1
Amino-acids

BY J. H. JONES

In one way or another, the whole of this Report is about the chemistry of amino-acid derivatives, but here we are concerned only with the amino-acids themselves. Emphasis is on α-amino-acids, and it is only for these that comprehensive coverage has been attempted. Furthermore, although many may think it retrogressive to draw demarcation lines between disciplines in these enlightened times, amino-acid biochemistry has been excluded. Amino-acid chemistry is under continuous scrutiny from every conceivable angle, and so papers on these compounds span a very diverse range of interests. The Reporter therefore makes no apology for the fact that this chapter is something of a miscellany.

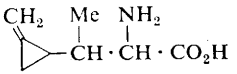
1 Naturally Occurring Amino-acids

A. Occurrence of Known Amino-acids.—Pipecolic acid (of unstated configuration, but presumably L) has been isolated from azuki beans (*Phaseolus angularis*) in a yield of about 0·05%.[1] *N*-Methyl-L-alanine, which has not been found in higher plants before, has been isolated from the leaves of *Dichapetalum cymosum*.[2] This plant is toxic and causes considerable cattle loss in southern Africa because it produces fluoroacetate. Young leaves contain remarkably large amounts of *N*-methyl-L-alanine (up to 5·6% by weight on a dry-weight basis) and it was suggested that the metabolism of the amino-acid and the toxin might be linked. *Erythro*-γ-methyl-L-glutamic acid has been isolated from seeds of *Lathyrus maritimus* and distinguished from the other possible methylglutamic acids by nuclear magnetic resonance (n.m.r.) spectrometry: chromatographic evidence indicates that this amino-acid also occurs in other *Lathyrus* species.[3] N^{ϵ}-Trimethyl-L-lysine has been isolated from seeds of *Reseda luteola*[4] and from chicken erythrocyte histones, where it occurs together with N^{ϵ}-methyl- and N^{ϵ}-dimethyl-L-lysine.[5,6] 6-Hydroxykynurenic acid has been obtained from tobacco leaves,[7] thus providing the first example of the occurrence of a kynurenic

[1] S. Hatanaka, *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, 1968, **17**, 219.
[2] J. N. Eloff and N. Grobbelaar, *J. S. African. Chem. Inst.*, 1967, **20**, 190.
[3] J. Przybylska and F. M. Strong, *Phytochemistry*, 1968, **7**, 471.
[4] P. O. Larsen, *Acta Chem. Scand.*, 1968, **22**, 1369.
[5] K. Hempel, H. W. Lange, and L. Birkhofer, *Z. Physiol. Chem.*, 1968, **349**, 603.
[6] K. Hempel, H. W. Lange, and L. Birkhofer, *Naturwiss.*, 1968, **55**, 37.
[7] P. K. Macnicol, *Biochem. J.*, 1968, **107**, 473.

acid derivative in plants. A survey of the distribution of fifteen non-protein amino-acids in about forty species of the genus *Acacia* shows that members of the Gummiferae series can be distinguished by their amino-acid content: they alone contain *N*-acetyldjenkolic acid, and they lack several amino-acids which are widely distributed among other *Acacia* species.[8] The possibility that the toxicity of various species of *Crotalaria* may not be due solely to the presence of pyrrolizidine alkaloids in these plants has been demonstrated by the isolation of the neurotoxin α-amino-β-oxalylaminopropionic acid from seeds of *C. incana* and *C. mucronata*.[9] *Trans*-3-hydroxy-L-proline has been isolated from seeds and vegetative tissue of *Delonix regia*, where it is a major component of the free amino-acid pool.[10]

B. New Naturally Occurring Amino-acids.—A number of new natural amino-acids have been characterised during the year: these are listed at the end of this section, together with their sources. Those whose structure has been confirmed by synthesis are included in the list of newly synthesised amino-acids in section 2. The increasing usefulness of physical methods for the characterisation of amino-acids is apparent: n.m.r. and mass spectrometry (applications of mass spectrometry in amino-acid and peptide chemistry have been reviewed[11]) have been particularly valuable. Especially noteworthy is the characterisation of the novel amino-acid (1) by spectroscopic methods.[12]



(1)

C. A List of New Naturally Occurring Amino-acids.—

Amino-acid	*Source*	*Ref.*
L-*N*-(3-amino-3-carboxypropyl)-β-carboxypyridinium betaine. (Nicotianine)	Tobacco leaves	13, 14
L-α-amino-ε-amidinocaproic acid. (Indospicine)	*Indigofera spicata*	15
β-(4-hydroxybenzothiazol-6-yl)alanine	Gallopheomelanins from chicken feathers	16
β-(2-methyl-4-hydroxybenzothiazol-6-yl)-alanine	Gallopheomelanins from chicken feathers	16
mixed disulphide of β-mercaptolactic acid and cysteine	Urine of mentally defective patient	17

