(Scheme 12; * indicates labelled atoms). The best catalyst for this reaction is $(Ph_3P)_2Ni(CO)_2$.[104] [3-^{11}C]-β-Alanine has been synthesized from chloracetic acid and $^{11}CN^-$ with subsequent catalytic reduction,[105] and [4-^{13}C]-1-methyl-2-amino-2-imidazolin-4-one from [1-^{13}C]glycine.[106]



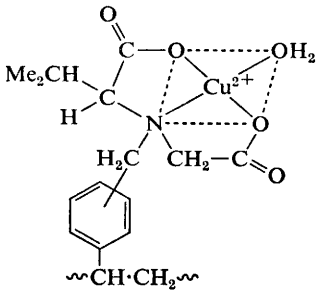
Scheme 12

An economical preparation of L-[^{35}S]methionine of high specific activity by extraction from yeast grown in the presence of $Na_2{}^{35}SO_4$ has been described,[107] and also a biosynthetic method.[108] *p*-[^{18}F]Fluorophenylalanine has been made by an exchange reaction with 4-(2′,2′-diethoxycarbonyl-2′-acetamidomethyl)phenyldiazonium tetrafluoroborate before carrying out a Schiemann reaction and subsequent hydrolysis.[109]

Resolution of α-Amino-acids.—Cyclohexyl *N*-trifluoroacetyl-L-α-aminobutyryl-L-α-aminobutyrate has been found useful as a stationary phase for the g.l.c. separation of the enantiomers of *N*-trifluoroacetyl-α-amino-acid n-propyl esters. Only glycine and L-threonine are not completely separated.[110] Separation of the enantiomers of t-leucine by g.l.c. of derivatives has not hitherto been achieved. Following up an idea that optically active stationary phases derived from α-amino-acids with less bulky side-chains might prove better for this purpose, it has been found that a resolution was possible using *N*-trifluoroacetyl-L-norvalyl-L-norvaline cyclohexyl ester.[111]

The labile metal complex (*N*-carboxymethyl-L-valine)copper(II) has been chemically bound to a polystyrene–0.8% divinylbenzene polymer to give a resin (21) which has been used to partially resolve several amino-acids. In all cases the D-isomers tend to elute first, and the degree of resolution increased as the bulkiness of the side-chain attached to the α-carbon increased.[112] D-Methionine-DL-*S*-oxide linked to a 5% cross-linked poly-

[104] L. Pichat, P. N. Liem, and J. P. Guermont, *Bull. Soc. chim. France*, 1972, 4224.
[105] H. Elias, H. F. Lotterbos, and K. Weiner, *Chem. Ber.*, 1972 **105**, 3754.
[106] G. L. Rowley and G. L. Kenyon, *J. Heterocyclic Chem.*, 1972, **9**, 203.
[107] R. Graham and W. M. Stanley, *Analyt. Biochem.*, 1972, **47**, 505.
[108] M. S. Bretscher and A. E. Smith, *Analyt. Biochem.*, 1972, **47**, 310.
[109] R. W. Goulding and A. J. Palmer, *Internat. J. Appl. Radiat. Isotopes*, 1972, **23**, 133.
[110] W. Parr and P. Y. Howard, *Angew. Chem. Internat. Edn.*, 1972, **11**, 529.
[111] W. Parr and P. Y. Howard, *J. Chromatog.*, 1972, **66**, 141.
[112] R. V. Snyder, R. J. Angelici, and R. B. Meck, *J. Amer. Chem. Soc.*, 1972, **94**, 260.



(21)

styrene and complexed with Cu^{2+} or Ni^{2+} has proved to be even more selective in the sorption of optical isomers. Conditions have been found which allow the L-isomers of isoleucine, proline, and *allo*threonine to be retained while the D-isomers are eluted.[113]

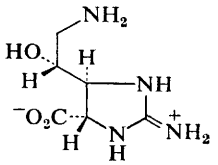
The preparation of all four stereoisomers of α,β-diaminobutyric acid from threonine has been reported.[114]

3 Physical and Stereochemical Studies of Amino-acids

Determination of Absolute Configuration.—A c.d. study of the *allo*threonine derived from the telomycin-related antibiotic LL-A-0341 has established that it has the D-configuration. This supports the 'rule of alpha epimerization' previously put forward by Bodanszky, and indicates that it originates from L-threonine. The 3-hydroxyproline and β-hydroxyleucine residues of this antibiotic were also investigated and found to have the L-configuration.[115] The peptide alkaloid lasiodine B contains a β-hydroxyleucine residue bound up as an aryl ether. The molecule can be degraded to (*p*-tolyloxy)leucine, and stereospecific syntheses of *threo*- and *erythro*-β-(*p*-tolyloxy)leucines have enabled the configuration of this residue in lasiodine to be determined.[16] The strongly basic amino-acid streptolidine, isolated on acid hydrolysis of the antibiotics streptolin, streptothricin, geomycin, roseothrycin, and racemomycin, has had its chirality established by a complete *X*-ray analysis of its dihydrochloride (22).[117]

Crystal Structures of Amino-acids.—A stereoscopic atlas of amino-acid structures has been published.[118] The *X*-ray crystal structure of β-(pyrazolyl-3)-L-alanine shows it to be, for most chemical purposes, isosteric

[113] S. V. Rogozkin, V. A. Davankov, I. A. Yamskov, and V. P. Kabanov, *J. Gen. Chem. U.S.S.R.*, 1972, **42**, 1605.
[114] E. Atherton and J. Meienhofer, *J. Antibiotics* (*Tokyo*), 1972, **25**, 539.
[115] A. Bodanszky, M. Bodanszky, K. L. Perlman, and D. Perlman, *J. Antibiotics* (*Tokyo*), 1972, **25**, 325.
[116] J. Marchand, F. Rocchiccioli, M. Pais, and F. X. Jarreau, *Bull. Soc. chim. France*, 1972, 4699.
[117] B. W. Bycroft and T. J. King, *J.C.S. Chem. Comm.*, 1972, 652.
[118] W. C. Hamilton, M. N. Frey, L. Golic, T. F. Koetzle, and M. S. Lehmann, *Mater. Res. Bull.*, 1972, **7**, 1225.



(22)

with histidine. However, it has been suggested that β-(pyrazolyl-4)-L-alanine may be a better substitute as a biological probe as it avoids the insertion of a hydrophobic region in a place where it is not present in Nature.[119] Other amino-acid crystal structures which have been established this year include homotaurine,[119a] γ-guanidobutyric acid hydrochloride,[120] L-histidine hydrochloride monohydrate,[121] the orthorhombic [122] and monoclinic [123] forms of L-histidine, and L-arginine phosphate monohydrate.[124] Amino-acid derivatives such as hippuric acid,[125] L-pyroglutamic acid *N*-methylamide,[126] *O*-(β-D-xylopyranosyl)-L-serine,[127] *N*-acetyl-L-histidine monohydrate,[128] and isostructural *N*-(halogenoacetyl)-L-phenylalanine ethyl esters [129] have also received attention.

The first papers in two projected series of neutron diffraction studies of α-amino-acids have appeared. These studies are aimed at producing more definitive stereochemical information on the hydrogen atom positions and better framework geometry than is available from *X*-ray studies. So far work concerning L-asparagine monohydrate,[130, 131] L-lysine hydrochloride dihydrate,[132, 133] L-glutamic acid hydrochloride,[134] L-histidine,[135] L-alanine,[136] and L-proline monohydrate [137] has been published.

