# 1
# Amino-acids

BY G. C. BARRETT

## 1 Introduction

More new amino-acids, and more new syntheses, reactions, and properties of amino-acids, have been reported in the year under review. However, the present chapter, intended to be a thorough coverage but excluding many biological aspects, is based on a similar number of references to its predecessors, and is about as long. The 'enormous increase in the rate of accumulation of knowledge' which feeds these Specialist Periodical Reports is apparent in some physical and analytical aspects of the study of amino-acids, and in methods of synthesis, but is far more evident in the biological aspects.

**Textbooks and Reviews.**—Series One of the MTP International Review of Science includes a Volume with reviews of amino-acids [1] partly overlapping the coverage of Volumes 3 and 4 of the present series; a textbook on microbial synthesis of amino-acids has appeared.[2] Various reviews are cited in the appropriate sections below.

## 2 Naturally Occurring Amino-acids

**Occurrence of Known Amino-acids.**—The implications of the distribution of non-protein amino-acids in plants, and their role, have been discussed.[1a] A more restricted review of physiologically active amino-acids in plants concentrates on mimosine and indospicine.[3] Paleobiochemical aspects of evolution are considered in a review of the amino-acid content of black cherts of different geological age.[4] Extraterrestrial distribution, in meteorites [5] and in the moon soil brought back by the Apollo 14 mission,[6] has been reviewed; improved analytical methods give more emphatic

[1] MTP International Review of Science, Organic Chemistry, Series One, Vol. 6, ed. D. H. Hey and D. I. John, 1973: (*a*) 'Amino-acids of Natural Origin', E. A. Bell, p. 1; (*b*) 'Synthesis, Structural Properties, and Reactions of Amino-acids', A. Thomson, p. 17.

[2] 'Microbial Production of Amino-acids', ed. K. Yamada, Kodansha, Tokyo, 1972.

[3] M. P. Hegarty, *Australas. J. Dermatol.*, 1973, **14**, 35.

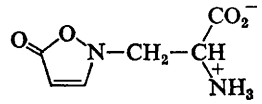
[4] W. Heller, *Naturwiss.*, 1973, **60**, 460.

[5] E. Anders, R. Hayatsu, and M. H. Studier, *Science*, 1973, **182**, 781.

[6] C. W. Gehrke, R. W. Zumwalt, K. Kuo, W. A. Aue, D. L. Stalling, K. A. Kvenvolden, and C. Ponnamperuma, 'Proceedings of the 3rd Lunar Science Conference', ed. D. Heymann, Massachusetts Institute of Technology, Cambridge, Mass., 1972, Vol. 2, p. 2119.

answers concerning the moon samples than reported [7] for samples from earlier missions (amino-acids are present to the extent of *ca.* 20—70 p.p.b.[7]). No amino-acids are present in moon soil [8] within the detection limits of ion-exchange and g.l.c. methods – between 300 pg and 1 ng for the protein amino-acids. The range of amino-acids in certain meteorites is more positively identified.[5]

Studies with pea seedlings (*Pisum sativum*) continue to provide information on early stages in biosynthetic processes. Isolation of *N*-malonyl-D-alanine and γ-L-glutamyl-D-alanine from this source has been reported,[8] the first time derivatives of D-alanine have been found in higher plants. Enzyme preparations from this source, or from *Pisum arvense*, convert *O*-acetyl-serine and isoxazolin-5-one into β-(2-isoxazolin-5-onyl)alanine (1).[9] T.l.c. of extracts of pea seedlings exposed to $H_2{}^{35}S$ reveals the presence



(1)

of a radioactive substance with all the properties of thio-threonine (α-amino-β-mercaptobutyric acid).[10] Pyroglutamic acid, found in large amounts in young pea seedling extracts, is nevertheless a glutamine artefact.[11] Maturing seeds of lima beans biosynthesize *Se*-methyl-selenocysteine, while selenomethionine accumulates as the principal product of selenate assimilation in the leaves of this plant.[12]

Other plant and fungal sources, and the more notable amino-acids present, are: *Sedum acre* ($N^{\varepsilon}$-methyl-lysine);[13] *Cannabis* seeds (L-isoleucine betaine);[14] a New Guinea *Boletus* (L-2-amino-4-methylhex-5-enoic acid).[15] (2*S*,3*R*,4*R*)-4-Hydroxyisoleucine, known as a component of γ-amanatin but not previously reported to be present in higher plants, is found in *Trigonella foenum-graecum* seeds [16] (the 2*R*,3*R*,4*R*-diastereoisomer is a minor component). *N*-Feruloylglycyl-peptides in barley seed globulins are considered to arise through the presence of *N*-feruloylglycine as a 'starter' of protein biosynthesis.[17]

[7] 'Amino-acids, Peptides, and Proteins', ed. G. T. Young (Specialist Periodical Reports), The Chemical Society, London, 1972, Vol. 4, p. 1.
[8] T. Ogawa, M. Fukuda, and K. Sasaoka, *Biochim. Biophys. Acta*, 1973, **297**, 60; M. Fukuda, A. Tokumura, T. Ogawa, and K. Sasaoka, *Phytochemistry*, 1973, **12**, 2593.
[9] I. Murakoshi, F. Kato, J. Haginiwa, and L. Fowden, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 918.
[10] J. Schnyder and K. H. Erismann, *Experientia*, 1973, **29**, 232.
[11] H. G. Wager and F. A. E. Porter, *J. Sci. Food Agric.*, 1973, **24**, 69.
[12] S. N. Nigam and W. B. McConnell, *Phytochemistry*, 1973, **12**, 359.
[13] E. Leistner and I. D. Spenser, *J. Amer. Chem. Soc.*, 1973, **95**, 4715.
[14] C. A. L. Bercht, R. J. J. C. Lousberg, F. J. E. M. Kuppers, and C. A. Salemink, *Phytochemistry*, 1973, **12**, 2457.
[15] E. Gellert, B. Halpern, and R. Rudzats, *Phytochemistry*, 1973, **12**, 689.
[16] L. Fowden, H. M. Pratt, and A. Smith, *Phytochemistry*, 1973, **12**, 1707.
[17] C. F. Van Sumure, H. de Pooter, H. Ali, and M. Degrauw-van Bussel, *Phytochemistry*, 1973, **12**, 407.

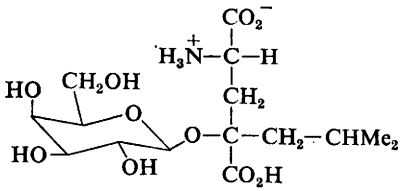
An increasing number of side-chain methylated arginines ($N^G$-monomethyl-, $N^GN^G$-dimethyl-, $N^GN^{G'}$-dimethyl-) and lysines ($N^\epsilon$-methyl-, $N^\epsilon$-dimethyl-, and $N^\epsilon$-trimethyl-) has been found in bovine brain proteins;[18] only the first two of these are present in myelin basic proteins from a wide variety of species.[19] Hypusine, $N^6$-(4-amino-2-hydroxybutyl)-2,6-diaminohexanoic acid, is present in bovine brain protein.[20] 2,4-Diaminobutyric acid is a component of the murein of seventeen strains of coryneform bacteria.[21] Formation of L-2-amino-4-methoxy-*trans*-but-3-enoic acid by *Pseudomonas aeruginosa* grown on straight-chain paraffins is notable amongst microbiological studies;[22] this amino-acid was previously detected in culture fluids from the same source grown on cerelose.[23] Subtilin, a peptide produced by *Bacillus subtilis*, contains two residues of dehydroalanine and one of β-methyldehydroalanine, and is similar in this respect to nisin.[24]

Microbial synthesis of amino-acids offers scope for large-scale production of compounds important in medicine.[2, 25] Of course, L-dopa production is pre-eminent in such studies, and its formation, with L-tyrosine, from DL-serine and catechol by *Erwinea herbicola*,[26] from pyruvate, ammonia, and catechol by the same organism,[27] or from tyrosine by *Vibrio tyrosinaticus*,[28] has been described. Other microbial syntheses recently reported concern tryptophan,[29] L-threonine,[30] and $N^\delta$-acetyl-L-ornithine.[31]

β-Alanine betaine is present in the adductor muscle of the fan mussel *Atrina pectinata japonica*.[32]

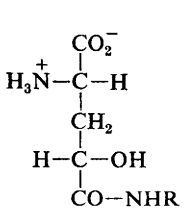
**New Natural Free Amino-acids.**—Many of the new amino-acids reported this year are hydroxylated versions of long-known amino-acids, *e.g.* (2*S*,4*R*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid (2) from flowers of *Reseda odorata*.[33] The assignment of 4*R*-configuration is

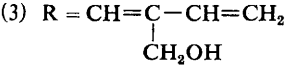
[18] Y. Matsuoka, *Seikagaku*, 1972, **44**, 353.
[19] G. E. Deibler and R. E. Martenson, *J. Biol. Chem.*, 1973, **248**, 2387.
[20] N. Imaoka and T. Nakajima, *Biochim. Biophys. Acta*, 1973, **320**, 97.
[21] F. Fiedler and O. Kandler, *Arch. Mikrobiol.*, 1973, **89**, 51.
[22] U. Sahm, G. Knobloch, and F. Wagner, *J. Antibiotics*, 1973, **26**, 389.
[23] L. H. Sello, T. Williams, and A. Stempel, *J. Antibiotics*, 1972, **25**, 122.
[24] E. Gross and H. H. Kiltz, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 559.
[25] S. Abe, *Kagaku Kogyo*, 1973, **24**, 389.
[26] H. Enei, H. Matsui, H. Nakazawa, S. Okumura, and H. Yamada, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 493.
[27] H. Enei, H. Nakazawa, S. Okumura, and H. Yamada, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 725.
[28] H. Yoshida, Y. Tanada, and K. Nakayama, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2121.
[29] H. Nakazawa, H. Enei, S. Okumura, and H. Yamada, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 2523.
[30] T. Hirakawa, T. Tanaka, and K. Watanabe, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 123; T. Hirakawa, *ibid.*, p. 243.
[31] K. Araki and J. Nakajima, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2639.
[32] S. Konosu and K. Watanabe, *Nippon Suisan Gakkaishi*, 1973, **39**, 645.
[33] P. O. Larsen, H. Soerensen, D. W. Cochran, E. W. Hagaman, and E. Wenkert, *Phytochemistry*, 1973, **12**, 1713.

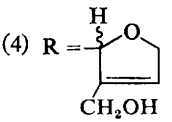


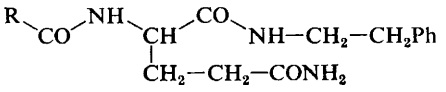
(2)

tentative. This is the first example of a glycoside from a higher plant in which the carbohydrate residue is linked to an aliphatic hydroxy-group of an amino-acid. Further details of structural studies reported for pinnatanine (3) in 1971 [34] are available,[35] together with details of a new analogue from the same source, seeds of *Staphylea pinnata* L., oxypinnatanine (4).[35] *N*-Acylated L-glutaminyl 2-phenylethylamines (5), and





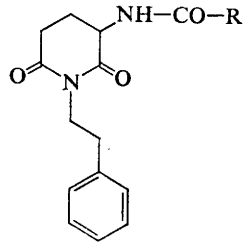




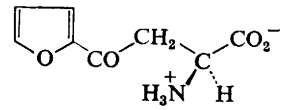
(5) a; R = $Pr^i$
b; R = (*R*)-EtCHMe

related glutarimides (6), have been isolated from *Croton humilis*,[36] the novel feature as far as the glutarimide is concerned being the *R*-configuration of the 2-methylbutanoyl substituent, the first natural appearance of the *R*-enantiomer of this acid; the *S*-analogue of (6b), julocrotine, has been known for some time.[37] $N^5$-(2′-Hydroxybenzyl)-allo-4-hydroxy-L-glutamine and $N^5$-(4′-hydroxybenzyl)-L-glutamine are new amino-acid amides from buckwheat seeds.[38] L-3-(2-Furoyl)alanine (7) from buckwheat seeds [39]

[34] M. D. Grove, M. E. Daxenbichler, D. Weisleder, and C. H. VanEtten, *Tetrahedron Letters*, 1971, 4477.
[35] M. D. Grove, D. Weisleder, and M. E. Daxenbichler, *Tetrahedron*, 1973, **29**, 2715.
[36] K. L. Stuart, D. McNeill, J. P. Kutney, G. Eigendorf, and F. K. Klein, *Tetrahedron*, 1973, **29**, 4071.
[37] T. Nakano, C. Djerassi, R. A. Corral, and O. O. Orazi, *J. Org. Chem.*, 1961, **26**, 1184.
[38] M. Koyama, Y. Tsujizaki, and S. Sakamura, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2749.
[39] A. Ichihara, H. Hasegawa, H. Sato, M. Koyama, and S. Sakamura, *Tetrahedron Letters*, 1973, 37.



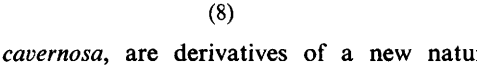
(6) a; R = $Pr^i$
b; R = (*R*)-EtCHMe



(7)

and from Green Gram seeds[40] may be an artefact since it is formed[41] from ascorbalamic acid during treatment with hot hydrochloric acid. The amino-acid (7) from buckwheat seeds has the L-configuration since it gives L-aspartic acid on ozonolysis,[39] and (7) is readily synthesized from 2-acetylfuran,[39] from L-3-chloroalanine and ascorbic acid,[41] or from furacyl bromide by the acetamidomalonate route.[41]

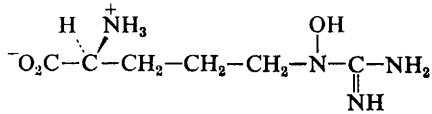
4-Hydroxy-3-hydroxymethylphenylalanine and 3-hydroxymethylphenylalanine are major constituents of seeds of *Caesalpinia tinctoria*.[42] *N*-Acylated 2-methylene-β-alanine methyl esters (8; $n$ = 13—17) from the sponge



(8)

*Fasciospongia cavernosa*, are derivatives of a new naturally occurring β-amino-acid.[43]

Amino-acids with fungal or bacterial origins have been reported. Another new acetylenic amino-acid from *Tricholomopsis rutilans* is 2-amino-3-hydroxyhex-4-ynoic acid, $MeC{\equiv}C{-}CH(OH){-}CH(NH_3^+){-}CO_2^-$ (stereochemistry not yet elucidated).[44] $N^5$-Hydroxy-L-arginine (9) from *Nannizzia gypsea*[45] and from an unidentified bacillus[46] may be a



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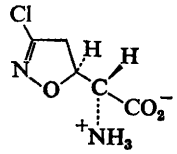
[40] T. Kasai, Y. Kishi, M. Sano, and S. Sakamura, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2923.
[41] R. Couchman, J. Eagles, M. P. Hegarty, W. M. Laird, R. Self, and R. L. M. Synge, *Phytochemistry*, 1973, **12**, 707.
[42] R. Watson and L. Fowden, *Phytochemistry*, 1973, **12**, 617.
[43] Y. Kashman, L. Fishelson, and I. Ne'eman, *Tetrahedron*, 1973, **29**, 3655.
[44] S. I. Hatanaka, Y. Niimura, and K. Taniguchi, *Z. Naturforsch.*, 1973, **28C**, 475.
[45] B. Fischer, W. Keller-Schierlein, H. Kniefel, W. A. Koenig, W. Loeffler, A. Mueller, R. Muntwyler, and H. Zaehner, *Arch. Mikrobiol.*, 1973, **91**, 203.
[46] H. Maehr, J. F. Blount, D. L. Pruess, L. Yarmchuk, and M. Kellett, *J. Antibiotics*, 1973, **26**, 284.