[8] A. S. Seneviratne and L. Fowden, *Phytochemistry*, 1968, **7**, 1039.
[9] E. A. Bell, *Nature*, 1968, **218**, 197.
[10] M. L. Sung and L. Fowden, *Phytochemistry*, 1968, **7**, 2061.
[11] J. H. Jones, *Quart. Rev.*, 1968, **22**, 302.
[12] D. S. Millington and R. C. Sheppard, *Phytochemistry*, 1968, **7**, 1027.
[13] M. Noguchi, H. Sakuma, and E. Tamaki, *Arch. Biochem. Biophys.*, 1968, **125**, 1017.
[14] M. Noguchi, H. Sakuma, and E. Tamaki, *Phytochemistry*, 1968, **7**, 1861.
[15] M. P. Hegarty and A. W. Pound, *Nature*, 1968, **217**, 354.
[16] L. Minale, E. Fattorusso, G. Cimino, S. de Stefano, and R. A. Nicolaus, *Gazzetta*, 1967, **97**, 1636.
[17] M. Ampola, E. M. Bixby, J. C. Crawhall, M. L. Efron, R. Parker, W. Sneddon, and E. P. Young, *Biochem. J.*, 1968, **107**, 16P.

Amino-acid	*Source*	*Ref.*
N^{ϵ}-(indole-3-acetyl)-L-lysine	*Pseudomonas savastanoi*	18
p-hydroxymethyl-L-phenylalanine	*Escherichia coli*	19
O-ethyl-L-homoserine	*Corynebacterium ethanolaminophilum*	20
O-n-propyl-L-homoserine	*Corynebacterium ethanolaminophilum*	20
O-n-butyl-L-homoserine	*Corynebacterium ethanolaminophilum*	20
L-5-methyl-2-aminohex-4-enoic acid	*Leucocortinarius bulbiger*	21
2-amino-4-methylhex-4-enoic acid	*Aesculus californicus*	22
2-amino-4-methylhexanoic acid	*Aesculus californicus*	22
2-amino-4-methyl-6-hydroxyhex-4-enoic acid	*Aesculus californicus*	12, 22
β-(methylenecyclopropyl)-β-methylalanine	*Aesculus californicus*	12, 22
β-acetamido-L-alanine	*Acacia armata*	8
α-(3-hydroxyphenyl)glycine	*Euphorbia helioscopa*	23
α-(3,5-dihydroxyphenyl)glycine	*Euphorbia helioscopa*	23
1-methyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid	*Euphorbia myrsinites*	24
L-*cis*(?)-2-amino-3-hydroxymethylpent-3-enoic acid	*Bankera fulgineoalba*	25
L-*cis*(?)-2-amino-3-formylpent-3-enoic acid	*Bankera fulgineoalba*	25

2 Chemical Synthesis and Resolution of Amino-acids

The majority of new syntheses reported this year were performed by variations of well-established routes or involved elaboration of available amino-acids. Therefore, only those syntheses which have points of particular interest will be discussed in the following outline, the remainder being merely mentioned or incorporated into the appendix to this section (see p. 13).

A. Protein Amino-acids.—New syntheses have been reported for DL-lysine,[26] DL-histidine,[27] DL-cystine,[28] and DL-tryptophan,[29] and reactions for the conversion of serine to DL-cystine,[30] L-tyrosine to L-phenylalanine,[31] and L-ornithine to L-proline [32] have been described. The resolution of racemic

[18] O. Hutzinger and T. Kosuge, *Biochemistry*, 1968, 7, 601.
[19] N. H. Sloane and S. C. Smith, *Biochim. Biophys. Acta*, 1968, **158**, 394.
[20] Y. Murooka and T. Harada, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 1035.
[21] G. Dardenne, J. Casimar, and J. Jadot, *Phytochemistry*, 1968, 7, 1401.
[22] L. Fowden and A. Smith, *Phytochemistry*, 1968, 7, 809.
[23] P. Müller and H. R. Schütte, *Z. Naturforsch.*, 1968, **23b**, 659.
[24] P. Müller and H. R. Schütte, *Z. Naturforsch.*, 1968, **23b**, 491.
[25] R. R. Doyle and B. Levenberg, *Biochemistry*, 1968, 7, 2457.
[26] S. Motoki, S. Satsumabayashi, and F. Minemura, *J. Org. Chem.*, 1968, **33**, 3667.
[27] J. Fernandez-Bolanos and D. Martinez-Ruiz, *Anales real Soc. espaň. Fis. Quim.*, 1968, **64**, 423.
[28] P. Rambacher, *Chem. Ber.*, 1968, **101**, 3433.
[29] N. I. Aboskalova, A. S. Polyanskala, and V. V. Perekalin, *Doklady Akad. Nauk S.S.S.R.*, 1967, **176**, 829.
[30] P. Rambacher, *Chem. Ber.*, 1968, **101**, 2595.
[31] T. Kishi, Y. Kato, and M. Tanaka, *J. Agric. Chem. Soc. Japan*, 1968, **42**, 238.
[32] S. Ohshiro, K. Kuroda, and T. Fujita, *J. Pharm. Soc. Japan.*, 1967, **87**, 1184.

glutamic acid [33-39] and alanine [40] by preferential crystallisation has been studied in detail by Japanese workers.