[119] N. C. Seeman, E. L. McGandy, and R. D. Rosenstein, *J. Amer. Chem. Soc.*, 1972, **94**, 1717.
[119a] S. Veoka, T. Fujiwara, and K. Tomita, *Bull. Chem. Soc. Japan*, 1972, **45**, 3634.
[120] T. Maeda, T. Fujiwara, and K. Tomita, *Bull. Chem. Soc. Japan*, 1972, **45**, 3628.
[121] K. Oda and H. Koyama, *Acta Cryst.*, 1972, **B28**, 639.
[122] J. J. Madden, E. L. McGandy, and N. C. Seeman, *Acta Cryst.*, 1972, **B28**, 2377.
[123] J. J. Madden, E. L. McGandy, N. C. Seeman, and M. M. Harding, *Acta Cryst.*, 1972, **B28**, 2382.
[124] W. Saenger and K. C. Wagner, *Acta Cryst.*, 1972, **B28**, 2237.
[125] W. Harrison, S. Rittig, and J. Trotter, *J.C.S. Perkin II*, 1972, 1036.
[126] A. Aubry, M. Marraud, J. Protas, and J. Neel, *Compt. rend.*, 1972, **274**, *C*, 1378.
[127] L. T. J. Delbaere, M. Higham, B. Kamenar, P. W. Kent, and C. K. Prout, *Biochim. Biophys. Acta*, 1972, **286**, 441.
[128] T. J. Kistenmacher, D. J. Hunt, and P. E. Marsh, *Acta Cryst.*, 1972, **B28**, 3352.
[129] C. H. Wei, D. G. Doherty, and J. R. Einstein, *Acta Cryst.*, 1972, **B28**, 907.
[130] J. J. Verbist, M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Acta Cryst.*, 1972, **B28**, 3006.
[131] M. Ramandham, S. K. Sikha, and R. Chidambaram, *Acta Cryst.*, 1972, **B28**, 3000.
[132] R. R. Bugagong, A. Sequiera, and R. Chidambaram, *Acta Cryst.*, 1972, **B28**, 3214.
[133] T. F. Koetzle, M. S. Lehmann, J. J. Verbist, and W. C. Hamilton, *Acta Cryst.*, 1972, **B28**, 3207.
[134] A. Sequiera, H. Rajagopal, and R. Chidambaram, *Acta Cryst.*, 1972, **B28**, 2514.
[135] M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Internat. J. Protein Res.*, 1972, **4**, 229.
[136] M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *J. Amer. Chem. Soc.*, 1972, **94**, 2657.
[137] J. J. Verbist, M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Nature*, 1972, **235**, 328.

Optical Rotatory Dispersion and Circular Dichroism.—The use of some chiroptical probes for the configurational analysis of α-amino-acids has been reviewed.[138] Several reports this year concern the N-derivatives of α-amino-acids. A u.v.–visible and c.d. appraisal of *N*-(3-nitro-2-pyridyl)-amino-acids has been made, and as a result of this work a method of correlating the sign of the 420 nm c.d. band and the absolute configuration of the *N*-(3-nitro-2-pyridyl)-terminal amino-acid liberated by cleavage of the *N*-terminal peptide bond under mild acidic conditions has been proposed. The 3-nitro-2-pyridyl chromophore exhibits its long-wavelength optically active transition in a spectral range which is transparent for all natural amino-acids.[139] Since the reaction between 2-fluoro-3-nitropyridine and cysteinyl residue side-chains is selective and quantitative if suitable conditions are chosen for the reaction, a c.d. method is available for the analytical determination of thiol groups in peptides.[140]

N-Dimedonyl-α-amino-acids, derivatives which possess a vinylogous amide chromophore, have been spectroscopically re-investigated. Some similarity to the o.r.d. curves of some *N*-(2-pyridyl *N*-oxide)amino-acids was noted.[141] Another o.r.d. study of *N*-2,4-dinitrophenyl (DNP) α-amino-acids has been reported. The magnitude of the Cotton effects of di-DNP amino-acids is sensitive to solvent as well as to the difference in the chain length which separates the two DNP-groups. The spatial arrangement of the two DNP-groups is also a very important factor.[142] The c.d. spectra of intramolecularly hydrogen-bonded *N*-acetyl amino-acids are reported to have characteristics reminiscent of α-helical polypeptides.[143] The c.d. spectra of sultam derivatives of amino-acids [144] and pyridinium analogues of α-amino-acids [145] have also received attention.

Temperature-dependence measurements of the c.d. of solutions of L-cystine suggest that a fixed cystine residue in a protein molecule can have a bigger c.d. intensity than that expected from the spectrum of L-cystine measured at about 20 °C.[146] C.d. and n.m.r. studies of the N-methylation, N-acylation, and amidation of L-cystine have been interpreted in terms of rotamer preferences.[147] A study of the effects of temperature and hydrogen-bonding on the u.v. and c.d. spectra of *N*-acetyl-L-tyrosine n-hexyl ester, its *O*-methyl derivative, and *N*-acetyl-*O*-methyltyrosine has appeared. Variable-temperature c.d. of this sort may enable the motility of the tyrosyl side-chains in proteins to be investigated.[148] Work on the effects of

[138] C. Toniolo and A. Signor, *Experientia*, 1972, **28**, 753.
[139] C. Toniolo, D. Nisato, L. Biondi, and A. Signor, *J.C.S. Perkin I*, 1972, 1179.
[140] C. Toniolo, D. Nisato, L. Biondi, and A. Signor, *J.C.S. Perkin I*, 1972, 1182.
[141] V. Tortorella, G. Bettani, B. Halpern, and P. Crabbé, *Tetrahedron*, 1972, **28**, 2991.
[142] U. Nagai, M. Kurumi, and T. Umemura, *Tetrahedron*, 1972, **28**, 4959.
[143] J. R. Cann, *Biochemistry*, 1972, **11**, 2654.
[144] G. Snatzke and S. H. Doss, *Tetrahedron*, 1972, **28**, 2539.
[145] T. Gronneberg and K. Undheim, *Acta Chem. Scand.*, 1972, **26**, 2267.
[146] T. Takagi and N. Iro, *Biochem. Biophys. Acta*, 1972, **257**, 1.
[147] J. P. Casey and R. B. Martin, *J. Amer. Chem. Soc.*, 1972, **94**, 6141.
[148] E. Strickland, M. Wilchek, J. Horwitz, and C. Billups, *J. Biol. Chem.*, 1972, **247**, 572.

alkaline pH on the difference spectra of *N*-acetyltyrosine and its ethyl ester now permits the differential spectrophotometric assay for chymotrypsin-like enzymes to be extended to this pH range.[149]

Nuclear Magnetic Resonance Spectra.—The 220 MHz ^1H n.m.r. spectra of the peptide alkaloids frangulanine and discorines A and B have been analysed to show that the β-hydroxyleucine residue is of the *erythro*-configuration. This assignment has been supported by chemical degradation studies.[150] The n.m.r. spectra of six sulphur-containing α-amino-acids have been interpreted in terms of the apparent fractional populations of individual rotamers;[151] similar studies have been made on aspartic acid and asparagine.[152] Use of an n.m.r. shift reagent has made it possible to establish that the configuration about the double bond in naturally occurring L-γ-ethylideneglutamic acid is *cis*,[153] and examination of the n.m.r. spectra of substituted β-lactams has shown that, in contrast to previous assumptions, they exist in four isomeric forms.[154]

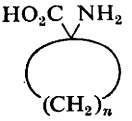
Mononuclear INDOR (internuclear double resonance) spectroscopy has now been applied to free amino-acids and their derivatives.[155] This technique simplifies the usual n.m.r. spectra and gives more chemical information. It is also a powerful method for examining total conformation in polypeptides. Deuterium nuclear quadrupole relaxation times have been obtained from proton lineshape analysis of the CHD resonances of [$^2H_1$]glycine and used to calculate correlation times for the molecular motions of the compound.[156] N.m.r. studies of polycrystalline aliphatic α-amino-acids have revealed two distinct second-moment transitions between about −180 and +110 °C. These transitions originate from hindering reorientation of CH_3 and NH_3^+ groups.[157]

Mass Spectrometry.—The mass spectral fragmentation mechanisms of twenty sulphur-containing amino-acids, including *S*-alkyl-L-cysteines, their sulphoxides, *S*-alkyl-2-methyl-DL-cysteines, and cyclic amino-acids, have been discussed. Using a direct inlet system, most compounds showed large molecular ion abundances and gave reproducible fragmentations.[158] Field-desorption mass spectrometry is a very sensitive method for the analysis of compounds of low volatility and thermal instability; it is therefore very suitable for the study of amino-acids. Recent work shows that, with the exception of methionine or cysteine, all amino-acids so far

[149] C. W. Ward, *Biochim. Biophys. Acta*, 1972, **271**, 87.
[150] M. G. Sierra, O. A. Moscaretti, F. J. Diaz, E. A. Ruveda, C. J. Chang, E. W. Hagaman, and E. Wenkert, *J.C.S. Chem. Comm.*, 1972, 915.
[151] K. D. Bartle, D. W. Jones, and R. L'Amie, *J.C.S. Perkin II*, 1972, 646.
[152] K. D. Bartle, D. W. Jones, and R. L'Amie, *J.C.S. Perkin II*, 1972, 650.
[153] J. R. Nulu and E. A. Bell, *Photochemistry*, 1972, **11**, 2573.
[154] H. Sterk, G. Uray, and E. Ziegler, *Monatsh.*, 1972, **103**, 544.
[155] W. A. Gibbons, H. Alms, J. Sogu, and H. R. Wyssbrod, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1261.
[156] J. P. Behr and J. M. Lehn, *J.C.S. Perkin II*, 1972, 1488.
[157] S. Ratkovic, *Chem. Phys. Letters*, 1972, **17**, 623.
[158] H. Nishimura, S. Takara, O. Okuyama, and T. Mitzutani, *Tetrahedron*, 1972, **28**, 4503.