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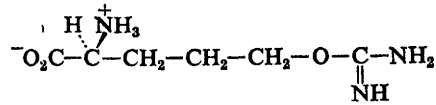
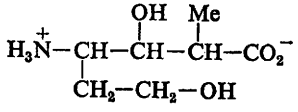


(11)

valuable antimicrobial agent, but even more important perhaps will be the antitumour property of the antibiotic (10) from *Streptomyces sviceus*;[47] 5-(*O*-isoureido)-L-norvaline (11) is a new arginine antagonist from a bacterial culture.[48]

**New Amino-acids from Hydrolysates.**—A review, covering amino-acids reported since 1931 to be components of native proteins, describes the less common protein constituents.[49]

Antibiotics, inhibitory peptides, and antimetabolites are major sources of new amino-acids. This year, Actinomycin $Z_1$ has come under structural scrutiny;[50] one of its constituent amino-acids is 3-hydroxy-4-oxo-5-methylproline. Actinomycin $Z_5$ contains *cis*-5-methylproline.[51] Antibiotics YA-56 of the phleomycin–bleomycin group contain an extraordinary variety of amino-acids;[52, 53] in addition to β-amino-β-(4-amino-6-carboxy-5-methyl-2-pyrimidinyl)propionic acid, β-aminoalanine, L-erythro-β-hydroxyhistidine,[54] and 2-[2-(2-aminoethyl)-$\Delta^2$-thiazolin-4-yl]thiazole-4-carboxylic acid, all previously reported, the presence of β-hydroxy-L-valine,[53] 4-amino-3,6-dihydroxy-2-methylhexanoic acid (12),[53] and several unidentified amino-acids, has been reported. The last-named amino-acid appears in hydrolysates as stereoisomers of (13), formed from it by cyclization.[53]



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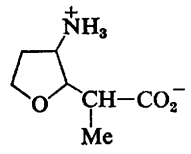

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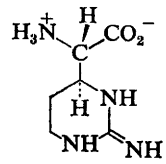
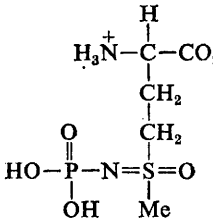


(14)

[47] D. G. Martin, D. J. Duchamp, and C. G. Chidester, *Tetrahedron Letters*, 1973, 2549.
[48] W. A. Koenig, H. Kneifel, E. Bayer, G. Mueller, and H. Zaehner, *J. Antibiotics*, 1973, **26**, 44.
[49] H. B. Vickery, *Adv. Protein Chem.*, 1972, **26**, 81.
[50] H. Brockmann and E. A. Stahler, *Tetrahedron Letters*, 1973, 3685.
[51] E. Katz, K. T. Mason, and A. B. Mauger, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 819.
[52] Y. Ohashi, H. Abe, and Y. Ito, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2277.
[53] Y. Ohashi, H. Abe, and Y. Ito, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2283.
[54] G. Koyama, H. Nakamura, Y. Muraoka, T. Takita, K. Maeda, H. Umezawa, and Y. Iitaka, *J. Antibiotics*, 1973, **26**, 109.

The chymostatins are a group of tetrapeptides of microbial provenance, important as inhibitors of chymotrypsin and papain. They all contain two modified phenylalanine residues and (2*S*,3*S*)-α-(2-iminohexahydro-4-pyrimidyl)glycine (14); chymostatin A also contains L-leucine, while chymostatin B contains L-valine, and chymostatin C contains L-isoleucine.[55] The basic amino-acid (14) is a diastereoisomer of capreomycidine, previously established[56] to be a component of capreomycins.

L-($N^5$-Phosphono)methionine-(*S*)-sulphoximine (15) is a component of the tripeptide (15)-L-Ala-L-Ala, an antimetabolite of L-glutamine produced by an unclassified *Streptomycete*.[57]



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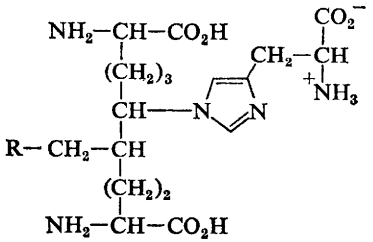


(16) a; R = OH

b; R = NH—$CH_2$—CH(OH)—$(CH_2)_2$—CH($^-O_2C$)—$H_3N^+$

Further information on the aldol-histidine cross-link (16a) of collagen (see Vol. 5, p. 6) is now available.[58] Histidino-hydroxymerodesmosine (16b), formed from aldol-histidine and hydroxylysine, is a newly discovered cross-link of collagen, capable of uniting three or four polypeptide chains. These cross-links, whose structures were largely established by mass

[55] K. Tatsuta, N. Mikami, K. Fujimoto, S. Umezawa, H. Umezawa, and T. Aoyagi, *J. Antibiotics*, 1973, **26**, 625.

[56] B. W. Bycroft, D. Cameron, L. R. Croft, and A. W. Johnson, *Chem. Comm.*, 1968, 1301.

[57] D. L. Pruess, J. P. Scannell, H. A. Ax, M. Kellett, F. Weiss, T. C. Demny, and A. Stempel, *J. Antibiotics*, 1973, **26**, 261.

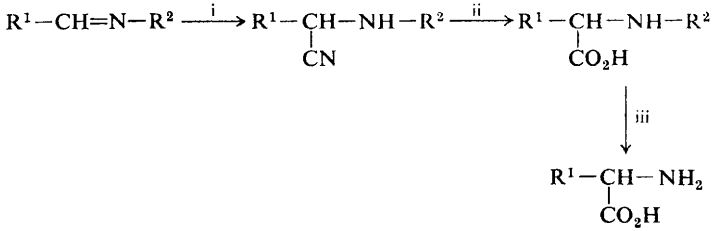
[58] M. L. Tanzer, T. Housley, L. Berube, R. Fairweather, C. Franzblau, and P. M. Gallop, *J. Biol. Chem.*, 1973, **248**, 393.

spectrometry, are surprising discoveries since the pyridinium cross-links present in elastin might have been expected.[58]

## 3 Chemical Synthesis and Resolution of Amino-acids

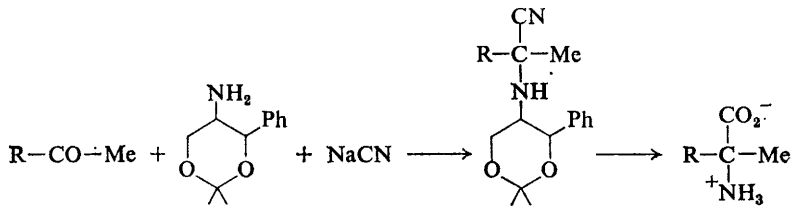
**General Methods of Synthesis.**—*Asymmetric Synthesis.* The ingenuity in approaches to novel asymmetric syntheses of α-amino-acids seems limitless, and more papers have come into the reckoning for this chapter. Further results on the hydrogenolytic asymmetric transamination approach, involving a resolved α-amino-acid and an α-keto-acid, have been reported.[59] D-α-Phenylglycine undergoes this reaction with pyruvic acid and with α-ketoglutaric acid to give D-alanine and D-glutamic acid, respectively, and a clearer picture of the structural requirements for maximum optical purity is emerging.[59] Schiff bases of aliphatic aldehydes with an optically active benzylamine give optically active N-substituted amino-nitriles with hydrogen cyanide[60,61] or with benzoyl cyanide[62] which, on hydrolysis, give predominantly D-amino-acids when the Schiff base involves the *R*-enantiomer of the α-alkylbenzylamine. Optical purities of the products are in the range 22—58% when hydrogen cyanide is used, and 15—37% when the benzoyl cyanide route is employed (Scheme 1).

A novel asymmetric Strecker synthesis[63] applied to the synthesis of α-methyl-α-amino-acids (Scheme 2) employs a methyl ketone and



Reagents: i, HCN; ii, $H_3O^+$; iii, $H_2$–$Pd(OH)_2$–C

**Scheme 1**

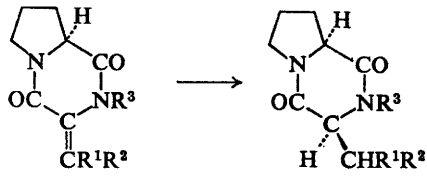


**Scheme 2**

[59] K. Harada, T. Iwasaki, and T. Okawara, *Bull. Chem. Soc. Japan*, 1973, **46**, 1901.
[60] K. Harada and T. Okawara, *J. Org. Chem.*, 1973, **38**, 707.
[61] K. Harada, T. Okawara, and K. Matsumoto, *Bull. Chem. Soc. Japan*, 1973, **46**, 1865.
[62] K. Harada and T. Okawara, *Bull. Chem. Soc. Japan*, 1973, **46**, 191.
[63] K. Weinges and B. Stemmle, *Chem. Ber.*, 1973, **106**, 2291.

(4*S*,5*S*)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxan. L-Amino-acids predominate in the product when the starting methyl ketone has an even number of carbon atoms.[63]

Dioxopiperazine formation from *N*-alkyl α-bromopropionamides containing a chiral *N*-alkyl group favours one enantiomer to a small extent, hydrolysis and hydrogenolysis of the dioxopiperazines giving alanine of optical purity up to 27%; chirality and optical purity depend upon the structure and chirality of the *N*-alkyl group.[64] In a related approach,[65] arylidene derivatives (17) of glycyl-L-proline anhydride give corresponding L-phenylalanyl-L-proline anhydrides (18) on hydrogenation, and the route




(17) (18)

has been used to prepare L-phenylalanine and L-dopa. *N*-Methylation of the starting compound to give (17; $R^3$ = Me) is readily achieved, and the synthesis then becomes a route to L-(*N*-methyl)phenylalanines. There is no asymmetric induction with alkylidene analogues (17; $R^1$ = $R^2$ = Me, or $R^1$ = H, $R^2$ = $CHMe_2$),[65] and no doubt this will arouse curiosity.

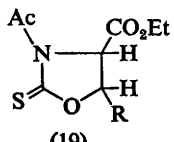
L-Phenylglycine, L-alanine, L-phenylalanine, and L-leucine are obtained with optical purities 27, 33, 62, and 75%, respectively, by Neber rearrangement of corresponding (−)-menthyl *N*-chloroimidates.[66]

β-Hydroxy-α-amino-acids prepared by the reaction of aldehydes with glycine derivatives at high pH are enriched in one enantiomer if the N-substituted glycine is part of a resolved cobalt(III) complex.[67] The (+)-enantiomer of potassium bis-(*N*-salicylideneglycinato)cobaltate(III), obtained by resolution of the racemate with brucine, reacts with acetaldehyde at pH 11.2 to give a mixture of D-threonine and D-allothreonine in asymmetric yields 19—46% and 55—64%, respectively.[67]

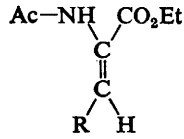
*Syntheses using α-Isocyano- and α-Isothiocyanato-esters.* Variations of the method (see Vol. 5, p. 12) using oxazolin-2-one-4-carboxylates to synthesize β-hydroxy-α-amino-acids have broadened the scope of the route. Like the oxazolin-2-ones, *N*-acetyl oxazolin-2-thiono-4-carboxylates (19) may be cleaved by base ($Bu^tOK$ in the present case [68]) to give derivatives of

[64] T. Okawara and K. Harada, *Bull. Chem. Soc. Japan*, 1973, **46**, 1869.
[65] H. Poisel and U. Schmidt, *Chem. Ber.*, 1973, **106**, 3408.
[66] Y. Nogami, Y. Kawazoe, and T. Taguchi, *Yakugaku Zasshi*, 1973, **93**, 1058.
[67] Y. N. Belokon, M. M. Dolgaya, N. I. Kuznetsova, S. V. Vitt, and V. M. Belikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 156.
[68] D. Hoppe, *Angew. Chem. Internat. Edn.*, 1973, **12**, 656.

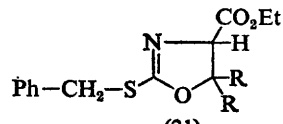
α-aminoacrylic acid (20) from which a variety of β-functionalized α-amino-acids may be prepared.[68] A similar route, starting likewise with α-isothiocyanato-acetates, but proceeding *via* 2-benzylthio-oxazolin-4-carboxylates (21), gives N-protected α-aminoacrylates.[69]



(19)

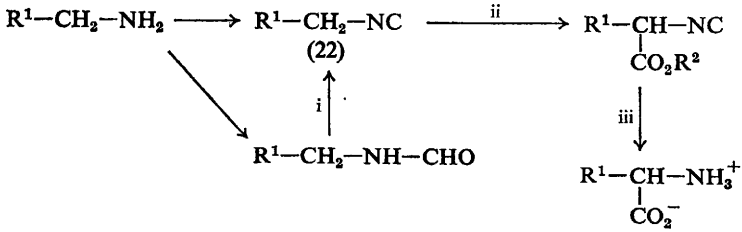


(20)



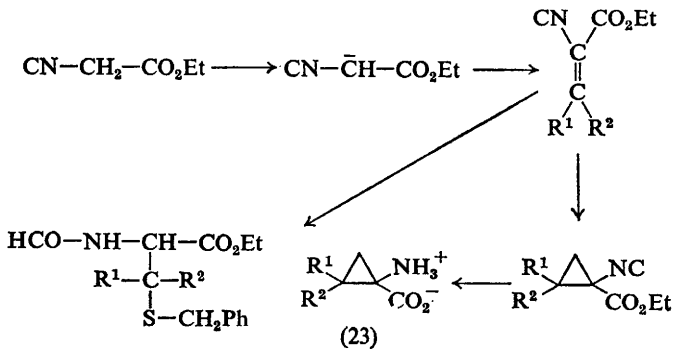
(21)

A new synthesis of α-amino-acids (Scheme 3) involving α-carboxylation of amines with chloroformates or dialkyl carbonates,[70, 71] after their conversion into isocyanides (22), should have useful applications in cases where side-chains can withstand the successive reactions. Ethyl isocyanoacetate $NCCH_2CO_2Et$ gives α-isocyanoacrylates by reaction with ketones, from which β,β-disubstituted *N*-formyl *S*-benzylcysteines (Scheme 4) may



Reagents: i, $COCl_2$–$NEt_3$; ii, $ClCOOR^2$ or $R^2OCOOR^2$; iii, aq. HCl

**Scheme 3**



(23)

**Scheme 4**

[69] D. Hoppe, *Angew. Chem. Internat. Edn.*, 1973, **12**, 658.
[70] W. Vaalburg, J. Strating, and M. G. Woldring, *Synthetic Comm.*, 1972, **2**, 423.
[71] K. Matsumoto, M. Suzuki, and M. Miyoshi, *J. Org. Chem.*, 1973, **38**, 2094.