B. Other α-Amino-acids.—A very brief preliminary description of a new general route to α-amino-acids has appeared:[41] treatment of an *NN*-bis(trimethylsilyl)glycine ester (2) with base followed by reaction with an alkyl halide results in alkylation of the α-carbon atom giving (3) which is very easily hydrolysed to an α-amino-ester with dilute acid.

$$[(Me)_3Si]_2N \cdot CH_2 \cdot CO_2R^1 \qquad [(Me)_3Si]_2N \cdot \underset{\displaystyle R^2}{\underset{|}{CH}} \cdot CO_2R^1$$

(2) (3)

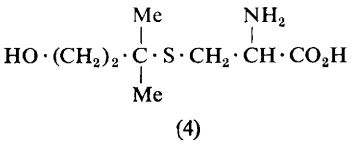
A convenient synthesis of DL-α-aminosuberic acid (by the diethyl acetamidomalonate route) has been reported:[42] resolution can be achieved enzymically or, preferably, by the method of Vogler *et al.*[43] using optically active tyrosine hydrazide. An improved method for the conversion of tyrosine (D and L) to 3,5-dichlorotyrosine has been described.[44] Synthesis of α,α′-diaminopimelic acid by the method of Work *et al.*[45] gives an equimolecular mixture of racemic and *meso* diaminodiacids: a simpler method for separating the isomers and resolving the racemate has been published.[46] DL-α-Acetamido-β-methylaminopropionic acid has been obtained by an improved procedure.[47] Stereospecific enzymic deacylation gave L-α-amino-β-methylaminopropionic acid which had the same specific rotation as a sample of the same amino-acid isolated in 1967 from *Cycas circinalis*, thus confirming the L configuration of the latter.

L-Felinine (4) can be prepared by acid-catalysed *S*-alkylation of L-cysteine with 2-methylbut-1-ene-4-ol or 2-methylbut-2-ene-4-ol.[48]

Birch reduction of phenylalanine gives 3,6-dihydrophenylalanine (5), which can be partially hydrogenated to 3,4,5,6-tetrahydrophenylalanine (6). The position of the double bond in (6) was confirmed by isolation of the

[33] T. Watanabe and G. Noyori, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1967, **70**, 2164.
[34] T. Watanabe and G. Noyori, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1967, **70**, 2167.
[35] T. Watanabe, H. Kurokawa, T. Koga, Y. Kawauchi, and G. Noyori, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1967, **70**, 2170.
[36] T. Watanabe and G. Noyori, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1967, **70**, 2174.
[37] T. Watanabe and G. Noyori, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1968, **71**, 676.
[38] N. Mizoguchi, *J. Agric. Chem. Soc. Japan.* 1967, **41**, 607.
[39] N. Mizoguchi, *J. Agric. Chem. Soc. Japan*, 1967, **41**, 616.
[40] I. Chibata, S. Yamada, M. Yamamoto, and M. Wada, *Experientia*, 1968, **24**, 638.
[41] K. Rühlmann and G. Kuhrt, *Angew. Chem. Internat. Edn.*, 1968, **7**, 809.
[42] S. Hase, R. Kivoi, and S. Sakakibara, *Bull. Chem. Soc. Japan*, 1968, **41**, 1266.
[43] K. Vogler and P. Lanz, *Helv. Chim. Acta*, 1966, **49**, 1348.
[44] K. R. Brody and R. P. Spencer, *J. Org. Chem.*, 1968, **33**, 1665.
[45] E. Work, S. M. Birnbaum, M. Winitz, R. J. Koegel, and J. P. Greenstein, *J. Amer Chem. Soc.*, 1957, **79**, 648.
[46] J. van Heijenoort and E. Bricas, *Bull. Soc. chim. France*, 1968, 2828.
[47] A. Vega, E. A. Bell, and P. B. Nunn, *Phytochemistry*, 1968, **7**, 1885.
[48] A. Schoeberl, J. Borchers, and D. Hantzsch, *Chem. Ber.*, 1968, **101**, 373.

lactone (7) after treatment with hydrochloric acid, and hydrolysis of (7) gave the new amino-acid (8).[49]



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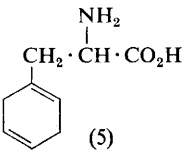

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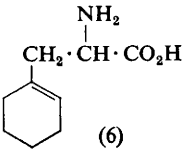

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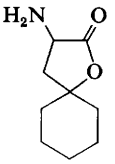

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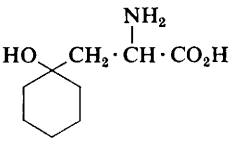

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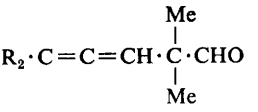
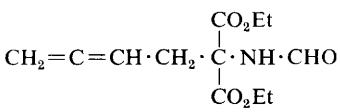


(9)