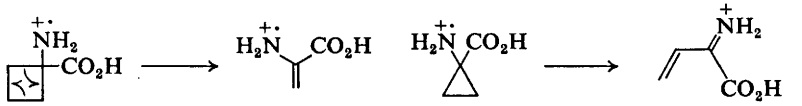
studied yield molecular or quasi-molecular ions. Arginine and cystine behave normally, in contrast to results obtained by electron-impact or chemical ionization studies. Less fragmention occurs in the field-desorption method, and rearrangement peaks are not found.[159]

The variation of fragmentation with ring size in a series of cyclic α-amino-acids (23; n = 3—6) has been investigated. In the compounds with the



(23)

larger ring sizes about half of the total ion current is carried by $M - CO_2H^+$ ions; this is analogous to the fragmentation of the open-chain naturally occurring amino-acids. However, in the cyclobutane and cyclopropane derivatives cleavage characteristic of the ring is observed (Scheme 13).[160]



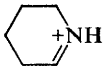
m/e 115 *m/e* 87 *m/e* 101

Scheme 13

Two further reports on the mass spectra of methyl- and phenyl-thiohydantoin derivatives have been published. One of these deals with the major metastable ions and points to their utility in identifying glycine, whose molecular ions are not unique, in the presence of other MTH's or PTH's. They also serve to clear up ambiguities occurring, for example, in mixtures of leucine and/or isoleucine.[161] The other paper discusses the use of low ionizing voltages; the spectra observed are less complex than the ones obtained by more conventional methods, and sensitivity is at a maximum at about 20 eV.[162]

An examination of derivatives of α-lysine, β-lysine, and various peptides containing these residues indicates that it is possible to distinguish between these isomeric amino-acids by m.s. The most intense peak for α-lysine occurs at *m/e* 84 and is assigned to (24); in contrast, β-lysine shows its most prominent peak at *m/e* 70 (25). β-Lysine peptides do not yield the aldimine fragments containing a *C*-terminal lysine that occur in the corresponding α-lysine compounds.[163]

[159] H. Winkler and H. D. Beckey, *Org. Mass Spectrometry*, 1972, **6**, 655.
[160] A. W. Coulter and C. C. Fenselau, *Org. Mass Spectrometry*, 1972, **6**, 105.
[161] T. Sun and R. E. Lovins, *Org. Mass. Spectrometry*, 1972, **6**, 39.
[162] T. Sun and R. E. Lovins, *Analyt. Biochem.*, 1972, **45**, 176.
[163] L. I. Rostovseva and A. A. Kiryushin, *Org. Mass. Spectrometry*, 1972, **6**, 1.


(24)


(25)

Other Physical and Stereochemical Studies.—Polarographic studies of cysteine in an ammoniacal buffer containing cobalt(III) have shown a catalytic double wave with markedly different peak potentials.[164] The dissociation constants of pipecolic acid, its 4-hydroxy-derivative, and dihydroxyphenylalanine (dopa) have been determined,[165] and a kinetic determination of α-amino-acid pK values based on their reactions with 1-fluoro-2,4-dinitrobenzene has been made. In this latter study the pH-dependent second-order rate constants were used to calculate the pK's; the amides of glycine and valine were found to have relatively high nucleophilicities.[166] A polarized i.r. study of the vibrational spectrum of a single crystal of (−)-alanine has been made; the spectrum was correlated with a C_2 molecular point group.[167] Ultrasound absorption measurements have been used to establish that a direct intramolecular proton transfer occurs between the protonated α-amino-group and the thiol anion of cysteine in weakly basic solution.[168] Other physical studies reported include an account of the effect of the dielectric constant of a solvent on the acoustic relaxation frequency in proline solutions,[169] and measurements of the optical absorption, conductivity, and photoemission of glycine sulphate.[170] Self-consistent-field calculations of glycine have also been made.[171]

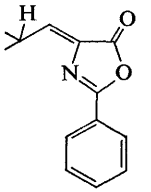
A solvent able to separate the *cis*- and *trans*-isomers of 4-hydroxymethylproline has been used to establish that loquat seeds contain not only the 'normal' *cis*-L-isomer but also the *trans*-D- and *trans*-L-forms.[172] A simple and efficient new resolution of racemic *N*-acetyl-β-(3,4-dimethoxyphenyl)-alanine with quinine has been reported,[173] and a quantitative estimation of the D- and L-enantiomers of leucine using a g.l.c. separation of *N*-trifluoroacetyl-L-prolyl-leucine methyl esters developed. Contrary to previous assertions, both the formation and use in the presence of triethylamine of *N*-trifluoroacetyl-L-prolyl chloride may be attended by extensive racemization. During coupling this can be prevented by adding the triethylamine slowly in very dilute solution in dichloromethane at the temperature of dry ice.[174]

[164] P. Anzerbacher and U. Kalous, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3209.
[165] A. Brun and R. Rossel, *Compt. rend.*, 1972, **274**, *C*, 1810.
[166] J. G. Ghazarian, *Arch. Biochem. Biophys.*, 1972, **150**, 72.
[167] R. Adamowicz and E. Fishman, *Spectrochim. Acta*, 1972, **28A**, 889.
[168] G. Maass and F. Peters, *Angew. Chem. Internat. Edn.*, 1972, **11**, 428.
[169] M. K. Ul'masova, *Izvest. Akad. Nauk S.S.S.R., Ser. fiz.-mat. Nauk*, 1972, **16**, **71**.
[170] G. Royal, B. Marlon, and G. Godfrey, *Compt. rend.*, 1972, **275**, *B*, 353.
[171] J. A. Ryan and J. L. Whittle, *J. Amer. Chem. Soc.*, 1972, **94**, 2396.
[172] D. O. Gray, *Phytochemistry*, 1972, **11**, 751.
[173] J. P. M. Houbiers, *Synthetic Comm.*, 1972, **2**, 143.
[174] W. A. Bonner, *J. Chromatog. Sci.*, 1972, **10**, 159.

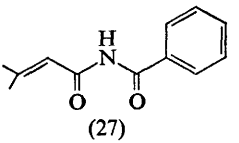
The physicochemical properties of several surface-active *N*-acyl glutamates and their sodium salts have been examined.[175]

4 Chemical Studies of Amino-acids

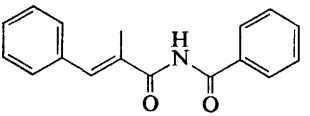
Oxidation and Reduction.—In the presence of mild bases such as triethylamine, 4-alkylidene-2-phenyl-2-oxazolin-5-ones, *e.g.* (26), rapidly absorb atmospheric oxygen and eliminate carbon dioxide, forming a mixture of imides (27) and (28). The formation of these and two other acidic by-products (29) and (30) can be accounted for by postulating the decomposition of a hydroperoxide intermediate.[176] The decomposition of tryptophan



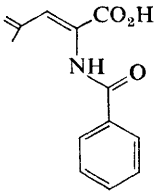
(26)



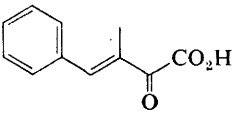
(27)



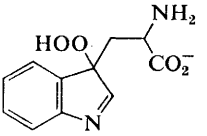
(28)



(29)



(30)



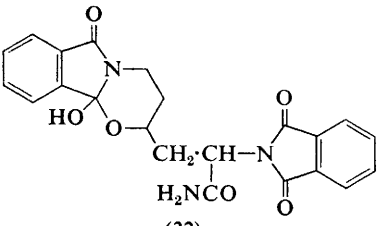
(31)

under both alkaline and acidic conditions has been re-evaluated. In alkali in the presence of oxygen a free-radical autoxidation involving the hydroperoxide (31) is proposed; this is supported by the relative stability of 1-methyltryptophan. Initiation of the reaction appears to be due to impurities in the sodium hydroxide and not to the base itself. This accords with the greater apparent stability of tryptophan in solutions of barium hydroxide.[177] The more extensive destruction that occurs in hydrochloric acid is probably due to autoxidation of the 1-protonated form initiated by impurities in the glass of the containing vessels.[178]