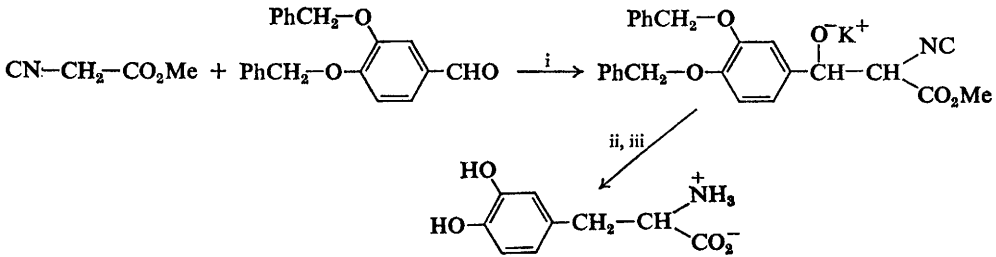
be obtained by addition of benzyl mercaptan;[72] alternatively, cyclopropane amino-acids (23) may be obtained by their reaction with dimethylsulphonium methylide (Scheme 4).[73]

The same general scheme has been employed in a new synthesis of DL-dopa (Scheme 5) in a one-pot process.[74] Methyl α-alkyl isocyanoacetates give oxazolines with aromatic aldehydes and α-acyl-α-amino-acids with acid chlorides, from which β-hydroxy-α-amino-acids are obtained, the two routes favouring *threo*- and *erythro*-isomers, respectively (Scheme 6).[75, 76]

*Syntheses by Classical Methods.* α-Halogeno-amines or -acids, on treatment with cyanide ion or amines, respectively, provide intermediates from which corresponding α-amino-acids may be obtained.[14, 77, 78] The former route, using $CF_3 \cdot CHCl \cdot NH \cdot CO \cdot CH_2Ph$, provides a new synthesis of trifluoroalanine.[79] Reaction of $p\text{-}ClC_6H_4 \cdot S \cdot NH \cdot CO \cdot OCH_2Ph$ with an α-bromo-acid gives the corresponding *N*-benzyloxycarbonyl-DL-amino-acid after removal of the *N*-(*p*-chlorophenylsulphenyl) group from the condensation product by treatment with *p*-chlorothiophenol.[80]

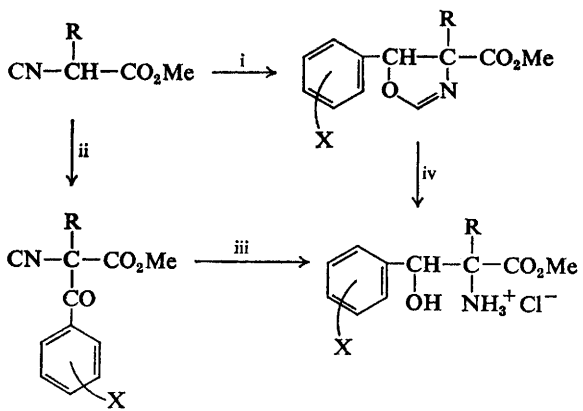
Alkylation of α-acylamino-malonic esters, then hydrolysis of the product, remains a standard route to α-amino-acids.[81–84] One modification includes a half-ester stage, permitting decarboxylation (in refluxing dioxan) to the *N*-acylamino-acid ester to be effected, so that an enzymic hydrolysis step (chymotrypsin or subtilisin) to give a mixture of *N*-acyl-L-α-amino-acid and *N*-acyl-D-α-amino-acid esters may be incorporated.[85] A modified Curtius reaction with malonic acid half-esters, $HO_2C \cdot CHR \cdot CO_2Et$, using diphenylphosphoryl azide with triethylamine, followed by addition of benzyl alcohol provides [86] *N*-benzyloxycarbonylamino-acid esters, including derivatives of α,α-disubstituted series not accessible directly through the acylamino-malonate route. Alkylation of methyl α-nitroacetate, $MeOCO\text{-}CH_2NO_2$, followed by reduction and hydrolysis, is a related route used to prepare amino-alkyl glycines and representative protein amino-acids;[87] *NN*-bis(trimethylsilyl)glycine trimethylsilyl esters, $(Me_3Si)_2NCH_2CO_2Si\text{-}$

[72] U. Schollkopf and D. Hoppe, *Annalen*, 1973, 799.
[73] U. Schollkopf, R. Harms, and D. Hoppe, *Annalen*, 1973, 611.
[74] R. Damico and J. M. Nicholson, *J. Org. Chem.*, 1973, **38**, 3057.
[75] M. Suzuki, T. Iwasaki, K. Matsumoto, and K. Okumura, *Chem. and Ind.*, 1973, 228.
[76] M. Suzuki, T. Iwasaki, M. Miyoshi, K. Okumura, and K. Matsumoto, *J. Org. Chem.*, 1973, **38**, 3571.
[77] J. E. Baldwin, J. Loliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. de la Higuera, *J. Amer. Chem. Soc.*, 1973, **95**, 3796; corrigendum, *ibid.*, p. 6511.
[78] M. Gacek and K. Undheim, *Tetrahedron*, 1973, **29**, 863.
[79] A. Uskert, A. Neder, and E. Kasztreiner, *Magyar Kém. Folyóirat*, 1973, **79**, 333.
[80] T. Taguchi and T. Mukaiyama, *Chem. Letters*, 1973, 1.
[81] Y. K. Lee and T. Kaneko, *Bull. Chem. Soc. Japan*, 1973, **46**, 2924.
[82] M. L. Sethi, G. S. Rao, and G. J. Kapadia, *J. Pharm. Sci.*, 1973, **62**, 1802.
[83] H. R. Bosshard and A. Berger, *Helv. Chim. Acta*, 1973, **56**, 1838.
[84] K. Balenovic and A. Deljac, *Rev. Trav. chim.*, 1973, **92**, 117.
[85] A. Berger, M. Smolarsky, N. Kurn, and H. R. Bosshard, *J. Org. Chem.*, 1973, **38**, 457.
[86] S. Yamada, K. Ninomiya, and T. Shioiri, *Tetrahedron Letters*, 1973, 2343.
[87] E. Kaji and S. Zen, *Bull. Chem. Soc. Japan*, 1973, **46**, 337.



Reagents: i, $Bu^tOK$–MeOH; ii, HCl–MeOH 17 h; iii, Pd–$H_2$–$H_3O^+$

**Scheme 5**



Reagents: i, $XC_6H_4CHO$; ii, $XC_6H_4COCl$; iii, $H_2$–Pd; iv, aq. HCl

**Scheme 6**

$Me_3$, may be alkylated as the α-sodio-derivatives, illustrated by the preparation of DL-2-amino-6-nitrocaproic acid, a masked lysine for use in peptide synthesis.[88]

Reductive cleavage of phenylhydrazones of α-keto-acids, readily available through the Japp–Klingemann reaction starting from active methylene compounds, would be more widely used with a convenient hydrogenolysis procedure. Whereas their treatment with zinc dust and acid at 0 °C is ineffective, hydrogenation of aqueous suspensions over palladium gives α-amino-acids in high yields.[89] An improved method for converting α-hydroxy-acids into *N*-phthaloylamino-acids with inversion of configuration employs phthalimide, triphenylphosphine, and diethyl azodicarboxylate.[90] Treatment of a bisulphite addition product of an aldoxime with sodium cyanide, followed by hydrolysis, can be vaunted as a new synthesis of amino-acids from amines,[91] since aldoximes can now be prepared from amines, *e.g.*

$$HOCH_2CH_2NH_2 \longrightarrow HOCH_2CH{=}NOH \longrightarrow HOCH_2CH(NH_2)CO_2H$$

Routes to amino-acids other than α-amino-acids are not easily generalized since various strategies must be chosen to provide the required location of the functional groups. 4*S*-Amino-3*S*-hydroxy-6-methylheptanoic acid $Me_2CH{\cdot}CH(NH_2){\cdot}CH(OH){\cdot}CH_2{\cdot}CO_2H$, a constituent of pepstatin A, may be synthesized from L-leucine,[92] all four stereoisomers being available by straightforward methods.[93] The Arndt–Eistert reaction gives optically active β-amino-acids starting from α-amino-acids.[94]

**Synthesis under Simulated Prebiotic Conditions.**—Reviews on abiogenic synthesis of amino-acids have appeared.[95, 96] A 20 mA silent discharge through a 0.14 : 0.21 : 1 mixture of methane, carbon dioxide, and nitrogen gives a 1% overall yield of glycine, alanine, norvaline, serine, aspartic acid, α-amino-isobutyric acid, and other unidentified amino-acids.[97] High temperatures reduce the number of amino-acids and favour the formation of β-amino-acids when a 2 : 1 mixture of water-saturated methane and ammonia is passed over quartz sand at temperatures between 900 and 1060 °C.[98] Amino-acid precursors (methane, nitrogen, carbon monoxide

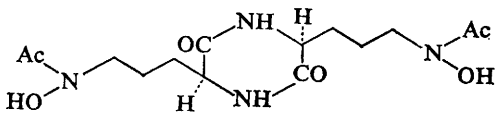
[88] E. Bayer and K. Schmidt, *Tetrahedron Letters*, 1973, 2051.
[89] N. H. Khan and A. R. Kidwai, *J. Org. Chem.*, 1973, **38**, 822.
[90] M. Wada, T. Sano, and O. Mitsunobu, *Bull. Chem. Soc. Japan*, 1973, **46**, 2833.
[91] G. Natta and I. Pasquon, *Chimie et Industrie*, 1973, **55**, 323.
[92] H. Morishima, T. Takita, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 115.
[93] M. Kinoshita, S. Aburaki, A. Hagiwara, and J. Imai, *J. Antibiotics*, 1973, **26**, 249.
[94] Y. Seto, T. Yamada, K. Niwa, S. Miwa, F. Tanaka, S. Kuwata, and H. Watanabe, *Chem. Letters*, 1973, 151.
[95] R. M. Lemmon, *Environ. Biol. Med.*, 1973, **2**, 1.
[96] F. Balestic, *J. Chim. phys.*, 1973, **70**, 169.
[97] E. F. Simonov and V. B. Lukyanov, *Vestnik Moskov Univ., Khim.*, 1973, **14**, 118 (*Chem. Abs.*, 1973, **79**, 5557c).
[98] J. G. Lawless and C. D. Boynton, *Nature*, 1973, **243**, 405.

and dioxide, carbon disulphide, hydrogen cyanide) are present in moon soil brought back by Apollo 14 and earlier missions, at 20—70 ng $g^{-1}$ levels.[99]

With a substantial accumulation of data on the precursors and conditions required for chance synthesis of α-amino-acids, interest is shifting towards the next stage, the chance coupling of such amino-acids to form peptides. Reaction of amino-acids with polyphosphates in the prebiotic soup in the presence of $Mg^{2+}$ can give phosphoramidates, $R^1O \cdot PO_2^- \cdot NH \cdot CHR^2 \cdot CO_2^-$, as derivatives suitably activated towards peptide bond formation.[100] Peptide bond formation induced by hydroxyapatite or orthophosphates is enhanced by cyanate,[101] formed as a consequence of oligomerization of hydrogen cyanide and subsequent hydrolysis of the resulting non-peptide polymer.[102] Acid hydrolysis of the polymer gives amino-acids, citrulline being a major constituent of the hydrolysate;[102] further study of the cyanide oligomerization should reveal new chemistry of more general interest,[103] and, already, the rate of oligomerization has been shown to be independent of added nucleophiles.[104]

**Protein and Other Naturally Occurring Amino-acids.**—New syntheses described in the preceding sections are illustrated by syntheses of some of the well-known protein amino-acids.

Syntheses of alanosine (β-hydroxynitrosamino-L-alanine) have been reported by two groups.[105, 106] One synthesis [105] starts with DL-β-chloroalanine, which on reaction with sodium *anti*-benzaldoximate gives the *N*-hydroxyamine, $HONHCH_2CH(NH_2)CO_2H$, after cleavage of the benzylidene group; DL-alanosine is obtained on nitrosation. The L-isomer is obtained [106] starting from ethyl 2,3-dibromopropionate and *N*-tosyl-*O*-benzylhydroxylamine, a resolution stage being included late in the synthesis. A further synthesis of δ-hydroxy-L-ornithine has been reported,[107] and $N^{\delta}$-acetyl-$N^{\delta}$-benzyloxy-L-ornithine for use in the synthesis of rhodo-

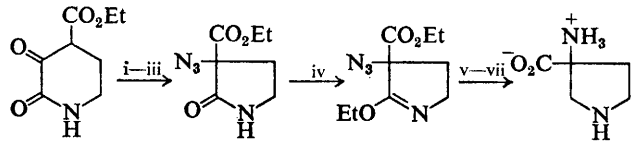



(24)

[99] S. W. Fox, K. Harada, and P. E. Hare, 'Proceedings of the 3rd Lunar Science Conference', ed. D. Heymann, Massachusetts Institute of Technology, Cambridge, Mass., 1972, Vol. 2, p. 2109.
[100] R. Lohrmann and L. E. Orgel, *Nature*, 1973, **244**, 418.
[101] J. J. Flores and J. O. Leckie, *Nature*, 1973, **244**, 436.
[102] J. P. Ferris, D. B. Donner, and A. P. Lobo, *J. Mol. Biol.*, 1973, **74**, 499.
[103] J. P. Ferris and T. J. Ryan, *J. Org. Chem.*, 1973, **38**, 3302.
[104] J. P. Ferris, D. B. Donner, and A. P. Lobo, *J. Mol. Biol.*, 1973, **74**, 511.
[105] C. N. Eaton, G. H. Denney, M. A. Ryder, M. G. Ly, and R. D. Babson, *J. Medicin. Chem.*, 1973, **16**, 289.
[106] Y. Isowa, H. Kurita, M. Ohmori, M. Sato, and K. Mori, *Bull. Chem. Soc. Japan*, 1973, **46**, 1847.
[107] G. Tomlinson and T. Viswanatha, *Canad. J. Biochem.*, 1973, **51**, 754.

torulic acid (24) has been prepared [108] from diethyl α-(3-bromopropyl)-α-acetamidomalonate by reaction with $PhCH_2ONHAc$ followed by conventional elaboration and deprotection steps.

In a new synthesis (Scheme 7) of (±)-cucurbitine (25), a novel ring-contraction provides ethyl 3-azido-2-oxopyrrolidine-3-carboxylate, reduc-




Reagents: i, $Br_2$; ii, $NaN_3$–boiling 1,2-dimethoxyethane; iii, peroxyacetic acid; iv, $Et_3O^+BF_4^-$; v, diborane; vi, $H_2$–Pt; vii, $H_2O$

**Scheme 7**

tion of which gives (±)-(25).[109] The (−)-isomer occurs in several species of Cucurbitaceae.