Allenic aldehydes such as (9) which are fully substituted at the 2-position give good yields in the Strecker synthesis, but poor yields of allenic amino-acids are obtained if this position is unsubstituted.[50a] The reaction of allenic bromides with diethyl formamidomalonate is a more general route to such amino-acids:[50b] the reaction is applicable to 1-bromoalka-1,2-, -2,3-, and -3,4-dienes. This reaction was used for the preparation of (10), which was treated with di-iodomethane and a zinc–copper couple followed



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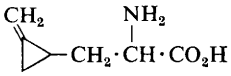


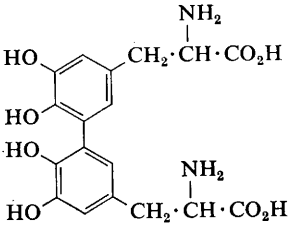
(11)

by hydrolysis and decarboxylation to yield (±)hypoglycin A (11), a naturally occurring hypoglycaemic amino-acid.[51] The final decarboxylation in this synthesis was stereoselective, and only one of the two racemates of (11) was obtained: this proved to be the required (±)amino-acid. The stereoselectivity was attributed to thermodynamic control, and models showed that the most stable racemate should consist of the (2*S* : 4*S*) and (2*R* : 4*R*) diastereoisomers. Since the susceptibility of natural hypoglycin A to enzymic oxidation had already established the configuration at the α-carbon as (*S*) it was predicted that the natural amino-acid would be found to have the absolute stereochemistry (2*S* : 4*S*), and this was confirmed by chemical correlation (see p. 15). The formamidomalonate route is also suitable for the preparation of aminoenynoic acids: thus alkylation of

[49] M. L. Snow, C. Lauinger, and C. Ressler, *J. Org. Chem.*, 1968, **33**, 1774.
[50a] D. K. Black and S. R. Landor, *J. Chem. Soc.* (*C*), 1968, 281. [b] D. K. Black and S. R. Landor, *J. Chem. Soc.* (*C*), 1968, 283.
[51] D. K. Black and S. R. Landor, *J. Chem. Soc.* (*C*), 1968, 288.

diethyl formamidomalonate with 5-bromopent-3-en-1-yne followed by hydrolysis and decarboxylation gave 2-aminohept-4-en-6-ynoic acid in good yield.[50b]

The diaminodiacid (12), which is one of the possible products of oxidative dimerization of 3,5-dihydroxyphenylalanine (DOPA), has been synthesised by the oxazolone route from the dialdehyde (13).[52] This compound ('DOPA dimer') was shown to polymerise in the presence of tyrosinase and oxygen at the same rate as DOPA itself, which is consistent with the possibility of (12) as an intermediate in the melanogenesis of DOPA.



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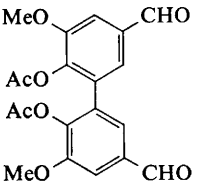

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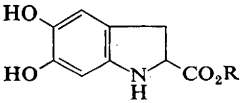
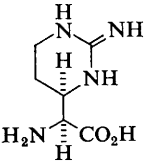


(14) R = Me
(15) R = H



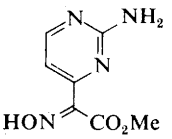
(16)

Oxidative cyclisation of DOPA methyl ester with ferricyanide followed by dithionite reduction gives (14) which can be converted to 'cycloDOPA' (15) by anaerobic hydrolysis after acetylation.[53]

DL-Capreomycidine (16), a guanidino-amino-acid obtained from acid hydrolysates of antibiotics of the capreomycin group, has been synthesised by catalytic reduction of the oxime (17) followed by saponification and separation of the mixture of diastereoisomers.[54]

C. α-Dialkyl-α-amino-acids.—The hydantoin route is in frequent use for the synthesis of α-dialkyl-α-amino-acids but difficulties due to the resistance of 5,5-disubstituted hydantoins to hydrolysis are sometimes encountered. It has been reported [55] that this difficulty can be circumvented by conversion of such hydantoins to their 3-tosyl derivatives (18). Alkaline hydrolysis (dilute sodium hydroxide) of (18) gives hydantoic acid derivatives (19) which

[52] Y. Omote, Y. Fujinuma, and N. Sugiyama, *Chem. Comm.*, 1968, 190.
[53] H. Wyler and J. Chiovini, *Helv. Chim. Acta.*, 1968, **51**, 1476.
[54] B. W. Bycroft, D. Cameron, L. R. Croft, and A. W. Johnson, *Chem. Comm.*, 1968, 1301.
[55] K. Hiroi, K. Achiwa, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 444.



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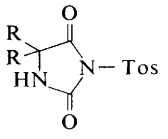

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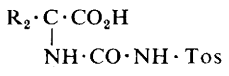
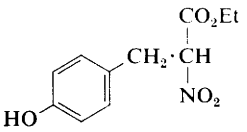


(19)

can be hydrolysed further to amino-acids with dilute hydrochloric acid. Although developed primarily for α-dialkyl-α-amino-acids, this method of hydrolysing hydantoins under mild conditions may find application in the synthesis of amino-acids which would not survive the usual vigorous hydrolysis conditions.