Attempts to prepare 4-oxolysine from 4-hydroxylysine, which is readily available from lysine by photochlorination and subsequent hydrolysis, have proved largely unsuccessful. Alkaline permanganate gave some of the desired product, but it seemed very susceptible to further oxidation;

[175] M. Takehara, H. Mariyuku, I. Yoshimura, and R. Yoshida, *J. Amer. Oil Chemists' Soc.*, 1972, **49**, 143.
[176] R. Bisson, R. B. Yeats, and E. W. Warnhoff, *Canad. J. Chem.*, 1972, **50**, 2851.
[177] M. Stewart and C. H. Nicholls, *Austral. J. Chem.*, 1972, **25**, 1595.
[178] M. Stewart and C. H. Nicholls, *Austral. J. Chem.*, 1972, **25**, 2139.

some glycine and aspartic acid are formed. *NN'*-Diphthaloyl-*threo*-4-hydroxy-L-lysineamide proved resistant to oxidation under acidic conditions, probably owing to its existence as the cyclic hemiacetal tautomer (32).[81] The oxidation of *N*-benzoylmethionine by 3-hydroperoxyindolenines, reagents thought likely to exist in biological systems, is more



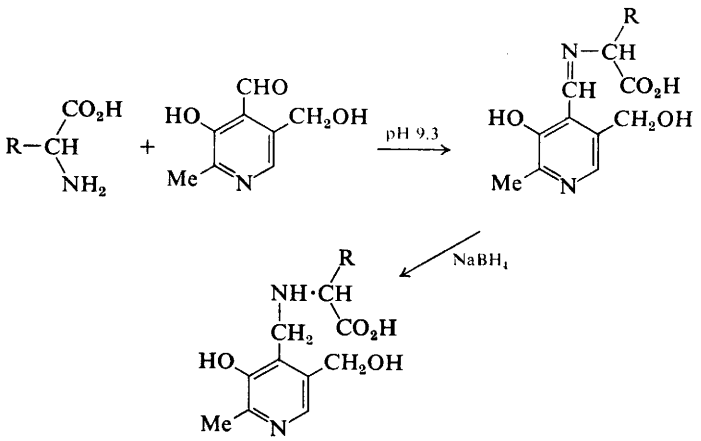
(32)

selective than that which occurs when hydrogen peroxide is used. Even if an excess of the reagent is used, oxidation goes no further than the corresponding sulphoxide.[179]

Both horseradish- and lacto-peroxidase are stereospecific in their oxidation of tyrosine. The product formed in both cases under initial rate conditions is the *oo'*-diphenyl-linked dimer. Lactoperoxidase couples the L-isomer more readily than the D, but horseradish peroxidase has the reverse specificity.[180] Kinetic studies of the reaction of β-chloroalanine with D-amino-acid oxidase, which involves both oxidation and dehydrohalogenation, have been reported.[181–183]

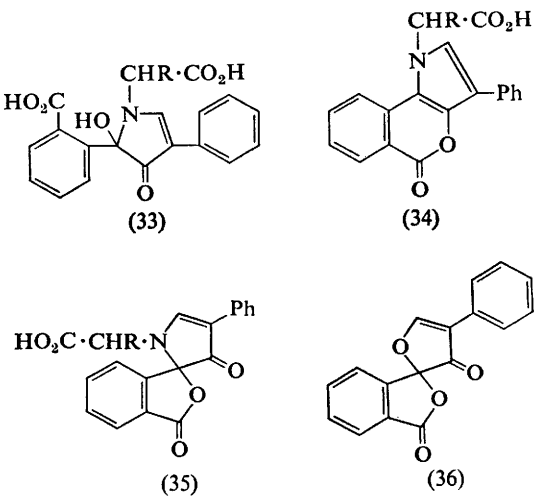
The formation of *N*-acyl-α-amino-aldehydes from *N*-acyl-α-amino-acids by hydrogenolysis, in the presence of palladium, of their mixed anhydrides with ethyl chloroformate has been investigated. There is considerable racemization, and the presence of a little acetic acid in the reaction mixture favours the highest yield of aldehyde. If triethylamine is present, however, much more of the corresponding alcohol is formed.[184] In an alkaline medium, amino-acids condense with pyridoxal to give Schiff bases which can be reduced to pyridoxyl amino-acids by sodium borohydride (Scheme 14). These derivatives can be used to determine the original amino-acid concentration. Spectrophotometry will detect 2×10^{-8} mol of the derivative, fluorimetry 5×10^{-10} mol, and, if $NaBT_4$ is used, quantitative determination on a picomole scale is possible.[185]

[179] M. Nakagawa, T. Suzuki, T. Kawashima, and T. Hino, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 2413.
[180] G. S. Bayse, A. W. Michaels, and M. Morrison, *Biochim. Biophys. Acta*, 1972, **284**, 30, 34.
[181] J. G. Voet, D. J. T. Porter, and H. J. Bright, *Z. Naturforsch.*, 1972, **27b**, 1054.
[182] Y. Miyaki, T. Abe, and T. Yamamo, *Z. Naturforsch.*, 1972, **27b**, 1376.
[183] D. J. T. Porter, J. G. Voet, and H. J. Bright, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 257.
[184] A. Seki, K. Koga, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 361.
[185] N. Lustenberger, H.-W. Lange, and K. Hempel, *Angew. Chem. Internat. Edn.*, 1972, **11**, 227.



Scheme 14

General Reactions.—Details of the investigation of the reaction of phenylalanine with ninhydrin to generate fluorescent compounds from amino-acids or amines have been published. The key intermediate involved is phenylacetaldehyde; this reacts with any amino-acid present to give the major fluophor (33), a minor fluophor (34), and a non-fluorescent minor component (35).[186, 187] Further work has established that the fluophor (33) can be generated directly by reaction of an amino-acid with 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (36; 'fluorescamine'); the reagent



(33) (34)

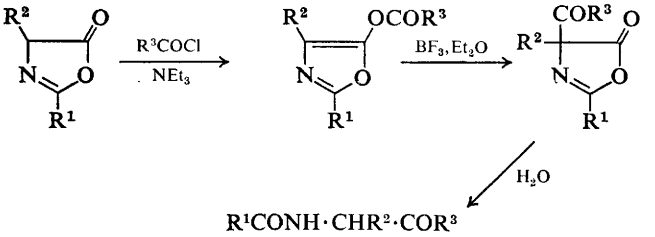
(35) (36)

[186] M. Weigele, J. F. Blount, J. P. Tengi, R. C. Czarjowski, and W. Leimgruber, *J. Amer. Chem. Soc.*, 1972, **94**, 4052.

[187] M. Weigele, S. C. De Bernardo, J. P. Tengi, and W. Leimgruber, *J. Amer. Chem. Soc.*, 1972, **94**, 5927.

itself is not fluorescent. When fluorescamine is added to an amino-acid in aqueous buffer at pH 7 reaction is complete in less than a second. Excess reagent is hydrolysed in less than a minute.[188] The application of this method to the automated assay of amino-acids has also been described; 50 pmol can be detected.[189]

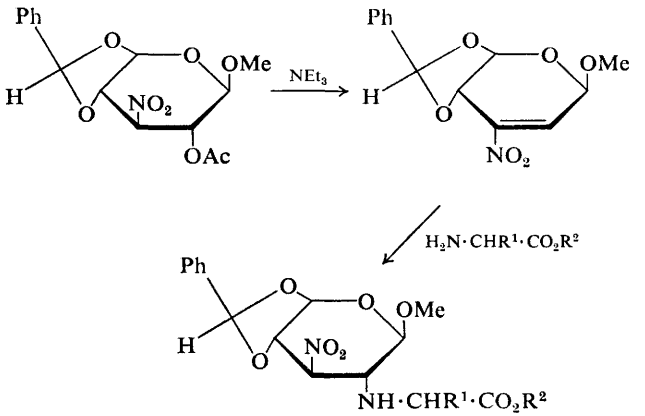
A method of preparing α-acylamino-ketones from α-amino-acids by C-acylation of azlactones has been developed. The enol ester initially formed with an acid chloride isomerizes on treatment with boron trifluoride etherate, and subsequent hydrolysis liberates the desired derivative (Scheme 15).[190]