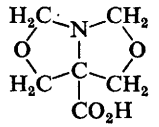
**α-Alkyl- and α-Aralkyl-α-amino-acids.**—Continued exploration in the synthesis of α-alkyl-α-amino-acids is referred to in preceding paragraphs.[63, 75, 86] *N*-Acetyl-α-benzylphenylalanine ethyl ester is obtained by Schmidt rearrangement of ethyl dibenzylacetoacetate, and converted without difficulty into its *N*-*o*-nitrophenylsulphenyl derivative for use in peptide synthesis.[110]

**α-Hydroxy-α-amino-acids and Amino-acids with Aliphatic Hydroxy-groups in the Side-chain.**—Amino-acids with α-hydroxy- or α-alkoxy-groups are of renewed interest, partly because of the possible value in medicine of 6-methoxy-penicillins and cephalosporin analogues. *N*-Phenylacetylamino-acid esters give *N*-chloro-derivatives with t-butyl hypochlorite, which on dehydrochlorination give acylimines $PhCH_2CON{=}CRCO_2Me$, to which an alcohol may be added to give α-alkoxy-α-amino-acids, *e.g.* $PhCH_2{\cdot}CO{\cdot}NH{\cdot}CPh(OMe){\cdot}CO_2Me$.[111, 112] *N*-Acyl dehydroalanine esters give analogous α-alkoxy-*N*-acylalanine esters through acid-catalysed addition of alcohols.[113]

β-Hydroxy-α-amino-acids may be obtained by reaction between a glycine derivative and an aldehyde or ketone, usually in alkaline solution, and asymmetric synthesis possibilities have been demonstrated.[67] A similar route uses α-isocyanoacetates.[75] The prebiotic significance of the analogous reactions of glycine itself, in mildly alkaline solution, to give

[108] T. Fujii and Y. Hatanaka, *Tetrahedron*, 1973, **29**, 3825.
[109] H. J. Monteiro, *J.C.S. Chem. Comm.*, 1973, 2.
[110] G. C. Barrett, P. M. Hardy, T. A. Harrow, and H. N. Rydon, *J.C.S. Perkin I*, 1972, 2634.
[111] J. E. Baldwin, F. J. Urban, R. D. G. Cooper, and F. L. Jose, *J. Amer. Chem. Soc.*, 1973, **95**, 2401.
[112] G. A. Koppel and R. E. Koehler, *J. Amer. Chem. Soc.*, 1973, **95**, 2403.
[113] G. Lucente and D. Rossi, *Chem. and Ind.*, 1973, 324.

serine, hydroxymethylserine, sarcosine, iminodiacetic acid, and alanine, has been discussed.[114] Bis-(L-serinato)copper(II) reacts with excess formaldehyde at pH 7—9 to give the bis(amino-acidato)copper(II) complex involving (26), from which α-hydroxymethylserine is obtained on treatment with hydrogen sulphide.[115] An optically active intermediate can be

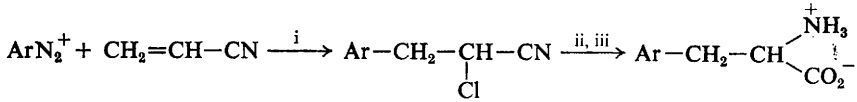


(26)

detected in the hydroxymethylation stage, indicating initial hydroxymethylation on nitrogen.

γ-Hydroxy-α-amino-acids are an important group of naturally occurring compounds for which improved synthetic methods are being developed. Photochlorination of α-amino-acids carrying a γ-hydrogen atom gives corresponding α-amino-γ-lactones after hydrolysis;[116] an alternative route to such lactones uses diethyl allyl acetamidomalonate as starting material,[81] which by addition of $Br_2$ and treatment with 48% hydrobromic acid gives diastereoisomeric 2-amino-5-bromo-4-valerolactone hydrobromides which after separation and hydrolysis give DL-γ-hydroxyproline and the allo-isomer.[81] Synthesis of all stereoisomers of γ-hydroxyglutamic acid is reported by the same workers.[117]

**Aromatic and Heterocyclic Amino-acids.**—Among numerous routes to the phenylalanine analogues which have been synthesized recently, that employing acrylonitrile (Scheme 8) is of wide potential use.[118] The oxazolone route,[119, 120] and the acetamidomalonate route [82] or its benzyloxycarbonylamino variant [83] have been used for phenylalanine analogues.



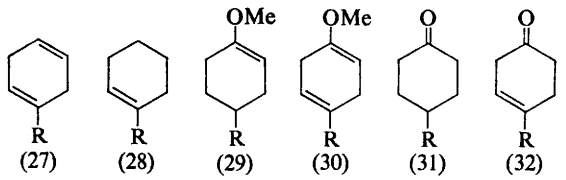
Reagents: i, $CuCl_2$–NaOAc; ii, $NH_3$; iii, $H_3O^+$

**Scheme 8**

[114] A. S. Subbaraman, Z. A. Kazi, and A. S. U. Choughuley, *Indian J. Biochem. Biophys.*, 1972, **9**, 268.
[115] J. R. Brush, R. J. Magee, M. J. O'Connor, S. B. Teo, R. J. Geue, and M. R. Snow, *J. Amer. Chem. Soc.*, 1973, **95**, 2034.
[116] H. Faulstich, J. Dolling, K. Michl, and T. Wieland, *Annalen*, 1973, 560.
[117] Y. K. Lee and T. Kaneko, *Bull. Chem. Soc. Japan*, 1973, **46**, 3494.
[118] M. Y. Mogilevskii, N. T. Morozova, and O. E. Antropova, *Zhur. obshchei Khim.*, 1973, **43**, 1822.
[119] K. Karpavicius, G. Prasmickiene, L. Gurviciene, and O. V. Kildisheva, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 1887.
[120] B. Sila, J. Wojtanis, and T. Lesiak, *Roczniki Chem.*, 1973, **47**, 1281.

Aryl-substitution procedures include a new synthesis of L-dopa from L-tyrosine;[121] Friedel–Crafts reaction of L-tyrosine with acetyl chloride gives 3-acetyl-L-tyrosine, from which L-dopa is obtained by treatment with alkaline $H_2O_2$. Previously described '2,4-dibromo-L-phenylalanine' is shown to be the 2,3-isomer;[122] separation of the bromination products of L-phenylalanine on Sephadex LH-20 using aqueous methanol gives 2,3-dibromo- (2%), 2,5-dibromo- (13%), 3,4-dibromo- (5%), 2-bromo- (29%), and 4-bromo-L-phenylalanine (36%).[122] 5-Fluoro-L-dopa (*i.e.* 3,4-dihydroxy-5-fluoro-L-phenylalanine) and its [5-$^{18}$F]-analogue have been synthesized *via* the Schiemann reaction,[123] and a variety of nitro-imidazole analogues of L-histidine is now available.[124] $N^{\alpha}$-Benzoyl-L-histidine gives 2,4-bis(arylazo) coupled products with arenediazonium salts, whereas the *N*-acetyl analogue gives predominantly the 2-arylazo-imidazole, which gives 2-amino-L-histidine by catalytic hydrogenolysis and hydrolysis.[125] Since the previously synthesized 4-fluoro-L-histidine showed interesting *in vivo* and enzymic properties, *N*-acetyl-2-amino-L-histidine methyl ester was diazotized and irradiated in $HBF_4$ solution, to give 2-fluoro-L-histidine after enzymic hydrolysis and enzymic deacylation.[126]

Birch reduction of L-phenylalanine gives L-3-[1-(cyclohexa-1,4-dienyl)]-alanine (27),[127] while *O*-methyl-L-tyrosine gives cyclohexenes (28) and (29), and the cyclohexadiene (30), by reduction with sodium in liquid ammonia;[128]




(27) (28) (29) (30) (31) (32)

R = $-CH_2-CH(NH_3^+)CO_2^-$

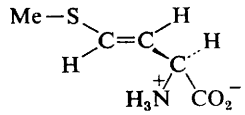
two of these, (29) and (30), are enol ethers, and give the cyclohexanone (31) and the cyclohexenone (32), respectively, on acid hydrolysis.

**N-Substituted Amino-acids.**—*N*-Carboxymethylation of α-amino-acids can be brought about by treatment with glyoxal in an acetate buffer at 100 °C during 30 min.[129] A useful *N*-methylation procedure is illustrated by the

[121] H. Bretschneider, K. Hohenlohe-Oehringen, A. Kaiser, and U. Wolcke, *Helv. Chim. Acta*, 1973, **56**, 2857.
[122] H. Faulstich, H. O. Smith, and S. Zobeley, *Annalen*, 1973, 765.
[123] G. Firnau, C. Nahmias, and S. Garnett, *J. Medicin. Chem.*, 1973, **16**, 416.
[124] W. Tantz, S. Teitel, and A. Brossi, *J. Medicin. Chem.*, 1973, **16**, 705.
[125] W. Nagai, K. L. Kirk, and L. A. Cohen, *J. Org. Chem.*, 1973, **38**, 1971.
[126] K. L. Kirk, W. Nagai, and L. A. Cohen, *J. Amer. Chem. Soc.*, 1973, **95**, 8389.
[127] G. R. Nagarajan, L. Diamond, and C. Ressler, *J. Org. Chem.*, 1973, **38**, 621.
[128] K. Kaminski and T. Sokolowska, *Roczniki Chem.*, 1973, **47**, 1091.
[129] N. V. Chuyen, T. Kurata, and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2209.

one-step conversion of an *N*-benzyloxycarbonylamino-acid into its *N*-methyl derivative with methyl iodide and sodium hydride.[130] Surprisingly, even *N*-benzyloxycarbonyl-methionine could be selectively *N*-methylated.[130] Use of $N^{in}$-formyl-L-tryptophan, as its $N^{\alpha}$-Boc-derivative, in solid-phase peptide synthesis has been studied.[131]

**Amino-acids containing Sulphur.**—Interest in α-mercapto-α-amino-acids is a consequence of the presence of the $-NH-\overset{|}{C}(S-)-CO-$ group in gliotoxins and related natural products. *N*-Acyl α-chloro-α-amino-acids, $R^1CONHCR^2ClCO_2H$, obtained by addition of hydrogen chloride in acetic acid to *N*-acyl αβ-dehydroamino-acids, give α-mercapto-, α-*S*-acylmercapto-, and α-*S*-benzhydrylthio-*N*-acylamino-acids.[132] An alternative approach[133] using methyl t-butyl 2-benzylthio-malonate, $Bu^tO_2C-CH(SCH_2Ph)CO_2Me$, as starting material, gives *N*-Boc DL-α-benzylthioglycine. The dipeptide of this amino-acid could be cyclized to the dioxopiperazine and debenzylated, but conversion into the 3,6-epi-dithiodioxopiperazinedione was not successful.[133]

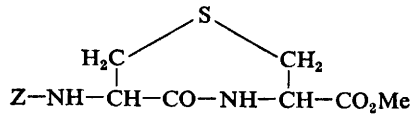


(33)

(+)-(*S*)-*E*-2-Amino-4-methylthiobut-3-enoic acid (33; dehydro-L-methionine) has been synthesized[84] starting with 3-acetamido-3,3-bis(ethoxycarbonyl)propionaldehyde, $AcNHC(CO_2Et)_2CH_2CHO$. The mercaptal, formed with methanethiol, was saponified, decarboxylated, and converted into the methyl ester $AcNHCH(CO_2Me)CH_2CH(SMe)_2$; an interesting use of the newer sulphur chemistry followed, whereby the mercaptal gives the monosulphoxide with sodium metaperiodate, which on pyrolysis gives dehydro-L-methionine as its *N*-acetyl methyl ester, as a mixture of *E*- and *Z*-isomers. Separation and resolution gave the (+)-*E*-isomer; no doubt there will be many potential amino-acid antagonists of this type synthesized in the future.

A lanthionine synthesis starting with *N*-benzyloxycarbonyl-*S*-benzoyl-L-cysteinyl-*O*-benzoyl-L-serine methyl ester, *via* the cyclo-lanthionine derivative (34) followed by hydrolysis, is also suitable for the synthesis of the *meso*-isomer.[134] *S*-Adenosyl-L-homo-cysteine and nucleoside analogues, and their sulphoxides, have been prepared as potential inhibitors of transmethylases.[135]

[130] J. R. McDermott and N. L. Benoiton, *Canad. J. Chem.*, 1973, **51**, 1915.
[131] D. Yamashiro and C. H. Li, *J. Org. Chem.*, 1973, **38**, 2594.
[132] S. M. Patel, J. O. Currie, and R. K. Olsen, *J. Org. Chem.*, 1973, **38**, 126.
[133] T. Petrzilka and C. Fehr, *Helv. Chim. Acta*, 1973, **56**, 1218.
[134] L. Zervas and N. Ferderigos, *Experientia*, 1973, **29**, 262.
[135] V. N. Rekunova, I. P. Rudakova, and A. M. Yurkevich, *Tetrahedron Letters*, 1973, 2811.



(34)

**A List of α-Amino-acids which have been Synthesized for the First Time** [a]

| *Compound* | *Ref.* |
|---|---|
| 3-(2-Furoyl)alanine [b] | 39, 41 |
| 2-Amino-6-nitrocaproic acid [b] | 88 |
| γ-Hydroxyglutamic acid [b] | 117 |
| 3,4-Dehydromethionine [b] | 84 |
| *threo*-β-Amino-DL-asparagine | 136 |
| *erythro*-β-Amino-DL-asparagine | 136 |
| *threo*-β-Phenyl-DL-asparagine | 136 |
| *erythro*-β-Phenyl-DL-asparagine | 136 |
| *threo*-β-(*N*-4-Hydroxyphenyl)amino-L-asparagine | 136 |
| *erythro*-β-(*N*-4-Hydroxyphenyl)amino-L-asparagine | 136 |
| *S*-Inosyl-L-homocysteine | 135 |
| *S*-Inosyl-L-homocysteine sulphoxide | 135 |
| *S*-Guanosyl-L-homocysteine | 135 |
| *S*-Guanosyl-L-homocysteine sulphoxide | 135 |
| *S*-Uridyl-L-homocysteine | 135 |
| *S*-Uridyl-L-homocysteine sulphoxide | 135 |
| *S*-Cystidyl-L-homocysteine | 135 |
| *S*-Cystidyl-L-homocysteine sulphoxide | 135 |
| DL-γ-Hydroxyproline | 81 |
| DL-Allo-γ-hydroxyproline | 81 |
| DL-2-Aminoadamantane-2-carboxylic acid | 137 |
| Pentamethylphenylalanine [b] | 138 |
| DL-3,4,5-Trimethoxyphenylalanine | 82 |
| DL-4-Hydroxy-3,5-dimethoxyphenylalanine | 82 |
| DL-3-Hydroxy-4,5-dimethoxyphenylalanine | 82 |
| DL-3,4-Dihydroxy-5-methoxyphenylalanine | 82 |
| L-(*p*-Pentafluorophenyl)phenylalanine | 83 |
| DL-(3,4-Dihydroxy-5-fluoro)phenylalanine | 123 |
| DL-(3,4-Dihydroxy-[5-$^{18}$F]fluoro)phenylalanine | 123 |
| L-*o*-Methylphenylalanine [b] | 85 |
| L-β-(2-Naphthyl)alanine | 85 |
| L-β-(6-Quinolyl)alanine | 85 |
| DL-*p*-Bis-(2-chloropropylamino)phenylalanine | 119 |
| DL-5,7-Disubstituted 2-coumaronylalanines | 120 |
| DL-3-Amino-1,2,3,4-tetrahydrocarbazole 3-carboxylic acid | 139 |
| DL-3-Amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole 3-carboxylic acid | 139 |

[136] P. K. Chang, L. J. Sciarini, and R. E. Handschumacher, *J. Medicin. Chem.*, 1973, **16**, 1277.
[137] H. T. Nagasawa, J. A. Elberling, and F. N. Shirota, *J. Medicin. Chem.*, 1973, **16**, 823.
[138] G. I. Tesser, H. G. A. Slits, and J. W. van Nispen, *Internat. J. Peptide Protein Res.*, 1973, **5**, 119.
[139] Y. Maki, T. Masugi, T. Hiramitsu, and T. Ogiso, *Chem. and Pharm. Bull.* (*Japan*), 1973, **21**, 2460.