A new synthesis of DL-α-methyltyrosine has been described:[56] *NN*-dimethyl-*p*-hydroxybenzylamine reacts with ethyl α-nitropropionate in the presence of a catalytic amount of sodium hydride *via* a quinone methide intermediate to give (20), which can be reduced and hydrolysed to DL-α-



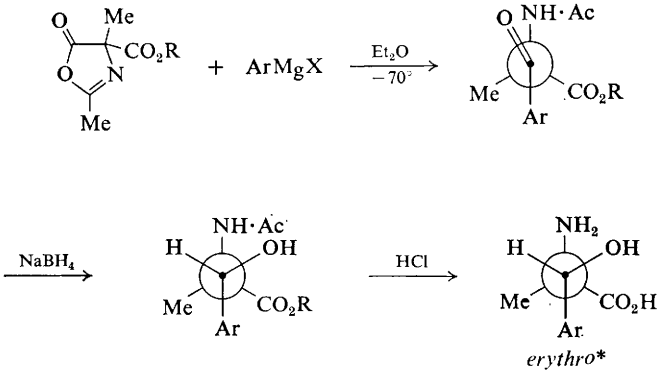
(20)

methyltyrosine. The resolution of α-methyl-α-amino-acids is a wasteful process if only one of the isomers is required, because the lack of an α-hydrogen atom prohibits racemisation and recycling of the isomer which is not required. This difficulty is avoided if resolution is performed at an earlier stage in the synthesis, and an example of this approach is provided by a new route to L-α-methylDOPA (α-methyl-3,4,-dihydroxy-L-phenylalanine).[57] The synthesis was performed by the Strecker method, with resolution at the aminonitrile stage: as the aminonitrile is formed in an equilibrium reaction, it is easily racemised, and it was therefore possible to recycle the D-aminonitrile.

The preparation of some α-methylserines is discussed in the next section.

D. Amino-acids with Aliphatic Hydroxyl Groups in the Side Chain.—A new synthesis[58] of β-aryl-α-methylserines is shown in Scheme 1. The synthesis comprises reaction of an aryl Grignard reagent with a 4-carboalkoxy-2,4-dimethyloxazol-5-one at low temperatures, followed by reduction with sodium borohydride and hydrolysis. This gives a mixture of diastereoisomers in which the *erythro* form predominates. The interconversion of the *erythro* and *threo* series is easily accomplished with thionyl chloride.[59]

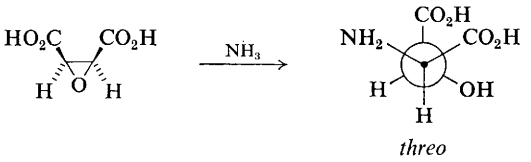
[56] W. S. Saari, *J. Org. Chem.*, 1967, **32**, 4074.
[57] D. F. Reinhold, R. A. Firestone, W. A. Gaines, J. M. Chemerda, and M. Sletzinger, *J. Org. Chem.*, 1968, **33**, 1209.
[58] S. H. Pines, S. Karady, and M. Sletzinger, *J. Org. Chem.*, 1968, **33**, 1758.
[59] S. H. Pines, S. Karady, M. A. Kozlowski, and M. Sletzinger, *J. Org. Chem.*, 1968, **33**, 1762.



* Some *threo* amino-acid is also formed.

Scheme 1

An attempt [60] to synthesise γ-hydroxyarginine by a multistage alternative to the direct preparation [61] from γ-hydroxyornithine has so far met with little success. Ammonolysis of *cis*-epoxysuccinic acid gives exclusively *threo*-β-hydroxy-DL-aspartic acid [62] (Scheme 2) but the *trans* epoxide was



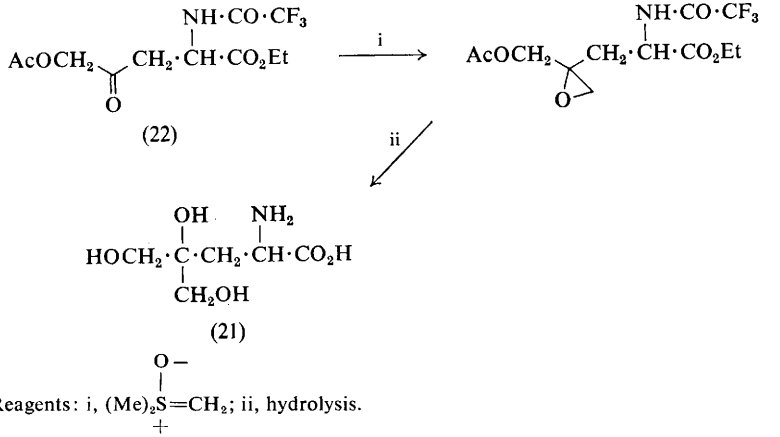
Scheme 2

found to give the *threo* and *erythro* isomers in about 1 : 2 proportions.[62] This last finding is surprising in view of the fact that the reaction of benzylamine with *trans*-epoxysuccinic acid is stereospecific,[63, 64] but it was admitted that the *trans*-epoxide used in the ammonolysis studies may have contained some *cis* isomer. *N*-Benzyl-*threo*-β-hydroxy-DL-aspartic acid is very conveniently (and almost quantitatively) resolved with ephedrine,[65] and hydrogenolysis after resolution gave L- and D-hydroxy-amino-acids of specific rotation slightly greater than previously reported. Optically pure *erythro*-β-hydroxy-D-aspartic acid has been obtained from optically pure *trans*-L-epoxysuccinic acid.[64] *Threo*- and *erythro*-β-hydroxy-DL-isoleucine