Scheme 15

A number of papers concerning further exploration of N-derivatives of general utility are most conveniently considered here. A novel route to carbohydrate derivatives involves generating α-nitro-olefinic sugars from β-nitroacetoxy precursors by treating with triethylamine. Amino-acid esters will add across the double bond to give the N-substituted product; the intermediate need not be isolated (Scheme 16).[191] Some *N*-(3,5-dinitro-2-thienyl) amino-acids have been examined. It is reported that the protecting group is of acid stability comparable to that of 2,4-dinitrophenyl except in the case of glycine and perhaps methionine.[192] The use of dipolar aprotic solvents such as dimethyl sulphoxide containing 30% water for the preparation of *N*-2,4-dinitrophenyl amino-acids is recommended. The rates of reaction are much faster than in the conventional aqueous ethanol and the yields are higher.[193] *p*-Aminobenzoyl amino-acids have been investigated as derivatives for isoelectric focusing; this acyl group has the advantage of absorbing u.v. light in a different region to the carrier ampholyte.[194] The preparation of *N*-pyrimidonyl amino-acids using methylmercaptopyrimidine has been reported.[195]

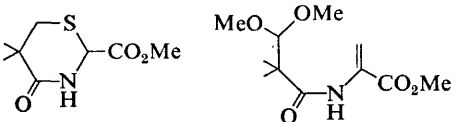
[188] S. Udenfriend, S. Stein, P. Bohlen, and W. Dairman, *Science*, 1972, **178**, 871.
[189] S. Udenfriend, *J. Res. Nat. Bur. Stand., Sect. A*, 1972, **76**, 637.
[190] N. I. Aronova, N. N. Makhova, and S. I. Zav'yalov, *Bull. Acad. Sci. U.S.S.R.*, 1972, **21**, 349.
[191] F. J. M. Rajabalee, *Synthesis*, 1972, 318.
[192] L. H. Hellberg, M. J. Prodanovich, and F. Stults, *J. Heterocyclic Chem.*, 1972, **9**, 401.
[193] J. A. Vinson and L. A. Pepper, *Analyt. Chim. Acta*, 1972, **56**, 245.
[194] N. Catsimpoolas and B. E. Campbell, *Analyt. Biochem.*, 1972, **46**, 674.
[195] S. Hoffmann, H. Schubert, and K. Nitsche, *Z. Chem.*, 1972, **12**, 21.



Scheme 16

A study of the solubility of amino-acids in aqueous guanidinium thiocyanate indicates that this solution is more effective than urea, guanidinium chloride, or organic solvents in decreasing the free energy of transfer of hydrophobic amino-acid side-chains from water to these solvents.[196] The standard enthalpies and entropies for protonating the anions of some biologically important amino-acids have been measured in 3M sodium perchlorate at 25 °C,[197] and a further study of the silylation of amino-acids has appeared.[198]

Specific Reactions.—The observation that the perhydro-1,4-thiazepine derivative (37) undergoes ring-opening on refluxing with silver oxide in



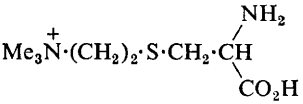
(37) (38)

methanol to form the α-acylamino-acrylate (38) has been extended to allow the preparation of α-acetamidoacrylates from various cysteine and penicillamine derivatives (Scheme 17). This reaction therefore appears to be a general one.[199] Trimethylaminoethylation of cysteine with (2-bromoethyl)trimethylammonium bromide leads to the new amino-acid thialaminine (39). This material was prepared in connection with protein modification studies; such derivatives of cysteine residues in proteins

[196] K. H. Dooley and F. J. Castellino, *Biochemistry*, 1972, **11**, 1870.
[197] R. D. Graham, D. R. Williams, and P. A. Yeo, *J.C.S. Perkin II*, 1972, 1876.
[198] H. A. Kricheldorf, *Annalen*, 1972, **763**, 17.
[199] D. Gravel, R. Gauthier, and C. Berse, *J.C.S. Chem. Comm.*, 1972, 1322.

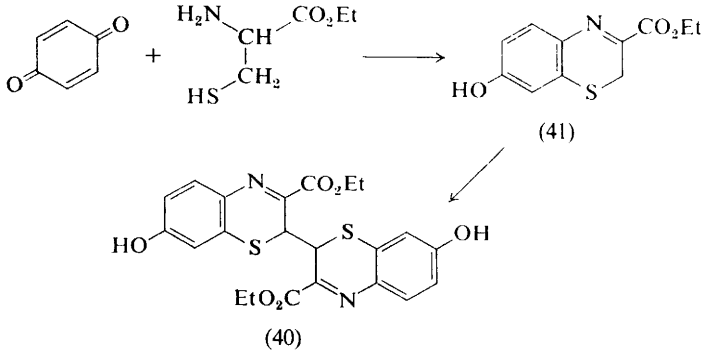
R = H or Me

Scheme 17



(39)

confer water solubility, in contrast to reduction or alkylation.[87] The two isomeric products obtained by treating L-cysteine methyl ester with *p*-benzoquinone are suggested to be the diastereoisomers of (40), which arise by oxidative coupling of (41) (Scheme 18).[200]

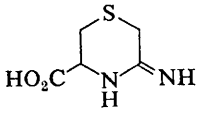


(41)

(40)

Scheme 18

Ethyl chloracetimidate has been proposed as a new bifunctional protein reagent; model reactions with glycine and cysteine yield chloracetamidinoacetic acid and (42), respectively.[201] 4-Chloro-3,5-dinitrophenacyl bromide has also been considered in this context. Reaction with thiol groups occurs readily, but the amino-groups of all amino-acids, except phenylalanine, tyrosine, and tryptophan, are not attacked. These exceptions are



(42)

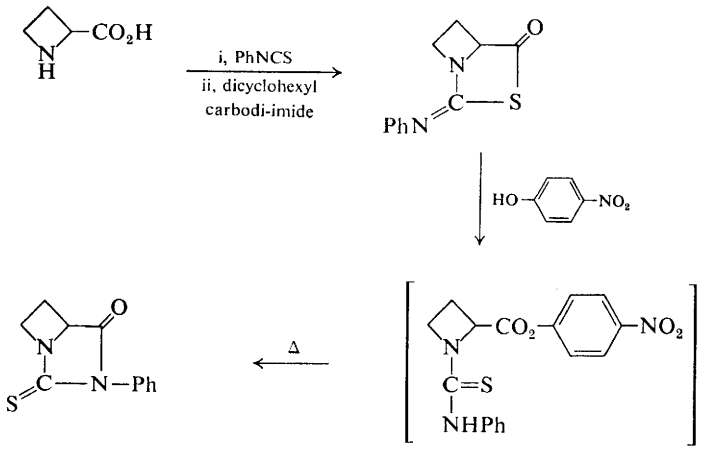
[200] G. Prota and E. Ponsiglione, *Tetrahedron Letters*, 1972, 1327.
[201] M. Olomucki and J. Diopak, *Biochim. Biophys. Acta*, 1972, **263**, 213.

ascribed to the formation of charge-transfer complexes between the aromatic rings and the reagent which facilitate reactions.[202] The modification of methionine with BBr_3 or BI_3 has been studied. After 167 h at 22 °C 94% of the methionine had reacted, largely to produce homocysteic acid, homoserine, and homoserine lactone. In experiments with proteins, other amino-acids were unaffected.[203] In the presence of ferrous or manganous ions and sulphite, methionine decomposes to free methanethiol and dimethyl sulphide. This degradation is thought to proceed through the initial formation of 3-(methylthio)propional.[204] A study of the reaction of methionylsulphonium salts with thiols has established that the methionyl residue can be regenerated in good yields.[205]

The influence of structure on the β-elimination reaction of protected *O*-tosyl β-hydroxy-α-amino-acids to give aziridinecarboxylic acid derivatives has been investigated. Ring formation of this sort is in general easier with threonine peptides.[206] It has been found possible to acylate selectively the hydroxy-groups of serine, threonine, and tyrosine without affecting the α-amino-groups by treating with carboxylic acid chlorides in anhydrous trifluoroacetic acid.[207] The conversion of DL-threonine into DL-*trans*-3-benzoyl-5-methyl-2-oxo-oxazolidine-4-carboxylic acid has been further detailed,[208] and a study has been made of the reaction products of thionyl chloride and a series of *erythro*- and *threo*-isomers of some *N*-benzoyl-β-arylserine methyl esters.[209]