**List of α-Amino-acids** (*cont.*)

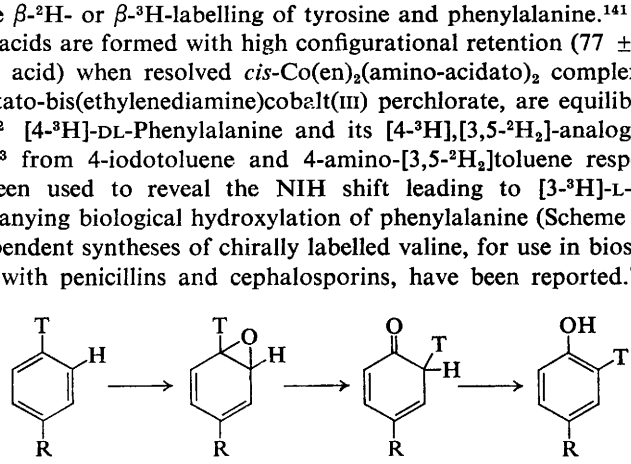
| *Compound* | *Ref.* |
|---|---|
| DL-3-Amino-6-methoxy-1,2,3,4-tetrahydrocarbazole 3-carboxylic acid | 139 |
| DL-3-Amino-6-benzyloxy-1,2,3,4-tetrahydrocarbazole 3-carboxylic acid | 139 |
| L-2-Aminohistidine | 125 |
| L-2-Fluorohistidine | 125 |
| DL-1-Methyl-2-nitrohistidine | 124 |
| L-1-Methyl-4-nitrohistidine | 124 |
| L-1-Methyl-5-nitrohistidine | 124 |

[a] Other new amino-acids, and labelled analogues of known amino-acids, are mentioned in this chapter.
[b] All stereoisomers synthesized and separated.

**Amino-acids with Amino-alkyl Side-chains.**—Further details of the synthesis of all four stereoisomers of 2,3-diaminobutyric acid from threonine and allothreonine have been given.[140] Reference is made elsewhere in this chapter to the synthesis of amino-acids with modified amino-alkyl side-chains.[105–108]

**Labelled Amino-acids.**—In some areas, the ability to conduct increasingly sophisticated biosynthetic studies is dependent upon the availability of specifically labelled α-amino-acids. Full details have been given of stereoselective β-$^2$H- or β-$^3$H-labelling of tyrosine and phenylalanine.[141] [2-$^2$H]-Amino-acids are formed with high configurational retention (77 ± 2% for aspartic acid) when resolved *cis*-Co(en)$_2$(amino-acidato)$_2$ complexes, *e.g.* L-aspartato-bis(ethylenediamine)cobalt(III) perchlorate, are equilibrated in $^2H_2O$.[142] [4-$^3$H]-DL-Phenylalanine and its [4-$^3$H],[3,5-$^2H_2$]-analogue, prepared[143] from 4-iodotoluene and 4-amino-[3,5-$^2H_2$]toluene respectively, have been used to reveal the NIH shift leading to [3-$^3$H]-L-tyrosine accompanying biological hydroxylation of phenylalanine (Scheme 9).

Independent syntheses of chirally labelled valine, for use in biosynthetic studies with penicillins and cephalosporins, have been reported.[77, 144, 145]




**Scheme 9**

[140] E. Atherton and J. Meienhofer, *Z. physiol. Chem.*, 1973, **354**, 689.
[141] G. W. Kirby and J. Michael, *J.C.S. Perkin I*, 1973, 115.
[142] W. E. Keyes and J. I. Legg, *J. Amer. Chem. Soc.*, 1973, **95**, 3431.
[143] W. R. Bowman, W. R. Gretton, and G. W. Kirby, *J.C.S. Perkin I*, 1973, 218.
[144] D. J. Aberhardt and L. J. Lin, *J. Amer. Chem. Soc.*, 1973, **95**, 7859; for related synthetic studies, see R. K. Hill, S. Yan, and S. M. Arfin, *ibid.*, p. 7857.
[145] H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1973, **95**, 6149.

A straightforward route (Scheme 10) to (2*RS*,3*S*)-[4,4,4-$^2H_3$]valine is notable for employing a 'mild' Strecker synthesis at the last stage.[144] Rather longer routes (Schemes 11[77] and 12[145]) give (2*RS*,3*R*)-[4-$^{13}$C]-valine[77] and (2*S*,3*S*)-[4-$^{13}$C]valine.[145]

[5-$^3$H]-L-Ornithine and [6-$^3$H]-L-lysine are available through catalytic tritiation of ω-cyano-L-aminobutyric acid and ω-cyano-L-norvaline, respectively.[146]

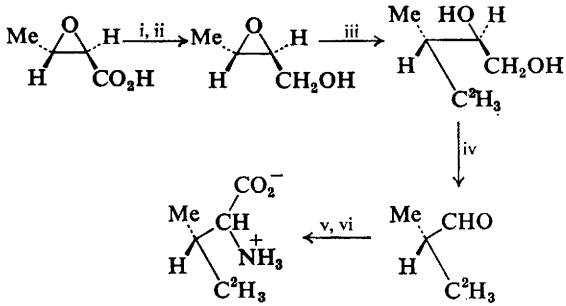
A clever application of the reversibility of the tyrosine phenol-lyase reaction allows [$^{14}$C]phenol to be incorporated into L-tyrosine in high yield, and using mushroom tyrosinase, [$^{14}$C]catechol can be prepared from [$^{14}$C]phenol and [$^{14}$C]-L-tyrosine can be converted into [$^{14}$C]-L-dopa. Preparation of [$^{14}$C]-L-dopa with two different labelling patterns is then possible, since [$^{14}$C]catechol can be incorporated into [$^{14}$C]-L-dopa using tyrosine phenol-lyase; ring [4-$^{14}$C]- and ring [3,4-$^{14}C_2$]-labelling of L-dopa are achieved in this way.[147]

A commercial sample of DL-phenylalanine, non-specifically labelled with $^3$H, gave $PhCH_2CO_2H$ retaining 96% of the label, on degradation;[148] electrophilic substitution studies indicated that 45% of this residual label was at the *p*-position, and 26% in each of the *o*-positions.[148]

**Resolution of Amino-acids.**—The claim[149] that preferential adsorption and polymerization of the L-enantiomer of a DL-amino-acid takes place on edge faces of kaolinite has been disputed for the original test case of DL-phenylalanine in aqueous solutions at pH 2 or 6.[150] Since the claim provides a start towards explaining differential availability of D- and L-enantiomers of amino-acids, and chance formation of proteins composed of L-amino-acid residues, no doubt its refutation will come under further scrutiny. Complete resolution of DL-tryptophan using bovine serum albumin attached to succinoylaminoethylagarose as an affinity chromatographic matrix has been reported;[151] as should be expected, the D-isomer emerges first from the column. The first optical resolution by liquid–liquid chromatography of a racemate by differential complexation has been demonstrated with DL-α-amino-acids.[152] The host–guest molecule principle is employed, based upon chiral cyclic polyethers; considerable planning is required to devise the right host molecule for a particular amino-acid, but then complete optical resolution of valine is easily achieved.[152, 153]

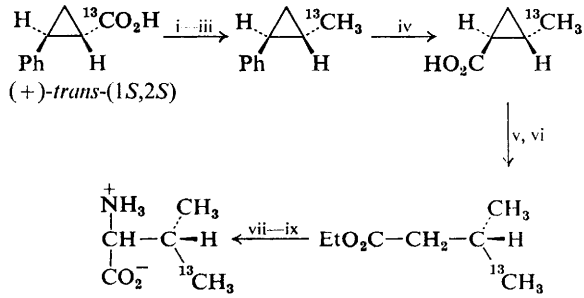
Resolution by analytical g.l.c. is discussed in Section 5 of this chapter. More conventional procedures include a preferential crystallization

[146] M. Havranek and I. Mezo, *Acta Chim. Acad. Sci. Hung.*, 1973, **77**, 341.
[147] B. E. Ellis, G. Major, and M. H. Zenk, *Analyt. Biochem.*, 1973, **53**, 470.
[148] R. B. Herbert and I. T. Nicholson, *J. Labelled Compounds*, 1973, **9**, 567.
[149] T. A. Jackson, *Experientia*, 1971, **27**, 242.
[150] W. A. Bonner and J. Flores, *Biosystems*, 1973, **5**, 103.
[151] K. K. Stewart and R. F. Doherty, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2850.
[152] R. C. Helgeson, K. Koga, J. M. Timko, and D. J. Cram, *J. Amer. Chem. Soc.*, 1973, **95**, 3021.
[153] R. C. Helgeson, J. M. Timko, D. J. and Cram, *J. Amer. Chem. Soc.*, 1973, **95**, 3023.



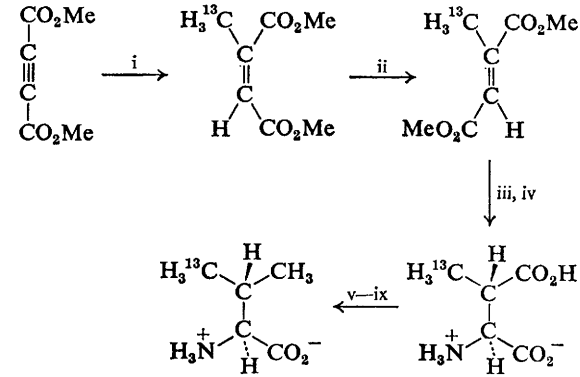
Reagents: i, $CH_2N_2$; ii, $NaBH_4$; iii, $C^2H_3Li$; iv, $NaIO_4$; v, $NH_4OH$–NaCN, 0 °C; vi, reflux conc. HCl

**Scheme 10**



Reagents: i, $LiAlH_4$; ii, mesyl chloride; iii, $LiAlH_4$; iv, $O_3$; v, diazoethane; vi, Li–$NH_3$; vii, $Br_2$; viii, $NH_3$; ix, $H_3O^+$

**Scheme 11**



Reagents: i, $^{13}CH_3Cu$; ii, *hν*/trace of $Br_2$; iii, $H_3O^+$; iv, β-methylaspartase; v, reduce *N*-trifluoroacetyl α-methyl ester with $B_2H_6$; vi, mesyl chloride; vii, NaI; viii, reduction; ix, $H_3O^+$

**Scheme 12**

procedure applied to DL-amino-acid arenesulphonate salts;[154] resolution of β-(2-pyridyl)-DL-alanine as the tartrate salt;[155] and enzymic resolution (using papain) of *N*-alkoxycarbonyl-DL-amino-acids.[155a]

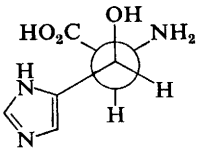
## 4 Physical and Stereochemical Studies of Amino-acids

Further study of 1-amino-cycloalkane-1-carboxylic acids has resolved disagreement in favour of the preferred adoption of equatorial and axial dispositions, respectively, by carboxy- and amino-groups in such compounds.[156]

**Crystal Structures of Amino-acids.**—A survey of published crystal structures of phenylalanine and tyrosine derivatives [156a] pays special attention to conformational regularities through the series.

Precision neutron diffraction studies of α-amino-acids (*i.e.* definitive hydrogen locations and conformational features) continue with structures assigned to L-arginine dihydrate,[157] L-tyrosine,[158] L-tyrosine hydrochloride,[158] L-glutamine,[159] 4-hydroxy-L-proline,[160] L-serine monohydrate,[161] and DL-serine.[161]

Absolute configurational assignments have been made to 4*S*-amino-3*S*-hydroxy-6-methylheptanoic acid, through *X*-ray analysis of its *N*-*p*-bromobenzoyl derivative,[162] confirming chemical correlation, and to *erythro*-β-hydroxy-L-histidine.[54] The *gauche* conformation (35), resulting from the presence of the hydroxy-group, is not adopted by other histidine derivatives in the crystal state.[54]




(35)

[154] S. Yamada, M. Yamamoto, and I. Chibata, *J. Org. Chem.*, 1973, **38**, 4408.
[155] L. N. Veselova and E. S. Chaman, *Zhur. obshchei. Khim.*, 1973, **43**, 1637.
[155a] J. L. Abernethy, R. Bobeck, A. Ledesma, and R. Kemp, *J. Org. Chem.*, 1973, **38**, 1286.
[156] Y. Maki, T. Masugi, and K. Ozeki, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2466.
[156a] V. Cody, W. L. Duax, and H. Hauptmann, *Internat. J. Peptide Protein Res.*, 1973, **5**, 297.
[157] M. S. Lehmann, J. J. Verbist, W. C. Hamilton, and T. F. Koetzle, *J.C.S. Perkin II*, 1973, 133.
[158] M. N. Frey, T. F. Koetzle, M. S. Lehmann, and W. C. Hamilton, *J. Chem. Phys.*, 1973, **58**, 2547.
[159] T. F. Koetzle, M. N. Frey, M. S. Lehmann, and W. C. Hamilton, *Acta Cryst.*, 1973, **B29**, 2571.
[160] T. F. Koetzle, M. S. Lehmann, and W. C. Hamilton, *Acta Cryst.*, 1973, **B29**, 231.
[161] M. N. Frey, M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Acta Cryst.*, 1973, **B29**, 876.
[162] H. Nakamura, H. Morishima, T. Takita, H. Umezawa, and Y. Iitaka, *J. Antibiotics*, 1973, **26**, 255.

Other *X*-ray studies concern the diethylphosphoric acid salt of arginine,[163] DL-aspartic acid,[164] orthorhombic L-cysteine,[165] L-dopa,[166] DL-isoleucine,[167] L-methionine,[168] L-norleucine,[168] $\gamma$-aminobutyric acid at low temperature,[169] L-penicillamine hydrochloride monohydrate,[170] $N^5$-hydroxy-L-arginine hydrobromide,[46] DL-penicillaminato-methylmercury(II),[171] $\alpha S,5S$-$\alpha$-amino-3-chloro-2-isoxazoline-5-acetic acid,[47] and the bis(amino-acidato)copper(II) complex of (26).[115]

**N.M.R. Spectrometry.**—Apart from the routine use of proton n.m.r. in support of structural assignments to amino-acids and their derivatives, most of the more notable results reported this year relate to conformational studies. Careful correlation with conformation of the solvent-dependent n.m.r. of the phenylalanine anion in mixed aqueous solvents as a function of temperature indicates the progressive disappearance of less-favoured conformers as the proportion of water in such solvents decreases.[172] Similar studies with histidine, and its *im*-benzyl, $N^{\alpha}$-acyl, and methyl ester derivatives in $^2H_2O$ at various pD, suggest a preferred conformation for histidine in basic solutions in which the imidazole and carboxylate groupings are close together.[173]

Stability constants for complexes of neodymium(III) with amino-acids have been determined by n.m.r. and by potentiometric titration, and are relevant to the use of $Nd^{3+}$ complexes as shift reagents for aqueous solution studies.[174]

Proton magnetic relaxation times, now increasingly recognized as capable of providing additional structural information, have been determined for aqueous solutions of glycine and a series of aliphatic amino-acids;[175] $T_1$ values are pH-independent, but $T_2$ values have a minimum value near pH 6 for this series. Proton spin–lattice relaxation times at 90 and 270 MHz for several amino-acids show no frequency dependence, while some $T_2$ values for certain peptides are frequency-dependent;[176] conformational inferences are discussed.