[60] B. A. Santa Rossa and T. Viswanatha, *Canad. J. Biochem.*, 1968, **46**, 725.
[61] Y. Fujita, *Bull. Chem. Soc. Japan.*, 1959, **32**, 439.
[62] H. Okai, N. Imamura, and N. Izumiya, *Bull. Chem. Soc. Japan*, 1967, **40**, 2154.
[63] Y. Liwschitz, Y. Rabinsohn, and A. Haber, *J. Chem. Soc.*, 1962, 3589.
[64] J. Oh-Hashi and K. Harada, *Bull. Chem. Soc. Japan*, 1967, **40**, 2977.
[65] Y. Liwschitz, Y. Edlitz-Pfeffermann, and A. Singermann, *J. Chem. Soc.* (*C*), 1967, 2104.

have been synthesised by stereospecific routes from *cis*- and *trans*-3-methylpent-2-enoic acids respectively: the diastereoisomers can be distinguished by n.m.r.[66]

The recent isolation[67] of γ,δ,δ'-trihydroxyleucine (21) has prompted the synthesis of the L-isomer[68] by epoxidation of (22) (which had earlier been synthesised[69] starting with L-asparagine), followed by hydrolysis (Scheme 3).



(22)

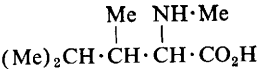
(21)

Reagents: i, $(Me)_2S{=}CH_2$; ii, hydrolysis.

Scheme 3

Dihydroxyprolines are discussed in the next section.

E. *N*-Substituted Amino-acids.—N^{α}-Methyl-L-histidine can be prepared[70] from L-histidine by a modification of the procedure of Quitt *et al.*[71] (formation of the N^{α}-benzyl derivative by reduction of the Schiff base, N^{α}-methylation by means of a Leuckart reaction, and finally removal of the N^{α}-benzyl group by hydrogenolysis). $N^{\alpha}N^{\alpha}$-Dimethyl-L-histidine has been prepared[70] for the first time by subjection of L-histidine to catalytic hydrogenation conditions in the presence of formic acid. A new synthesis[72] of *N*,β-dimethylleucine (23) has made use of the *N*-methylation procedure

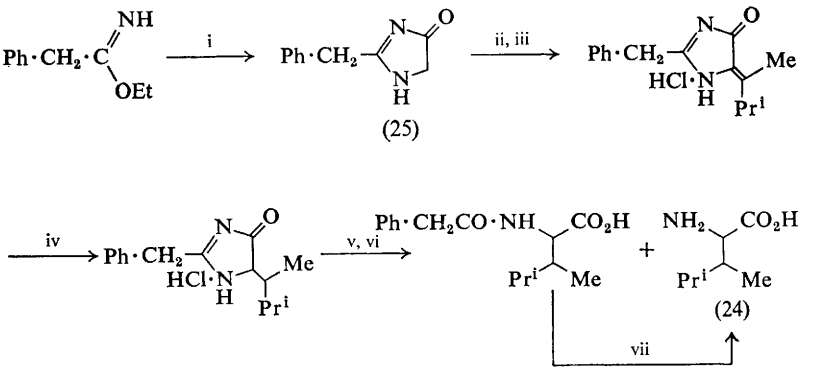


(23)

of Quitt *et al.*[71] The synthesis of β-methylleucine (24) from the imidazolone

[66] T. A. Dobson and L. C. Vining, *Canad. J. Chem.*, 1968, **46**, 3007.
[67] U. Gebert, T. Wieland, and H. Boehringer, *Annalen*, 1967, **705**, 227.
[68] F. Weygand and F. Mayer, *Chem. Ber.*, 1968, **101**, 2065.
[69] F. Weygand, P. Klinke, and I. Eigen, *Chem. Ber.*, 1957, **90**, 1896.
[70] V. N. Reinhold, Y. Ishikawa, and D. B. Melville, *J. Medicin. Chem.*, 1968, **11**, 258.
[71] P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, 1963, **46**, 327.
[72] H. Kotake, T. Saito, and K. Okubo, *Tetrahedron Letters*, 1968, **24**, 2015.

(25) (which is readily available [73]) shown in Scheme 4 gave both diastereoisomers, which were separated by fractional crystallisation and *N*-methylated.