A detailed investigation of the nitrous acid deamination of (*R*)-α-methylphenylalanine methyl ester in acetic acid has revealed products of elimination (68%), substitution (21%), hydrogen migration (8%), and phenyl migration (3%). The stereochemical courses of these reactions have also been examined.[210] Under the same conditions, L-valine benzyl ester gives a similar spectrum of products, but free L-valine yields only the substitution product.[211] The normal conditions used for the preparation of phenylthiohydantoins fail when applied to azetidine-2-carboxylic acid. This derivative can, however, be obtained in good yield by heating the *p*-nitrophenyl ester of the corresponding 2-phenyliminothiazolidin-5-one (Scheme 19).[212] Contrary to the views of earlier workers, the interaction

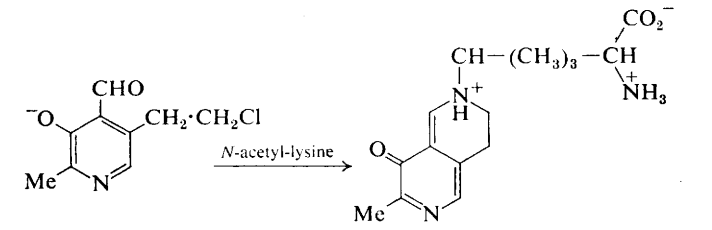
[202] J. Diopak and M. Olomucki, *Biochim. Biophys. Acta*, 1972, **263**, 220.
[203] M. Z. Atassi and M. T. Perlstein, *Tetrahedron Letters*, 1972, 1861.
[204] T. Wainwright, J. F. McMahon, and T. McDowell, *J. Sci. Food Agric.*, 1972, **23**, 911.
[205] F. Naider and Z. Bohak, *Biochemistry*, 1972, **11**, 3208.
[206] Y. Nakagawa, T. Tsuno, K. Nakajima, M. Iwai, H. Kawai, and K. Okawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 1162.
[207] A. Previero, L.-G. Barry, and M.-A. Coletti-Previero, *Biochim. Biophys. Acta*, 1972, **263**, 7.
[208] T. Inui, *Bull. Chem. Soc. Japan*, 1972, **45**, 1254.
[209] S. H. Pines and M. A. Kozlowski, *J. Org. Chem.*, 1972, **37**, 292.
[210] M. Kobayashi, K. Koga, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 1898.
[211] M. Taniguchi, K. Koga, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 1438.
[212] H. T. Nagasawa, P. S. Fraser, and J. A. Elberling, *J. Org. Chem.*, 1972, **37**, 516.



Scheme 19

between proline and other imino-acids and benzoquinone has been found to be due to charge-transfer complexation rather than chemical addition.[213] The photochemical formation of 2-isobutylpyrido[2,3-*d*]imidazole 1-oxide from 3-nitro-2-pyridyl-DL-leucine has been investigated spectrophotometrically.[214] Nicotinylglycine can be conveniently prepared by acylating glycine methyl ester using nicotinic anhydride and then saponifying. The nicotinic acid produced in the first stage is insoluble in non-polar solvents and readily separated.[215] L-Proline has been used for the spectrophotometric determination of Cu^{II}.[216]

A new cyclic imino-acid derivative of homopyridoxal can be formed from *N*-α-acetyl-L-lysine and 5-(2-chloroethyl)-3-hydroxy-2-methylpyridine-4-carboxaldehyde (Scheme 20). This product serves as a useful model of pyridoxal-P enzymes in which the cofactor is usually bound in an imine link with the ε-amino-group of a lysine residue.[216a]



Scheme 20

[213] G. H. Moxon and M. A. Slifkin, *J.C.S. Perkin II*, 1972, 1159.
[214] G. G. Aloisi, E. Bordignan, and A. Signor, *J.C.S. Perkin II*, 1972, 2218.
[215] M. T. Wu and R. E. Lyle, *J. Pharm. Sci.*, 1972, **61**, 141.
[216] E. Campi and P. Mirti, *Analyt. Chim. Acta*, 1972, **58**, 239.
[216a] E. W. Miles, H. M. Fales, and J. B. Gin, *Biochemistry*, 1972, **11**, 4945.

An improved method for preparing L-aspartic anhydride directly from L-aspartic acid using phosphorus halides has been evolved,[217] and the facile cyclization of glutamic acid α-t-butyl derivatives re-emphasized.[218] Two studies on the hydrolysis of amino-acid esters have been reported this year; one concerns the cobalt(II)-catalysed hydrolysis of diethyl aspartate and some dipeptide ethyl esters,[219] and the other deals with the chymotrypsin-catalysed decompositions of L-tryptophan and L-tyrosine methyl esters. The mechanism of the latter reactions appears to be the same as that for acylamino-esters, but the ionizability of the free α-amino-group produces a markedly different pH dependency.[220] Although the imino-groups of peptides react with t-butyl hypochlorite, the α-amino-groups of amino-acids are largely unaffected by this reagent.[221] The thermal decomposition of a solid complex of aminomalonic acid and β-alanine has been studied in detail.[222]

Effects of Electromagnetic Radiation on Amino-acids.—The radiation-induced reactions of amino-acids have been reviewed.[223] ENDOR studies of the effect of ionizing radiation on single crystals of acetylglycine show that the peptide link is involved in the reduction process thus initiated.[224] A similar study of DL-serine at 77 K indicated that the initial species produced are anion and cation radicals. At room temperature these decay to form two stable radicals.[225] Three investigations of the effect of u.v. radiation on specific amino-acids have been made. Irradiation of single crystals of cystine dihydrochloride with monochromatic u.v. is thought to lead to two processes (Scheme 21),[226] and photolysis of aqueous solutions

$$\mathrm{RSSR} + h\nu \longrightarrow \mathrm{RS\overset{+}{S}R} + \mathrm{e^-}$$
$$\mathrm{e^-} + \mathrm{RSSR} \longrightarrow \mathrm{RS\cdot} + \mathrm{RS^-}$$

Scheme 21

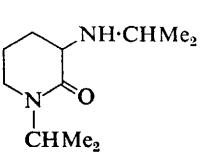
of arginine leads to fourteen ninhydrin-positive products, including ornithine, aspartic acid, and glycine.[227] The primary products of the flash photolysis of tryptophan have also been investigated.[228]

[217] Y. Arioyoshi, T. Yamatomi, N. Uchiyama, and N. Sato, *Bull. Chem. Soc. Japan*, 1972, **45**, 2208.
[218] M. Hollósi, M. Kajtár, Z. Ráthanyi, and J. Tomasz, *Acta Chim. Acad. Sci. Hung.*, 1972, **71**, 101.
[219] A. Y. Girgis and J. I. Legg, *J. Amer. Chem. Soc.*, 1972, **94**, 8420.
[220] F. J. Kézdy, S. P. Jindal, and M. L. Bender, *J. Biol. Chem.*, 1972, **247**, 5746.
[221] A. Matsushima, S. Yamazaki, K. Shibata, and Y. Imada, *Biochim. Biophys. Acta*, 1972, **271**, 243.
[222] Y. Nishijo, I. Imanishi, and G. Hashizume, *Bull. Chem. Soc. Japan*, 1972, **45**, 2070.
[223] G. M. Warren, *Radiat. Res. Rev.*, 1972, **3**, 305.
[224] H. C. Box, E. E. Budzinski, and K. T. Lilga, *J. Chem. Phys.*, 1972, **57**, 4295.
[225] B. W. Castleman and G. C. Moulton, *J. Chem. Phys.*, 1972, **57**, 1095.
[227] H. D. Pathak, P. C. Joshi, and U. N. Pande, *Indian J. Biochem. Biophys.*, 1972, **9**, 221.
[228] R. Santus and L. I. Grossweiner, *Photochem. and Photobiol.*, 1972, **15**, 101.