$^{19}F$ N.m.r. reveals preferential binding of ring-substituted *N*-trifluoroacetyl-DL-phenylalanines to chymotrypsin; separate resonances for the

[163] S. Furberg and J. Solbakk, *Acta Chem. Scand.*, 1973, **27**, 1226.
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[167] E. Benedetti, C. Pedone, and A. Sirigu, *Acta Cryst.*, 1973, **B29**, 730.
[168] K. Torii and Y. Iitaka, *Acta Cryst.*, 1973, **B29**, 2799.
[169] E. G. Steward, R. B. Player, and D. Warner, *Acta Cryst.*, 1973, **B29**, 2038, 2825.
[170] S. N. Rao, R. Parthasarathy, and F. E. Cole, *Acta Cryst.*, 1973, **B29**, 2373.
[171] Y. S. Wong, P. C. Chieh, and A. J. Carty, *J.C.S. Chem. Comm.*, 1973, 741.
[172] J. M. Purcell, J. E. Ramirez, and J. R. Cavanaugh, *J. Phys. Chem.*, 1973, **77**, 1501.
[173] R. J. Weinkam and E. C. Jorgensen, *J. Amer. Chem. Soc.*, 1973, **95**, 6084.
[174] A. D. Sherry, C. Yoshida, E. R. Birnbaum, and D. W. Darnall, *J. Amer. Chem. Soc.*, 1973, **95**, 3011.
[175] D. D. Eley, A. S. Fawcett, and M. J. Hey, *J.C.S. Faraday I*, 1973, **69**, 399.
[176] H. B. Coates, K. A. McLauchlan, I. D. Campbell, and C. E. McColl, *Biochim. Biophys. Acta*, 1973, **310**, 1.

D- and L-enantiomers appear in the presence of the enzyme, with a chemical shift difference related to the inhibitor : enzyme ratio.[177] Assignment of an envelope conformation, with axial fluorine, for *cis*- and *trans*-4-fluoro-L-proline in aqueous solution has been made using $^{1}H$ and $^{19}F$ n.m.r. spectra.[178]

All six carbon resonances suffer shifts with varying ionization patterns of the amino, carboxy, and imidazole groupings of L-histidine;[179] related $^{13}C$ n.m.r. studies with 1-methyl- and 3-methyl-histidines in comparison with histidine itself indicate the predominance of the 1-H-tautomer for L-histidine in basic solutions.[180]

Tentative interpretation is made of $^{15}N$ chemical shift data for several 95%-enriched $^{15}N$-amino-acids;[181] an indication of the pH-dependence of lineshapes, chemical shifts, nuclear Overhauser enhancements, and nuclear relaxation rates has been obtained for aqueous solutions of $^{15}N$-glycine and its ethyl ester.[182]

**O.R.D. and C.D. Spectra.**—The 210 nm $n \rightarrow \pi^*$ Cotton effect, diagnostic of absolute configuration at the α-carbon atom in simple α-amino-acids (positive Cotton effect = L-configuration) has been used to assign absolute configuration to α-methyl-α-amino-acids prepared by asymmetric Strecker synthesis.[63] The same relationship between sign of Cotton effect and absolute configuration holds also for simple *N*-methyl-,[183] *NN*-dimethyl-L-amino-acids,[184] and L-amino-acid betaines.[78] Calculations using semi-empirical CNDO–MO methods for various conformations of L-alanine and related α-amino-acids give non-empirical support[185] to Jorgensen's sector rule,[186] but some parameters used in the calculations are not sufficiently accurate to permit more precise c.d.–conformation correlations.[185]

Prototype c.d. spectrometers are capable of reaching to *ca.* 160 nm,[187] and have shown the presence of a negative Cotton effect in the range 168—172 nm, in addition to features at longer wavelengths, for five aliphatic L-amino-acids.[187] Proline differs from open-chain analogues in showing sigmoid $n \rightarrow \pi^*$ c.d.[187] Thin films of aliphatic α-amino-acids show four absorption bands in the 140—200 nm wavelength region, deriving from transitions in the carboxylate chromophore;[188] band locations are somewhat structure-dependent.[188]

[177] B. C. Nicholson and T. M. Spotswood, *Austral. J. Chem.*, 1973, **26**, 135.
[178] J. T. Gerig and R. S. McLeod, *J. Amer. Chem. Soc.*, 1973, **95**, 5725.
[179] M. H. Freedman, J. R. Lyerla, I. M. Chaiken, and J. S. Cohen, *European J. Biochem.*, 1973, **32**, 215.
[180] W. F. Reynolds, I. R. Peat, M. H. Freedman, and J. R. Lyerla, *J. Amer. Chem. Soc.*, 1973, **95**, 328.
[181] J. A. Sogn, W. A. Gibbons, and E. W. Randall, *Biochemistry*, 1973, **12**, 2100.
[182] R. A. Cooper, R. L. Lichter, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1973, **95**, 3724.
[183] J. Shoji, *J. Antibiotics*, 1973, **26**, 302.
[184] C. J. Hawkins and G. A. Lawrence, *Austral. J. Chem.*, 1973, **26**, 1801.
[185] J. Webb, R. W. Strickland, and F. S. Richardson, *Tetrahedron*, 1973, **29**, 2499.
[186] E. C. Jorgensen, *Tetrahedron Letters*, 1971, 863.
[187] P. A. Snyder, P. M. Vipond, and W. C. Johnson, *Biopolymers*, 1973, **12**, 975.
[188] T. Inagaki, *Biopolymers*, 1973, **12**, 1353.

The already extensive study of the c.d. of the perturbed indole chromophore, as it exists in L-tryptophan and its derivatives, is supplemented by extensive data on the series of negative Cotton effects in the 180—215 nm region.[189] There are four or five Cotton effects in this region, none of which arises by chromophore coupling between indole and carboxylate moieties. The magneto-c.d. spectra of tyrosine and tryptophan have been compared,[190] with special reference to pH-dependence.

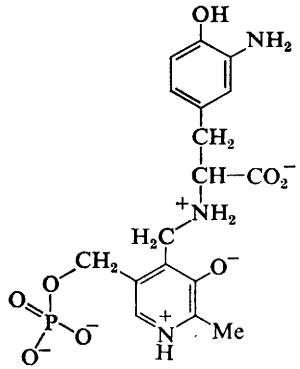
The c.d. spectra of several common sulphur-containing amino-acids have been studied in an attempt to correlate various features with conformation-dependent c.d. characteristics of the sulphur chromophores.[191] The temperature-dependent c.d. of *NN'*-diacetyl-L-cystine bis(dimethylamide) has been analysed in terms of the chirality of the disulphide chromophore.[192]

β-Amino-acids adopt different spatial relationships of carboxy chromophore to substituents at the asymmetric centre, compared with α-amino-acids, and a less secure relationship between sign of $n \rightarrow \pi^*$ Cotton effect and absolute configuration is found. The pH-dependence of the o.r.d. of compounds of this series is a satisfactory basis for absolute configurational assignments,[94] while the sign of the $n \rightarrow \pi^*$ Cotton effect of their *N*-dithioalkoxycarbonyl derivatives fails to correspond unambiguously with absolute configuration.

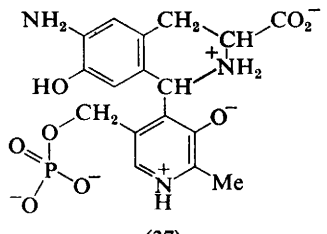
Hydantoins derived from L-α-amino-acids show a negative Cotton effect in the 230 nm wavelength region,[193] while *N*-methylthiohydantoins have some value in the assignment of absolute configuration to the α-amino-acids from which they are derived, provided that racemization can be avoided during their preparation.[194]

**Combined Use of Physical Methods; Amino-acid Derivatives in Solution.**—Solution conformation and antibody studies of the haptens *N*-(5-phosphopyridoxyl)-3'-amino-L-tyrosine (36) and its cyclic analogue (37) take account of fluorescence spectrometric, n.m.r., and c.d. data.[195] A thorough appraisal of the conformational behaviour in solution of $N^{\alpha}$-acetylamino-acid *N*-methylamides (38) and proline analogues involves i.r.,[196] n.m.r.,[197] dipole moment,[198] effective molecular weight,[199] and u.v., c.d., and o.r.d.

[189] H. E. Auer, *J. Amer. Chem. Soc.*, 1973, **95**, 3003.
[190] M. Gabriel, D. Larchier, H. Rinnert, and C. Thirion, *Compt. rend.*, 1973, **276**, *B*, 39.
[191] G. Jung, M. Ottnad, and M. Rimpler, *European J. Biochem.*, 1973, **35**, 436.
[192] T. Takagi, R. Okano, and T. Miyazawa, *Biochim. Biophys. Acta*, 1973, **310**, 11.
[193] T. Suzuki, K. Igarashi, K. Hase, and K. Tuzimura, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 411.
[194] T. Suzuki and K. Tuzimura, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 689.
[195] V. Raso and B. D. Stollar, *J. Amer. Chem. Soc.*, 1973, **95**, 1621.
[196] E. S. Efremov, L. B. Senyavina, V. N. Zheltova, A. G. Ivanova, P. V. Kostetskii, V. T. Ivanov, E. M. Popov, and Y. A. Ovchinnikov, *Khim. prirod Soedinenii*, 1973, **9**, 322.
[197] V. T. Ivanov, P. V. Kostetskii, T. A. Balashova, S. L. Portnova, E. S. Efremov, and Y. A. Ovchinnikov, *Khim. prirod Soedinenii*, 1973, **9**, 339.
[198] E. S. Efremov, P. V. Kostetskii, V. T. Ivanov, E. M. Popov, and Y. A. Ovchinnikov, *Khim. prirod Soedinenii*, 1973, **9**, 348.
[199] E. S. Efremov, P. V. Kostetskii, V. T. Ivanov, E. M. Popov, and Y. A. Ovchinnikov, *Khim. prirod Soedinenii*, 1973, **9**, 354.



(36)



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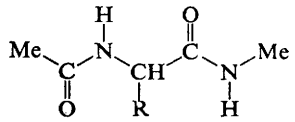


(38)

studies.[200] $N^{\alpha}$-Acetyl-$N^{\alpha}$-methyl-alanine *N*-methylamide exists predominantly in the *trans*-acetamide form in all solvents, but 15% in the *cis*-form in $CCl_4$ and 26% in the *cis*-form in $(C^2H_3)_2SO$.[197] The i.r. study reveals a high degree of coiling through intramolecular hydrogen-bonding in $CHCl_3$ and $CCl_4$,[196] while osmometry[199] indicates a tendency to form hydrogen-bonded dimers in concentrated solutions.

**Mass Spectrometry.**—Pyrolytic fragmentation of amino-acid betaines $Me_3N^+(CH_2)_nCO_2^-$ ($n$ = 1—5) in the mass spectrometer has been rationalized in terms of formation of $Me_2N^+{=}CH_2$ (base peak at *m/e* 58) together with cyclized fragments depending upon structure.[201] Identification of ten of the amino-acids present in soil samples by quadrupole mass spectrometry has been demonstrated for nanogram quantities of their *N*-trifluoroacetyl n-butyl esters, with results comparable with those from the amino-acid analyser.[202] Chemical ionization mass spectra of amino-acids and peptides and their ester and amide derivatives have been reported using either isobutane[203] or methane[204] as reactant plasma. Free amino-acids show loss of $HCO_2H$ with isobutane at 440—630 °C, in addition to fragments $MH^+$, $M_2H^+$, $(M + 57)^+$, and $(M + 39)^+$;[203] lower temperatures cause insufficient fragmentation.

[200] V. T. Ivanov, P. V. Kostetskii, E. A. Meshcheryakova, E. S. Efremov, E. M. Popov, and Y. A. Ovchinnikov, *Khim. prirod. Soedinenii*, 1973, **9**, 363.
[201] K. Undheim and T. Laerum, *Acta Chem. Scand.*, 1973, **27**, 589.
[202] W. E. Pereira, Y. Hoyano, W. E. Reynolds, R. E. Summons, and A. M. Duffield, *Analyt. Biochem.*, 1973, **55**, 236.
[203] M. Meot-Ner and F. H. Field, *J. Amer. Chem. Soc.*, 1973, **95**, 7207.
[204] P. A. Leclercq and D. M. Desiderio, *Org. Mass Spectrometry*, 1973, **7**, 515.

Where amino-acid sequences in peptides are not obtainable by mass spectrometric study of derivatized peptides, then mass spectrometry can be employed to identify the amino-acid derivative released in the cleavage step of a conventional peptide-sequencing procedure. The last residue in the Edman degradation of a peptide is obtained as its *N*-phenylthiocarbamoyl derivative, or as its methyl analogue, and these isomerize thermally in the mass spectrometer to corresponding *N*-phenyl- or *N*-methyl-thiohydantoins, and can be identified as such.[205] Neopentylidene amino-acid ethyl esters are useful volatile derivatives for mass spectrometry of amino-acids.[15]

**Determination of Absolute Configuration.**—In addition to results noted in preceding paragraphs, assignment of *S*-configuration to (+)-2-amino-2-phenylbutyric acid by chemical correlation with (−)-(*S*)-2-amino-2-phenylbutane has been confirmed by molecular rotation data,[206] and L-configuration has been assigned to the β-centre in the β-methyllanthionine component of nisin.[207] *N*-Methyl-alloisoleucine found in quinoxaline antibiotics is the L-enantiomer, as found in the Actinomycins.[183]

## 5 Chemical Studies of Amino-acids

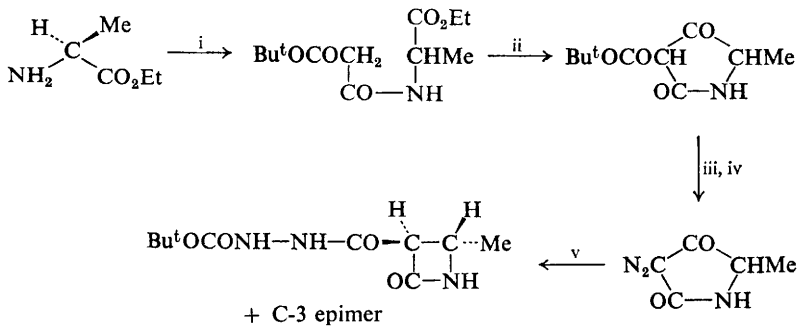
**Racemization and Inversion.**—The stirring of interest last year in the geochronological information which can be inferred from the degree of racemization suffered by fossil amino-acids has been developed into a subject in its own right with its own jargon. The half-life for the racemization of L-isoleucine giving D-allo-isoleucine (more than 100 000 years at ambient temperature) has been determined as a function of temperature; if the age of a fossil can be assessed by radiocarbon dating, then the average temperature to which the fossil has been subjected can be calculated from the L-isoleucine : D-allo-isoleucine ratio. That for a particular bone of age 40 000 years is between 0.42 and 0.46, suggesting that the average temperature during this period at the site of the fossil was 26.5°C (the average temperature in recent times is 28 °C).[208] Rates of racemization of amino-acids in modern bone samples are in the order aspartic acid > alanine ~ glutamic acid > isoleucine ~ leucine,[209] so that more accurate data should be obtainable for paleotemperature studies with aspartic acid; the rise in temperature during the Earth's last glaciation period was about 4 °C for the Mediterranean coast, and 5—6 °C in East Africa, based upon the amount of D-aspartic acid found in fossil bones from these regions.[210] Temperatures deduced are claimed to be reliable to within 1 °C, and,

[205] T. Fairwell, S. Ellis, and R. E. Lovins, *Analyt. Biochem.*, 1973, **53**, 115.
[206] J. A. Garbarino, J. Sierra, and R. Tapia, *J.C.S. Perkin I*, 1973, 1866.
[207] J. L. Morell and E. Gross, *J. Amer. Chem. Soc.*, 1973, **95**, 6480.
[208] J. L. Bada, R. Protsch, and R. A. Schroeder, *Nature*, 1973, **241**, 394.
[209] J. L. Bada and R. Protsch, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 1331.
[210] R. A. Schroeder and J. L. Bada, *Science*, 1973, **182**, 479.

although the 'right' answers are being derived from these studies, uncertainties concerning solid-state catalysis or inhibition of the racemization process may need to be considered.