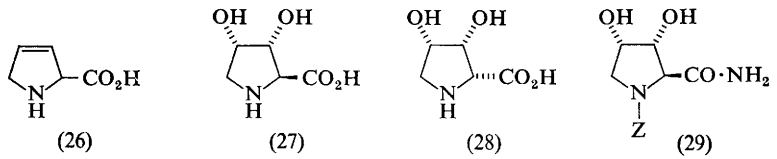


Reagents: i, Gly·OEt; ii, Me·CO·Pr^i; iii, HCl—EtOH; iv, H_2—PtO_2; v, $Ba(OH)_2$; vi, Et_3N; vii, HCl

Scheme 4

These two diastereoisomers of (23) were identified as *N*,γ-dimethylisoleucine and *N*,γ-dimethylalloisoleucine by spectroscopic examination, and the latter was resolved by way of its benzyloxycarbonyl derivative with ephedrine.[72]

Treatment of 3,4-dehydro-DL-proline (26) with alkaline permanganate gives an equimolecular mixture of 2,3-*trans*-3,4-*cis*-3,4-dihydroxy-DL-proline (27) and the 2,3-*cis*-isomer (28).[74] Separation of these isomers was



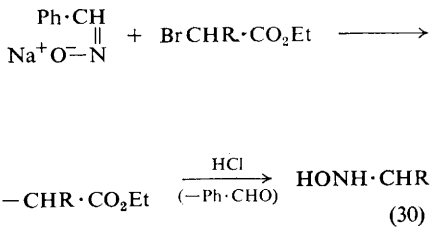
(26) (27) (28) (29)

difficult but was achieved by fractional crystallisation of their copper salts, and the two new amino-acids were characterised. When *N*-substituted 3,4-dehydroprolines are treated with osmium tetroxide, however, glycolation occurs exclusively from the less hindered side: *e.g.* benzyloxycarbonyl-3,4-dehydroprolineamide gave a single glycol (29), which could be converted to (27) by hydrogenation and hydrolysis, also in good yield.

N-Hydroxy-α-amino-acids (30) can be synthesised by a number of routes which were summarised in 1967,[75] but the most general route is *via*

[73] H. Lehr, S. Karlan, and M. W. Goldberg, *J. Amer. Chem. Soc.*, 1953, **75**, 3640.
[74] C. B. Hudson, A. V. Robertson, and W. R. J. Simpson, *Austral. J. Chem.*, 1968, **21**, 769.
[75] E. Buehler and G. B. Brown, *J. Org. Chem.*, 1967, **32**, 265.

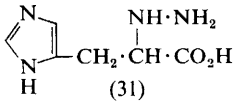
N-alkylation of the *anti*-benzaldoxime anion with an α-bromoester followed by hydrolysis of the resulting nitrone,[75, 76] as shown in Scheme 5. The



Scheme 5

syn-benzaldoxime anion undergoes alkylation on oxygen.[75] Hydrazine can also be used for cleavage of the nitrone.[76] This general route has been used for the synthesis of N^{α}-hydroxy-DL-asparagine,[77] *N*-hydroxy-[α-^{14}C]-DL-phenylalanine,[78] and also some *N*-hydroxy-β-amino-acids.[79]

The L, D, and DL forms of the *N*-amino derivative (31) of histidine have



(31)

been prepared by reaction of hydrazine with the chloroacids obtained from D, L, and DL-histidine respectively by deamination in concentrated hydrochloric acid.[80]

F. β-Amino-acids.—A large number of *N*-benzoyl-β-amino-acids have been prepared by means of a Ritter reaction (treatment with acetonitrile and sulphuric acid) starting with 3-hydroxypropanoic esters.[81] Aminopivalic acid is produced in poor yield when hydroxypivalic acid is catalytically hydrogenated in aqueous ammonia at high temperature and pressure.[82]

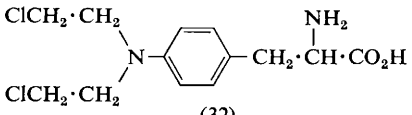
G. Labelled Amino-acids.—A short route to [1-^{14}C]-DL-arginine involving a Strecker synthesis with γ-guanidinobutyraldehyde has been reported: the synthesis uses radioactive intermediates only in the last two stages.[83] Alternative syntheses of [γ-^{14}C]-DL-aspartic and [δ-^{14}C]-DL-glutamic acids [84] have been claimed to be more convenient and less expensive than

[76] E. Bellasio, F. Parravicini, T. La Noce, and E. Testa, *Ann. Chim.* (*Italy*), 1968, **58**, 407.
[77] E. Falco and G. B. Brown, *J. Medicin. Chem.*, 1968, **11**, 142.
[78] H. Kindl and E. W. Underhill, *Phytochemistry*, 1968, **7**, 745.
[79] E. Ballasio, F. Parravicini, A. Vigevani, and E. Testa, *Gazzetta*, 1968, **98**, 1014.
[80] M. Sletzinger, R. A. Firestone, D. F. Reinhold, C. S. Rooney, and W. H. Nicholson, *J. Medicin. Chem.*, 1968, **11**, 261.
[81] C. Ivanov and A. Dobrev, *Monatsh.*, 1967, **98**, 2001.
[82] D. H. Johnson, *J. Chem. Soc.* (*C*), 1968, 126.
[83] L. Pichat, J. -P. Guermont, and P. N. Liem, *J. Labelled Compounds*, 1968, **4**, 251.
[84] R. J. Correla, C. P. Arciprete, and A. E. A. Mitta, *Anales Asoc. quim. argentina*, 1967, **55**, 173.