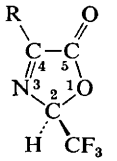
5 Analytical Methods

Gas–Liquid Chromatography.—Analysis of biological substances and geochemical samples requires such sensitivity that contamination is an important problem. A g.l.c. study of necessary chemical reagents and other sources of contamination has been made, and rigorous techniques are suggested so that analysis of samples containing 1—10 mg of amino-acids per gram is readily achievable. Analysis of 3—4 billion-year-old Onverwacht chert has been used to demonstrate the application of the techniques to a geochemically important sample.[229] An improved procedure for extraction after lyophilization has allowed as little as 10^{-11} mol of protein amino-acids to be determined in sea-water samples. The extracted samples were converted into their trimethylsilyl (TMS) derivatives for g.l.c. analysis.[230] A separation of twenty amino-acids by g.l.c. of their *N*-TMS-*O*-n-butyl esters in less than thirty-five minutes has been described. Numerous extraneous peaks detected along the base line are due to incomplete esterification of the amino-acids. This occurs because of some water produced under the conditions of esterification affecting the equilibrium position.[231] Using these same protecting groups, the elution positions of twenty-six non-protein amino-acid derivatives have been established. Only in two cases does co-elution with a protein amino-acid occur; *N*-amidinoalanine overlaps cystine, and 6-aminohexanoic acid is not resolved from aspartic acid.[232]

A g.l.c.–m.s. study of the isopropylation of amino-acids shows that, under the conditions used, the *N,O*-di-isopropyl derivatives are not always obtained in high yield. Some amino-acids do not react, and others show multiple peaks. Arginine forms a derivative thought to be the lactam (43).[233] The molar responses of *N*-trifluoroacetyl amino-acid methyl esters with a flame ionization detector have been determined.[234] Various oxazolin-5-one derivatives of leucine have been examined for their potential in g.l.c. They all proved thermally stable and can be prepared from the free amino-acids in a single step.[235] The successful separation by g.l.c. of a mixture of



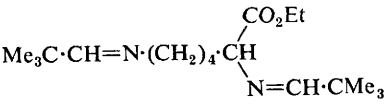
(43)



(44)

[229] J. J. Rosh, C. W. Gehrke, R. W. Zumwalt, K. C. Kuo, K. A. Kvenvolden, and D. L. Stalling, *J. Chromatog. Sci.*, 1972, **10**, 444.
[230] R. Pocklington, *Analyt. Biochem.*, 1972, **45**, 403.
[231] J. P. Hardy and S. L. Kerrin, *Analyt. Chem.*, 1972, **44**, 1497.
[232] F. Raulin, P. Shapshak, and B. N. Khare, *J. Chromatog.*, 1972, **73**, 35.
[233] B. Blessington and N. I. Y. Fiagbe, *J. Chromatog.*, 1972, **68**, 259.
[234] A. Islam and A. Darbre, *J. Chromatog.*, 1972, **71**, 223.
[235] O. Grahl-Nielson and E. Solheim, *J. Chromatog.*, 1972, **69**, 366.

ten amino-acid 2-trifluoromethyloxazolin-5-ones has subsequently been reported; the chirality of the α-C atom is lost in these derivatives but C-2 of the ring becomes asymmetric (44).[236] An improved g.l.c. analysis of methyl- and phenyl-thiohydantoins of amino-acids has been described,[237] and it has proved possible to assign configurations to amino-acid enantiomers by analysis of the g.l.c. elution positions of their *N*-trifluoroacetyl esters when the optically active stationary phase carbonylbis-(*N*-L-valine isopropyl ester) is used.[238] Lysine in acid hydrolysates of wheat and rice seeds can be conveniently determined by g.l.c. as its bisneopentylidene ethyl ester derivative (45). This is prepared by treating lysine ethyl ester with pivaldehyde.[239]



(45)

Ion-exchange Chromatography.—A study of the influence of acidity on the reaction of ninhydrin with amino-acids indicates that the optimum pH for colour development depends only on the pK_2 of the amino-acid. According to an equation proposed, histidine and tryptophan are anomalous in aqueous methyl cellosolve but not in aqueous dimethyl sulphoxide. In the latter solvent hydrogen-bonding is thought to prevent interaction of the NH of the rings with the α-amino-groups. The authors conclude that the pH of 5 as normally used represents the best compromise.[240] It has been reported that the specific radioactivity of individual ^{14}C-labelled amino-acids isolated by ion-exchange chromatography is different in consecutive fractions containing the same amino-acid. This is attributed to the resolution of [^{14}C]- and [^{12}C]-amino-acids during the ion-exchange. The specific radioactivity increases on progressive elution from cation-exchange resins, but decreases from anion-exchange resins. [^{14}C]-γ-Aminobutyric acid is not quantitatively eluted from ion-exchange resins by citrate buffers; this is attributed to adsorption on to the glass walls of containers. Recovery varies with the pH of the buffer.[241]

The hydrolysis of proteins and peptides by a mixture of sepharose-bound peptidases gives results comparable with acid hydrolysis for the acid-stable amino-acids, and has the advantage that good values are obtained also for tryptophan, asparagine, and glutamine. The peptidases involved are prolidase, aminopeptidase M, trypsin, and chymotrypsin.[242]

[236] O. Grahl-Nielson and E. Solheim, *J.C.S. Chem. Comm.*, 1972, 1092.
[237] J. J. Pisano, T. J. Bronzert, and H. B. Brewer, *Analyt. Biochem.*, 1972, **45**, 43.
[238] B. Feibush, E. Gil-Av, and T. Tamari, *J.C.S. Perkin II*, 1972, 1197.
[239] F. P. Zscheile and B. L. Brannaman, *Analyt. Biochem.*, 1972, **49**, 442.
[240] P. J. Lamotte and P. G. McCormick, *Analyt. Chem.*, 1972, **44**, 821.
[241] M. K. Gaitande and R. W. K. Nixey, *Analyt. Biochem.*, 1972, **50**, 416.
[242] H. P. J. Bennett, D. F. Elliott, B. E. Evans, P. J. Lowry, and C. McMartin, *Biochem. J.*, 1972, **129**, 695.

Further information on the elution positions of non-protein amino-acids relative to protein amino-acids on ion-exchange chromatography has been published.[243] An automated chromatographic system for the combined analysis of amino-acids and urinary peptides has been recommended,[244] and an automatic computer-compatible digital data-acquisition system for amino-acid analysis has been developed.[245]

An improved resolution of sulphur-containing amino-acids in physiological fluids on a new 10% cross-linked resin has been described. The longer elution times occurring as a result of this high degree of cross-linking can be offset by using a higher temperature and six buffers on a two-column system.[246] A urinary hydroxyproline assay involving adsorption on a strong cation-exchange resin, a washing step, and then hydrolysis of the resin-bound peptides by simply raising the temperature before eluting and estimating by conventional amino-acid analysis has been described as well suited for routine clinical assays.[247] A thirty-minute automated assay of hydroxyproline has also been advocated.[248] Two single-column systems for the separation of desmosine from other amino-acids have been reported;[249, 250] a one-column system for the determination of ^{14}C-labelled lysine and hydroxylysine is also now available.[251]

About 8% of the *trans*-4-hydroxyproline in collagen is epimerized on acid hydrolysis. Hitherto this has not been detected, as *cis*-4-hydroxyproline elutes on amino-acid analysis at the same time as threonine. However, if the primary amino-acids in the hydrolysate are deaminated with nitrous acid before carrying out ion-exchange chromatography the *cis*-isomer can be determined.[252] Addition of 3-(3-indolyl)propionic acid to proteins or peptides before acid hydrolysis has been found to improve the recovery of tryptophan. The use of reducing agents such as thioglycollic acid in a similar way, however, affects the recovery of some other amino-acids.[253] An ion-exchange system which separates the oxidation products of tryptophan in a two-hour run has been described.[254]

Thin-layer Chromatography.—A t.l.c. method for determining the optical purity of labelled amino-acids by converting them into dipeptides with the *N*-carboxanhydride of L-leucine has proved generally useful for at least

[243] P. Shapshak and M. Okaji, *J. Chromatog.*, 1972, **64**, 178.
[244] J. A. Klosse, D. Y. Huistra, P. K. de Bree, S. K. Wadman, and J. F. G. Vliegenthart, *Clin. Chim. Acta*, 1972, **42**, 409.
[245] M. L. Johnson, E. A. Khairallah, and D. A. Yphantis, *Analyt. Biochem.*, 1972, **50**, 364.
[246] J. O. Jeppsson and I. M. Karlsson, *J. Chromatog.*, 1972, **72**, 93.
[247] B. C. Goverde and F. J. N. Veenkamp, *Clin. Chim. Acta*, 1972, **41**, 29.
[248] P. X. Callahan, J. A. Shepard, and S. Ellis, *Analyt. Biochem.*, 1972, **49**, 155.
[249] G. E. Gerbb and G. D. Kemp, *J. Chromatog.*, 1972, **71**, 361.
[250] D. P. Thornhill, *Analyt. Biochem.*, 1972, **46**, 119.
[251] R. S. Askenasi and N. A. Kefalides, *Analyt. Biochem.*, 1972, **47**, 67.
[252] D. D. Dzietviatowski, V. C. Haxall, and R. L. Riolo, *Analyt. Biochem.*, 1972, **49**, 550.
[253] L. C. Gruen and P. W. Nicholls, *Analyt. Biochem.*, 1972, **47**, 348.
[254] P. W. Nicholls and D. E. Rivett, *J. Chromatog.*, 1972, **65**, 565.

nineteen labelled amino-acids.[255] Methods for the determination of methionine and cysteine in legume seeds have been developed. Methionine is converted into its sulphone and, after t.l.c. separation, developed with ninhydrin and quantitated by reflectance densitometry. Cysteine is subjected to hydrazinolysis and the hydrogen sulphide liberated estimated colorimetrically as bismuth sulphide.[256] The separation of free amino-acids on cellulose sulphate-impregnated cellulose [257] and silica layers bound with agar-agar [258] have also been detailed.