Conversion of (+)-*R*-α-methylphenylalanine into its enantiomer has been achieved[211] *via* the *R*-isocyanide after conversion of the carboxy-group into $-CH_2OAc$; the *R*-isocyanide gives the *S*-cyanide with 75.6% retention when heated in diphenyl ether at 280 °C, and further elaboration gives the (−)-*S*-amino-acid.

**General Reactions.**—Pride of place should go to an efficient synthesis of optically active *N*-benzyloxycarbonyl-aziridin-2-ones from *N*-benzyloxycarbonyl-L-amino-acids, using phosgene, thionyl chloride, or phosphorus oxychloride in THF at −20 °C to −30 °C, with addition of triethylamine to maintain neutral conditions.[212] Some of the derivatives are crystalline; they may be used in peptide synthesis, aminolysis proceeding without racemization.[213] The other small-ring system of major importance is azetidin-2-one (β-lactam); the series is accessible through addition of *N*-benzyloxycarbonylglycyl chloride to imines in the presence of triethylamine,[214] or through a novel ring-contraction route (illustrated in Scheme 13 for the 4-methyl series obtainable from L-alanine ethyl ester).[215]




Reagents: i, $Bu^tO_2CCH_2CO_2H$–DCCI; ii, $Bu^tOK$; iii, heat in xylene, 1.5 h; iv, $MeSO_2N_3$–$NEt_3$, −10 °C; v, *hν*, $Bu^tO_2CNHNH_2$

**Scheme 13**

Reactions at the α-amino- or α-imino-group of representative compounds include *N*-nitrosation of L-proline and other imino-acids with nitrosyl tetrafluoroborate,[216] and conversion of phenylalanine into its α-diazo-ester

[211] M. Shibasaki, S. Terashima, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 552.
[212] M. Miyoshi, *Bull. Chem. Soc. Japan*, 1973, **46**, 212.
[213] M. Miyoshi, *Bull. Chem. Soc. Japan*, 1973, **46**, 1489.
[214] A. K. Bose, H. P. S. Chawla, B. Dayal, and M. S. Manhas, *Tetrahedron Letters*, 1973, 2503.
[215] G. Lowe and D. D. Ridley, *J.C.S. Perkin I*, 1973, 2024.
[216] H. T. Nagasawa, P. S. Fraser, and D. L. Yuzon, *J. Medicin. Chem.*, 1973, **16**, 583.

*en route* to *cis*- or *trans*-cinnamate esters, for which a stereoselective route has been demonstrated; treatment of the diazo-ester with a sodium alkoxide gives the *trans*-series whereas the *cis*-series is reached using boron trifluoride diethyletherate.[217] Silylurethanes $Me_3SiOCONH \cdot CHR^1CO_2R^2$ are obtained by reaction between an amino-acid ester and t-butyl trimethylsilyl carbonate;[218] the same reaction under forcing conditions gives corresponding trimethylsilyl esters.

Side-chain studies include reaction of alkylating agents with *N*-acetyl methylamides of amino-acids with side-chain nucleophilic centres,[219] and intramolecular reactions accompanying N-protection of 4-chloro- and 4-hydroxy-lysines.[220]

Significant observations have been made concerning the formation of peptide esters through the condensation of amino-acid esters in the presence of $Cu^{II}$ [221] or $Pt^{II}$.[222]

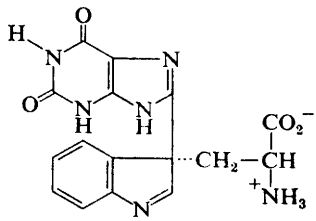
**Specific Reactions and Interactions.**—Discussion of side-chain protection and deprotection procedures with amino-acids is appropriate in this chapter where there is a broad significance in the chemistry involved. Removal of Boc groups with trifluoroacetic acid from protected peptides is commonly conducted in the presence of mercaptoethanol to prevent modification of tryptophan residues, but 1,2-ethanedithiol is superior for the purpose.[223] *S*-(*p*-Methoxybenzyl)-protected cysteine residues elsewhere in the protected peptide are unaffected by this Boc-removal procedure. *O*-(*o*-Bromobenzyloxycarbonyl) protection for tyrosine is recommended (removal by HF),[224] *O*-benzyl-protection being unsatisfactory since Boc-removal at the *N*-terminus can be accompanied by conversion of the tyrosine residue into 3-benzyl-tyrosine.[225] Blocking of the guanidino function by $N^{\delta},N^{\omega}$-bis(isobornyloxycarbonyl)ation is advocated when the arginine side-chain must be protected.[226]

A kinetic study of deuteriation at position 2 of the imidazole ring of histidine and related compounds, as models for the exchange behaviour in proteins, shows that the $NH_3^+$ group increases the rate of deuteriation three times compared with that for glycylhistidine, and seven times compared with the rate for histidine in alkaline solution (unprotonated $NH_2$).[227] Mono-*N*-hydroxymethylation of the imidazole ring of *N*-acetyl-

[217] N. Takamura, T. Mizoguchi, and S. Yamada, *Tetrahedron Letters*, 1973, 4267.
[218] Y. Yamamoto, D. S. Tarbell, J. R. Fehlner, and B. M. Pope, *J. Org. Chem.*, 1973, **38**, 2521.
[219] C. C. Price, H. Akimoto, and R. Ho, *J. Org. Chem.*, 1973, **38**, 1538.
[220] S. Clarke, R. C. Hider, and D. I. John, *J.C.S. Perkin I*, 1973, 230.
[221] S. Terashima, M. Wagatsuma, and S. Yamada, *Tetrahedron*, 1973, **29**, 1487, 1497.
[222] W. Beck, B. Purucker, and E. Strissel, *Chem. Ber.*, 1973, **106**, 1781.
[223] J. J. Sharp, A. B. Robinson, and M. D. Kamen, *J. Amer. Chem. Soc.*, 1973, **95**, 6097.
[224] D. Yamashiro and C. H. Li, *J. Org. Chem.*, 1973, **38**, 591.
[225] B. W. Erickson and R. B. Merrifield, *J. Amer. Chem. Soc.*, 1973, **95**, 3750.
[226] G. Jager and R. Geiger, *Annalen*, 1973, 1928.
[227] J. H. Bradbury, B. E. Chapman, and F. A. Pellegrino, *J. Amer. Chem. Soc.*, 1973, **95**, 6139.

histidine is brought about with formaldehyde in alkaline solution; further *N*-hydroxymethylation can occur in acidic solution.[228]

L-Tryptophan forms a complex with inosine, with equilibrium constant $10^3$ larger than that for other tryptophan nucleoside complexes;[229] L-tryptophan reacts with the powerful oncogen 3-acetoxy-xanthine to give the condensation product (39) and its C-3 epimer,[230] adding a further

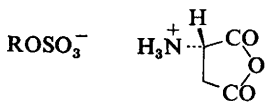


(39)

example to the list of compounds potentially capable of modifying this amino-acid irreversibly when it is a component of peptides and proteins. *trans*-4,5-Dehydro-lysine, prepared from ethyl *trans*-2-acetamido-2-ethoxycarbonyl-6-phthalimidohex-4-enoate, resists lactonization in acidic solution,[231] correcting a report that diastereoisomeric 4-hydroxy-DL-lysine lactones are formed during attempted synthesis.

Difficulties in the preparation of phenylthiohydantoins of β-hydroxy-α-amino-acids were noted in the early development of the Edman sequencing procedure; β-elimination during the preparation of methylthiohydantoins can be avoided by keeping the pH of solutions high.[232]

The latest improved route to L-aspartic acid anhydride uses concentrated sulphuric acid in ethyl acetate at room temperature during 48 h as a means of taking L-aspartic acid into solution, and is followed by treatment with an alcohol, ROH, then cyclization with acetic anhydride; the anhydride is isolated as its alkyl hydrogen sulphate salt (40).[233]

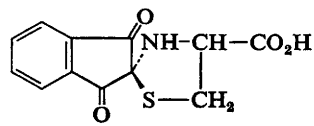


(40)

[228] P. Dunlop, M. A. Marini, H. M. Fales, E. Sokoloski, and C. J. Martin, *Bio-org. Chem.*, 1973, **2**, 235.
[229] I. Ibanez, M. Pieber, and J. Toha, *Z. Naturforsch.*, 1973, **28c**, 385.
[230] G. Stohrer, G. Salemnick, and G. B. Brown, *Biochemistry*, 1973, **12**, 5084.
[231] A. L. Davis, M. B. Cavitt, T. J. McCord, P. E. Vickrey, and W. Shive, *J. Amer. Chem. Soc.*, 1973, **95**, 6800.
[232] M. M. Amirkhanyan and V. M. Stepanov, *Zhur. obshchei Khim.*, 1973, **43**, 1630.
[233] Y. Ariyoshi, T. Yamatani, and Y. Adachi, *Bull. Chem. Soc. Japan*, 1973, **46**, 2611.

Sulphur functional groups in amino-acid side-chains have received attention. Oxidation of methionine to the sulphone with trichloroisocyanuric acid is rapid, but all other amino-acids are more or less rapidly degraded by this reagent.[234] Aqueous chlorine or bromine reacts with cysteine, cystine, alanine-3-sulphinic acid, and cystine *SS*-dioxide to give cysteic acid and cysteinylcysteic acid.[235] L-Methionine gives the L$^{\alpha}$-(*S*)-sulphoxide with an equimolar amount of $HAuCl_4$ within a few minutes;[236] the mechanism of this stereospecific oxidation involving $Au^{III} \rightarrow Au^{I}$ is not clear, but rapid formation of a methionine–$AuCl_3$ complex may be followed by stereospecific attack of a second molecule of methionine before oxidation. Hydrolysis rates of *NS*-diacetylcysteinamide and *N*-acetyl-*S*-benzoylcysteinamide are some twenty times faster than expected on the basis of p*K* values, and the rate for *N*-benzyloxycarbonyl-*S*-acetyl-L-cysteinyl-L-threonine ethyl ester is five to six times faster still, implying anchimeric assistance by functional groups neighbouring the thiolester grouping.[237] Electrochemical reduction of *S*-methyl-methionine gives mainly α-amino-butyric acid and methionine, with α-amino-γ-butyrolactone as minor product.[238]

The ninhydrin colour reaction is a reliable work-horse for amino-acid analysis, but there are still aspects for study, such as the dependence of colour formation upon hydrindantin concentration.[239] The hitherto accepted 1,4-thiazine structure for the cysteine–ninhydrin condensation product must now be replaced by the spiro-isomer (41);[240] analogous products are formed with penicillamine and cysteamine.



(41)

**Effects of Electromagnetic Radiation on Amino-acids.**—Recent work in this area concerns photochemical transformations of aromatic amino-acids, particularly tryptophan and tyrosine, the processes being relevant to possible mechanisms for the photoinactivation of enzymes. Photo-oxidation of tyrosine and histidine in the presence of tryptophan is shown to involve singlet oxygen, with formylkynurenin as the most probable photosensitizer.[241] Photo-oxidation of tryptophan is dependent upon

[234] M. Z. Atassi, *Tetrahedron Letters*, 1973, 4893.
[235] P. G. Gordon, *Austral. J. Chem.*, 1973, **26**, 1771.
[236] E. Bordignon, L. Cattalini, G. Natile, and A. Scatturin, *J.C.S. Chem. Comm.*, 1973, 878.
[237] D. G. Clark and E. H. Cordes, *J. Org. Chem.*, 1973, **38**, 270.
[238] T. Iwasaki, M. Miyoshi, M. Matsuoka, and K. Matsumoto, *Chem. and Ind.*, 1973, 1163.
[239] P. J. Lamothe and P. G. McCormick, *Analyt. Chem.*, 1973, **45**, 1906.
[240] G. Prota and E. Ponsiglione, *Tetrahedron*, 1973, **29**, 4271.
[241] P. Walraut, R. Santus, and M. Bazin, *Compt. rend.*, 1973, **276**, *C*, 149.

solvent, and the effects of co-solutes (3,4-benzpyrene, caffeine, sodium dodecyl sulphate) have been studied.[242] Photoconversion of tyrosine into bityrosine can be followed by fluorescence intensity changes at 400 nm, permitting the different mechanisms operating in acidic and alkaline solutions to be studied.[243] Data on quantum yields and fluorescence lifetimes of tyrosine and tryptophan in $^2H_2O$–$H_2O$ and glycerol–$H_2O$ have been reported;[244] a comparative study of the photochemistry of phenylalanine, tyrosine, and dopa shows phenylalanine to be the most easily photolysed in dilute aqueous solution at 254 nm, giving alanine, glycine, and four other amines in the absence of air, and giving tyrosine, and its *o*- and *m*-isomers, under aeration.[245] Tyrosine and dopa, under the latter conditions, are converted into melanin.[245]

Photodecarboxylation of *N*-phthaloyl amino-acids in acetone solution,[246] and of amino-acids themselves in the presence of transition-metal ions,[247] has been studied. The range of products (stereoisomers of propenyl sulphides) formed from *S*-(*cis*-1-propenyl)-L-cysteine under $\gamma$-irradiation in oxygen-free aqueous solutions[248] is different from that (numerous thiophens, aldehydes, thiols) formed under u.v.-irradiation,[249] although 1-propenylthiyl radicals are implicated in both processes.

## 6 Analytical Methods

As is customary in this Section, many of the reports are cited without discussion, since they derive from methodology established in recent years, which will be generally familiar to readers.

**Gas–Liquid Chromatography.**—The range of uses to which quantitative g.l.c. is now being applied illustrates the slowly evaporating conservatism which has prevented many analysts from considering anything but their ion-exchange amino-acid analyser for routine amino-acid assay. Comparisons of results from the two techniques are reported and the special potential of g.l.c. in the determination of enantiomeric composition of small samples is illustrated.

Volatile derivatives of amino-acids in current use are *N*-trifluoroacetyl methyl esters[250, 251] or n-butyl esters[6, 202, 252–254] following earliest prece-

[242] G. Reske and H. Bauer, *Z. Naturforsch.*, 1973, **28c**, 390.
[243] O. Shimizu, *Photochem. and Photobiol.*, 1973, **18**, 125.
[244] R. McGuire and I. Feldman, *Photochem. and Photobiol.*, 1973, **18**, 119.
[245] C. Hasselmann and G. Laustriat, *Photochem. and Photobiol.*, 1973, **17**, 275.
[246] Y. Sato, H. Nakai, T. Mizoguchi, M. Kawanishi, and Y. Kanaoka, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1164.
[247] R. Poupko, I. Rosenthal, and D. Elad, *Photochem. and Photobiol.*, 1973, **17**, 395.
[248] H. Nishimura and J. Mizutani, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 213.
[249] T. Hanzawa, H. Nishimura, and J. Mizutani, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2393; *Tetrahedron Letters*, 1973, 343.
[250] W. S. Gardner and G. F. Lee, *Environ. Sci. Technol.*, 1973, **7**, 719.
[251] A. J. Cliffe, N. J. Berridge, and D. R. Westgarth, *J. Chromatog.*, 1973, **78**, 333.
[252] C. W. Gehrke and H. Takeda, *J. Chromatog.*, 1973, **76**, 63.
[253] H. Hediger, R. L. Stevens, H. Brandenberger, and K. Schmid, *Biochem. J.*, 1973, **133**, 551.
[254] C. W. Gehrke and H. Takeda, *J. Chromatog.*, 1973, **76**, 77.

dent, though *N*-heptafluorobutyroyl n-propyl esters,[255] isoamyl esters,[256] and *N*-acetyl n-butyl esters [257] have their proponents. The twenty protein amino-acids can be separated on a single column with Apiezon M as stationary phase.[252] Use of 2.7M-HCl in n-butanol as an anhydrous reagent for esterification of amino-acids has been studied; it is the routine method as part of the derivatization procedure, but should not be conducted above 100 °C [258] since substantial amounts of water, chlorobutane, and dibutyl ether are formed in the reagent at higher temperatures. Pyrolysis of *N*-neopentylidene amino-acid trimethylanilinium salts in the injector port of the gas chromatograph gives volatile derivatives characteristic of the parent amino-acid;[259] Schiff bases of amino-acid esters with benzaldehyde or with pentane-2,4-dione have been proposed [260] for quantitative g.l.c. Acetylation of phenylthiohydantoins gives derivatives more suited to g.l.c. analysis,[261] while silylated thiohydantoins are also readily identified by g.l.c. and by mass spectometry.[262] Short glass capillary columns permit the separation of silylated methylthiohydantoins and phenylthiohydantoins, nineteen of twenty protein amino-acids being separated during 35 min in one pass when cysteine is *S*-methylated and arginine converted into ornithine in the sample preparation sequence.[263] The twentieth amino-acid, histidine, can be eluted from the column as its silylated *N*-methyl- or *N*-phenyl-thiohydantoin using a rather higher oven temperature.[263] Trimethylsilyl derivatives of glycine, lysine, and serine are suitable for g.l.c.–mass spectrometric analysis,[264] and comparison of results obtained by this technique with those of conventional amino-acid analysis for these amino-acids in Devonian black slate has been reported.[264]

Improvements in the g.l.c. technique have been proposed. Losses during sample preparation and transfer may be avoided using an aluminium or gold micro-reactor for all steps.[265] Problems with tryptophan have been discussed,[254] and qualitative use of the technique to identify asparagine, glutamine, and pyroglutamic acid in total enzymic hydrolysates, distinguishing them from their parent amino-acids by esterification rates, has been explored.[253] The sensitivity of the method (0.1 ng) [256] is well established, though progress still has to be made in terms of simpler, possibly automated, sample preparation procedures.

Separation of enantiomers of amino-acids as their *N*-trifluoroacetylisopropyl esters using *N*-trifluoroacetyl-L-α-aminobutyryl-L-α-aminobutyric

[255] J. Jonsson, J. Eyem, and J. Sjoquist, *Analyt. Biochem.*, 1973, **51**, 204.
[256] J. P. Zanetta and G. Vincendon, *J. Chromatog.*, 1973, **76**, 91.
[257] P. G. Vincent and J. Kirksey, *J. Assoc. Offic. Analyt. Chemists*, 1973, **56**, 158.
[258] J. P. Hardy, S. L. Kerrin, and S. L. Manatt, *J. Org. Chem.*, 1973, **38**, 4196.
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[260] P. W. D. Mitchell, *J. Chromatog.*, 1973, **76**, 236.
[261] A. S. Inglis and P. W. Nicholls, *J. Chromatog.*, 1973, **86**, 117.
[262] M. Rangarajan, R. E. Ardrey, and A. Darbre, *J. Chromatog.*, 1973, **87**, 499.
[263] J. Eyem and J. Sjoquist, *Analyt. Biochem.*, 1973, **52**, 255.
[264] W. Heller, W. A. Koenig, and K. Schmidt, *Chromatographia*, 1973, **6**, 327.
[265] B. Kolb and W. Hoser, *Chromatographia*, 1973, **6**, 28.

acid cyclohexyl ester as stationary phase has been demonstrated;[266] correspondingly substituted L-alanine, L-norvaline, or L-norleucine dipeptides were less satisfactory as optically active stationary phases. The first successful use of an optically active mesophase, smectic carbonyl bis-(D-leucine isopropyl ester) at 55—100 °C, for g.l.c. separation of enantiomers has been reported.[267] The alternative approach, conversion of the DL-amino-acid into a diastereoisomeric derivative, *e.g.* its *N*-trifluoroacetyl s-butyl ester,[268] has been further exploited in a novel quantitative analysis of the extent of degradation of solid amino-acid samples by photolysis, radiolysis, or electron bombardment; a known weight of one enantiomer is added and the mixture converted into a volatile diastereoisomer mixture for g.l.c. analysis.[269]

**Ion-exchange and Partition Chromatography.**—Progress continues towards automation [270–272] and computer-controlled data-acquisition [271] of the ion-exchange amino-acid analyser. Single-column sub-micro analysis of all protein amino-acids can be achieved with a pH-gradient technique,[272, 273] or by employing three sodium citrate buffers [274, 275] (*e.g.* [275] pH 5.25 for the third buffer) to give improved resolution of basic amino-acids. Lithium citrate buffers permit the separation of more than fifty amino-acids on a single column.[276] Modifications to standard techniques permit quantitative analysis of $N^{\epsilon}$-methyl-lysine [277] and other methylated basic amino-acids,[19] and permit simultaneous analysis of three samples in a little over 6 h.[278] A modified commercial amino-acid micro-analyser can be operated with picomole amounts of amino-acids.[279]

An amino-acid analyser based upon the fluorescamine detection method, which is two orders more sensitive than the ninhydrin colorimetric method,[280] has been developed. The procedure has been illustrated with one microgram of protein.[274, 280] The failure of imino-acids (prolines) to respond directly to the fluorescence-forming reaction can be circumvented by their oxidative decarboxylation into imines with halogenating

[266] W. Parr and P. Y. Howard, *Analyt. Chem.*, 1973, **45**, 711.
[267] C. H. Lochmuller and R. W. Souter, *J. Chromatog.*, 1973, **87**, 243.
[268] F. Raulin and B. N. Khare, *J. Chromatog.*, 1973, **75**, 13.
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[273] J. L. Young and M. Yamamoto, *J. Chromatog.*, 1973, **78**, 349.
[274] A. G. Georgiadis and J. W. Coffey, *Analyt. Biochem.*, 1973, **56**, 121.
[275] L. G. Gurtler, *J. Chromatog.*, 1973, **76**, 255.
[276] J. L. Young and M. Yamamoto, *J. Chromatog.*, 1973, **78**, 221.
[277] H. W. Lange, R. Lower, and K. Hempel, *J. Chromatog.*, 1973, **76**, 252.
[278] J. P. Ellis and J. B. Garcia, *J. Chromatog.*, 1973, **87**, 419.
[279] A. M. Gressner, *Analyt. Biochem.*, 1973, **56**, 532.
[280] S. Stein, P. Bohlen, J. Stone, W. Dairman, and S. Udenfriend, *Arch. Biochem, Biophys.*, 1973, **155**, 203.

agents;[281, 282] these give primary amines on hydrolysis, which are susceptible to the fluorescamine reaction. Thus, all natural amino-acids can now be analysed using the fluorimetric amino-acid analyser, and wide adoption of the technique can be expected. Modified fluorescence reactions are being considered; *e.g.* reaction of the amino-acid mixture with *o*-phthalaldehyde and 2-mercaptoethanol.[283]

A rapid ligand-exchange chromatographic method for the separation of amino-acids from peptides, employing Chelex 100, should be useful for studies of partial hydrolysates or for solving problems due to incomplete acid hydrolysis.[284] The effect which residual hydrochloric acid can have in modifying elution times and resolution of many amino-acids on ion-exchange columns has been demonstrated.[285]

Liquid chromatography of amino-acids in aqueous solution is feasible using poly(glycine) bonded to resin-coated glass beads, Porasil C, or Corasil II as stationary support.[286]

**Thin-layer and Paper Chromatography.**—The use of preparative t.l.c. on 1 mm cellulose layers for isolating amino-acids from mixtures is illustrated well for the isolation of cyclopent-2-en-1-yl glycine, a potent growth inhibitor from *Hydnocarpus anthelminthica*.[287]

A stringent test of analytical chromatographic methods is the separation of leucine and isoleucine. Ascending paper chromatography using n-butanol–acetic acid–water (4 : 1 : 5), the doyen of solvents for amino-acid paper chromatography, can separate these amino-acids if a temperature gradient of 5.88 °C $cm^{-1}$ is applied;[288] their dansyl derivatives can be resolved on silica gel layers by multiple development with $CHCl_3$–MeOH (95 : 5).[289] Chromatographic data for ion-exchange and paper chromatography of *N*-methylamino-acids have been reported.[290]

Phenylthiohydantoins can be separated by short-run two-dimensional t.l.c. on silica gel and detected on t.l.c. plates at $10^{-10}$ mol $l^{-1}$ levels.[291] Separations of histidine and arginine phenylthiohydantoins [292] and diphenylindonyl-substituted thiohydantoins [293] have been described. Dansylation of amino-acid mixtures, followed by two-dimensional polyamide t.l.c.

[281] M. Weigele, S. de Bernardo, and W. Leimgruber, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 352.
[282] A. M. Felix and G. Terkelsen, *Analyt. Biochem.*, 1973, **56**, 610; *Biochem. Biophys. Res. Comm.*, 1973, **50**, 352.
[283] M. Roth and A. Hampai, *J. Chromatog.*, 1973, **83**, 353.
[284] J. F. Bellinger and N. R. M. Buist, *J. Chromatog.*, 1973, **87**, 513.
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[287] F. Spener and M. Dieckhoff, *J. Chromatog. Sci.*, 1973, **11**, 661.
[288] C. Liteanu and A. Constantinescu, *Rev. Roumaine Chim.*, 1973, **18**, 155.
[289] R. S. Fager and C. B. Kutina, *J. Chromatog.*, 1973, **76**, 268.
[290] T. K. Audhya and D. W. Russell, *J. Chromatog.*, 1973, **84**, 361.
[291] M. C. Solal and J. L. Bernard, *J. Chromatog.*, 1973, **80**, 140.
[292] T. Inagami, *Analyt. Biochem.*, 1973, **52**, 318.
[293] C. P. Ivanov and I. N. Mancheva, *J. Chromatog.*, 1973, **75**, 129.

and elution and quantitation by fluorimetry and scintillation counting, is sensitive to $3 \times 10^{-12}$ mol $l^{-1}$ levels.[294]

**Other Analytical Methods.**—Fluorophotometric methods continue to show great promise, and recent papers, in addition to those cited in preceding paragraphs, cover highly fluorescent derivatives obtained from amino-acids by treatment with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole,[295] and with pyridoxal.[296] However, the latter technique is not significantly more sensitive than ninhydrin colorimetry, and is not suitable for assay of proline, hydroxyproline, or tryptophan.[296]

A kinetic method based upon the retardation by an amino-acid of the $Cu^{II}$-catalysed oxidation of catechol violet at pH 8.65 with hydrogen peroxide allows several amino-acids to be assayed at the 2 $\mu$mol level.[297] At the other extreme, 3—8 mg samples of amino-acids can be assayed by treatment with 2-nitrobenzenesulphenyl thiocyanate, followed by iodometric titration of the released thiocyanate ion.[298] A microbiological assay [299] and further studies of assays based on the potential developed in a cell with immobilized L-amino-acid oxidase electrodes [300] have been reported.

An isotope dilution principle is employed for the determination of amino-acids in biological samples, where the amino-acid to be determined competes with the added labelled amino-acid for binding to a tRNA, catalysed by an amino-acyl tRNA-synthetase.[301] Independent studies have been reported [302, 303] of a sensitive double-isotope derivative assay using $^{3}H$-labelled dansyl chloride and $^{14}C$-labelled amino-acids as internal standards, so that the $^{3}H : {}^{14}C$ ratio in the dansyl derivatives depends upon the ratio of added $^{14}C$-labelled amino-acid to the natural amino-acid concentration in the sample. The method needs no more than 20 pmol of amino-acid,[302] and has been used [303] to measure the release of amino-acids from microgram samples of brain tissue.

The most sensitive method yet reported appears to be the least likely to be routinely applied; tunnelling measurements of vibrational spectra of amino-acids give characteristic features with less than a monolayer of sample on an area 0.1—1.0 $mm^2$.[304]

[294] J. Airhart, S. Sibiga, H. Sanders, and E. A. Khairallah, *Analyt. Biochem.*, 1973, **53**, 132.
[295] R. S. Fager, C. B. Kutina, and E. W. Abrahamson, *Analyt. Biochem.*, 1973, **53**, 290.
[296] M. Maeda, A. Tsuji, S. Ganno, and Y. Onishi, *J. Chromatog.*, 1973, **77**, 434.
[297] T. J. Janjic and G. A. Milovanovic, *Analyt. Chem.*, 1973, **45**, 390.
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**Determination of Specific Amino-acids.**—Titration methods have been reported for glutamic acid [305] and cystine,[306] and fluorimetric assays for dopa [307] and phenylalanine.[308] The dopa assay (1 ng $ml^{-1}$) is one hundred times more sensitive than existing methods. Determinations of cysteine or cystine in intracellular fluids have been described,[309] and details given for microbiological assay of the antitumour agent L-alanosine;[310] L-canaline [311] and *O*-ureido-L-homoserine [312] have been studied from the aspects of isolation, synthesis, and assay.

[305] K. Bahadur and I. Saxena, *Microchem. J.*, 1973, **18**, 358.
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[312] G. A. Rosenthal, *Analyt. Biochem.*, 1973, **56**, 435.