The quantitative determination down to 10^{-14} moles of dansyl amino-acids prepared from ^{14}C-labelled dansyl chloride has been claimed. The derivatives are run on polyamide layers and the spots visualized by micro-autoradiography followed by scanning microscope photometry.[259] The separation of dansyl amino-acids on a layer composed of three sorbents using a single solvent system has been described,[260] and the t.l.c. of diphenyl-indenonesulphonyl amino-acids investigated.[261]

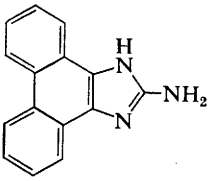
Other Methods.—Two systems for separating the cross-link amino-acids from acid hydrolysates of proteins such as elastin have been reported. Column chromatography on a polyacrylamide gel is recommended for large-scale separations,[262] and high-voltage paper electrophoresis has proved reliable and reproducible but does not separate desmosine and isodesmosine.[263] The separation of dansyl amino-acids on a polyamide column [264] and the copper complexes of amino-acids on a Sephadex anion-exchange column [265] have also proved successful. The latter system was developed for the qualitative analysis of cheese.

Determination of Specific Amino-acids.—Cysteine can be estimated by reacting it with ninhydrin at pH 10. The colour is due to the formation of hydrindantin, with a maximum at 470 nm. Other amino-acids do not interfere, but reducing substances such as glucose do if present at high concentration.[266] α-Substituted cysteines and cystines can be determined titrimetrically with *N*-bromosuccinimide using Bordeaux red as the indicator.[267] Aspartic acid and glutamic acid can be determined using guanidine carbonate,[268] and a radiometric technique for measuring L-asparagine in

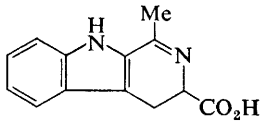
[255] A. V. Barooshian, M. J. Lautenschlager, and J. M. Greenwood, *Analyt. Biochem.*, 1972, **49**, 602; A. V. Barooshian, M. J. Lautenschlager, and W. G. Harris, *ibid.*, p. 569.
[256] H. E. Herrick, J. M. Lawrence, and D. R. Coalvan, *Analyt. Biochem.*, 1972, **48**, 353.
[257] K. Nagasawa, A. Ogamo, and M. Sekiguchi, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 1006.
[258] M. Jellinek, *J. Chromatog.*, 1972, **69**, 402.
[259] M. Weise and G. M. Eisenbach, *Experientia*, 1972, **28**, 245.
[260] Z. Deyl and J. Rosmus, *J. Chromatog.*, 1972, **67**, 368.
[261] Ch. P. Ivanov and Y. Vladovska-Yukhanovska, *J. Chromatog.*, 1972, **71**, 111.
[262] D. P. Thornhill, *Biochim. Biophys. Acta*, 1972, **279**, 1.
[263] R. B. Moczar and L. Robert, *Analyt. Biochem.*, 1972, **45**, 422.
[264] Z. Deyl and J. Rosmus, *J. Chromatog.*, 1972, **69**, 129.
[265] K. P. Polzhofer and K. H. Ney, *Tetrahedron*, 1972, **28**, 1721.
[266] M. B. Patil, S. O. Jeyakumar, S. Roberts, and G. D. Kalyankov, *Indian J. Biochem. Biophys.*, 1972, **9**, 217.
[267] P. O. Schneider, R. J. Thibert, and R. J. Walton, *Mikrochim. Acta*, 1972, 925.
[268] A. K. Saxena, *Microchem. J.*, 1972, **17**, 91.

picomole quantities has been described. This is designed for samples of biological origin. Enzymic decarboxylation is first carried out to remove L-glutamate and L-aspartate, then incubation with L-asparaginase generates L-aspartic acid from the asparaginine. Subsequent enzymic transamination with 2-oxo[1-^{14}C]glutarate gives L-[1-^{14}C]glutamate, which is determined by scintillation counting after removing excess reagent.[269]

Histidine can now be determined by titration with lead nitrate using xylenol orange or pyrogallol red as indicator,[270] and an automated procedure for determining the fluophor produced by the interaction of histidine and *o*-phthalaldehyde in alkaline solution has been developed for blood analysis.[271] A sensitive fluorimetric assay of lysine has been based on its reaction with *o*-diacetylbenzene in the presence of 2-mercaptoethanol at pH 10; ornithine and glycine also undergo this reaction.[272] Whereas lysine and ornithine give a similar colour yield with ninhydrin at pH 5, at an acid pH ornithine develops five or six times as much colour.[273] The fluorescent product of the reaction of phenanthrene quinone with arginine has now been identified as 2-amino-1*H*-phenanthro[9,10-*d*]imidazole (46).[274]



(46)



(47)

Carboxylic acid chlorides and anhydrides have been found to C-acylate the indole ring of tryptophan efficiently in anhydrous trifluoroacetic acid. Acetyl chloride, for example, yields 1-methyl-3,4-dihydro-β-carboline-3-carboxylic acid (47). This reaction has been used as the basis for a new spectrophotometric technique for the analysis of tryptophan.[275] Phosphoric acid can be used for detecting tryptophan; after four minutes at 110 °C a purple colour develops, but the mechanism is not known. Indole itself gives an orange colour.[276] Tryptophan is also the only naturally occurring amino-acid which shows a positive m.c.d. band. Furthermore, this absorption is almost completely free from overlapping contributions by other bands. This method of assay is likely to find its principal use in determining tryptophan in intact proteins. Tyrosine gives an intense m.c.d.

[269] D. A. Cooney and H. A. Milman, *Biochem. J.*, 1972, **129**, 953.
[270] O. C. Saxena, *Microchem. J.*, 1972, **17**, 210.
[271] H. D. Hill, G. K. Summer, and D. A. Newton, *Clin. Chim. Acta*, 1972, **36**, 105.
[272] M. Roth and L. Jeanneret, *Z. physiol. Chem.*, 1972, **353**, 1607.
[273] R. L. Davies, *J. Chromatog.*, 1972, **71**, 564.
[274] H. A. Itano and S. Yamada, *Analyt. Biochem.*, 1972, **48**, 483.
[275] A. Previero, G. Prota, and M.-A. Colleti-Previero, *Biochim. Biophys. Acta*, 1972, **285**, 269.
[276] F. N. Boctor, *J. Chromatog.*, 1972, **67**, 371.

band, but the error this can cause through overlap is small, *e.g.* 1% when the tryptophan : tyrosine ratio is 1 : 1.[277] A fluorimetric assay now enables 5-hydroxytryptophan to be determined in the presence of 5-hydroxyindoleacetic acid.[278] Other methods of estimation published include ones for pipecolic acid in serum and urine,[279] α-aminolaevulic acid in urine,[280] and *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine at the picomole level in transfer RNA.[281]

[277] G. Barth, W. Voelter, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, 1972, **94**, 1293.
[278] C. A. Fischer and H. H. Aprison, *Analyt. Biochem.*, 1972, **46**, 67.
[279] R. F. Grimble, *Clin. Chim. Acta*, 1972, **38**, 113.
[280] Gy. Berkó and I. Durkó, *Clin. Chim. Acta*, 1972, **37**, 443.
[281] J. P. Miller and M. P. Schweizer, *Analyt. Biochem.*, 1972, **50**, 3275.