previous methods. Synthesis of isoleucine from [$^{14}C_4$]-2-bromobutane by the acetamidomalonate route gave [$\beta,\gamma,\gamma',\delta$-^{14}C]-DL-isoleucine with a completely labelled side-chain, and this was obtained free of the allo-isomer by preparative paper chromatography.[85] The methods previously described for the 'cold' amino-acids have been used for the preparation of [α-^{14}C]-β-(1-pyrazolyl)-DL-alanine, [α-^{14}C]-β-(3-pyrazolyl)-DL-alanine, and [α-^{14}C]-β-(2-furyl)-DL-alanine.[86] A rapid and simple enzymic method for the resolution of [ε-^{14}C]-α-aminoadipic acid has been briefly described.[87] Syntheses of [α-^{14}C]- and [^{15}N]-DL-α-allylglycine and also [α-^{14}C]- and [^{15}N]-DL-homomethionine have been reported.[88] The preparation of [γ-^{14}C]-γ-aminobutyric acid (starting with radioactive cyanide) and its conversion to [4-^{14}C]-DL-azetidine-2-carboxylic acid have been published.[89]

Iodine monochloride (liberated *in situ* by the action of chloramine-T on radioactive iodide solutions) has been used in the preparation of [^{131}I]-[90, 91] and [^{125}I]-[90] 3-iodo-L-tyrosine and 3,5-di-iodo-L-tyrosine. The iodinated tyrosines were obtained with high specific activities: *e.g.* [^{131}I]-3-iodo-L-tyrosine prepared by this method had a specific activity of *ca.* 2C/μmole.[90] The chemical stability of radioactive iodotyrosines has been studied under a variety of experimental conditions under which they might be used as tracers:[91] they are stable only in the dark or in red light, and in the presence of traces of cupric ions (which might, for example, be present in chromatographic materials) they undergo rapid decomposition, even in the dark. Details of simplified small-scale preparations of [^{131}I]-labelled 3,3′,5-tri-iodo-L-thyronine [92, 93] and L-thyroxine [92] have been published, and the optimal conditions for the labelling of 3,3′,5-tri- and 3,3′,5,5′-tetra-iodo-L-thyronine by exchange have been studied.[93]

[$^2H_5$]-L-Phenyl-[α,β,β-$^2H_3$]-alanine has been synthesised in 18% overall yield from [$^2H_6$]-benzene by the oxazolone route: resolution was achieved *via* the *N*-acetyl derivative using renal acylase.[94] Melphalan (32) with



(32)

tritium in the aromatic ring has been obtained by a synthesis involving iodination and catalytic reduction with tritium.[95] Incubation of glycine

[85] G. Pascal, L. Pichat, and C. Baret, *Bull. Soc. chim. France*, 1968, 1481.
[86] V. Tolman, J. Hanuš, and K. Vereš, *J. Labelled Compounds*, 1968, **4**, 243.
[87] J. Mizon, *J. Labelled Compounds*, 1968, **4**, 278.
[88] M. Matsuo, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1030.
[89] L. Pichat, P. N. Liem, and J.-P. Guermont, *Bull. Soc. chim. France*, 1968, 4079.
[90] B. L. Brown and W. S. Reith, *Biochim. Biophys. Acta*, 1967, **148**, 423.
[91] A. E. A. Mitta and G. B. de Salas, *Anales Asoc. quim. argentina*, 1967, **55**, 85.
[92] Å. Høye, *Acta Chem. Scand.*, 1968, **22**, 695.
[93] G. N. B. de Salas and A. E. A. Mitta, *Anales Asoc. quim. argentina*, 1967, **55**, 89.
[94] A. T. Blomquist and R. J. Cedergreen, *Canad. J. Chem.*, 1968, **46**, 1053.
[95] R. Wade and T. S. Murthy, *J. Chem. Soc.* (*C*), 1968, 2564.

with serine hydroxymethylase in a system containing tetrahydrofolate and pyridoxal phosphate (but lacking formaldehyde, which is required for the normal reaction of the enzyme) brings about rapid stereospecific exchange of one of the two α-hydrogen atoms of the glycine. Treatment of [α-$^3H_2$]-glycine under these conditions thus gives one enantiomer of [α-$^3H_1$]-glycine, whereas exchange of ordinary glycine with tritiated water gives the other.[96]

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

Compound	*Ref.*
L-*N*-(3-amino-3-carboxypropyl)-β-carboxypyridinium betaine. (Nicotianine)	13, 14
γ,δ,δ′-trihydroxy-L-leucine	68
N^{ϵ}-(indole-3-acetyl)-L-lysine	18
p-hydroxymethylphenylalanine (L, D and DL)	97
O-ethylhomoserine (L and DL)	98
DL-5-methyl-2-aminohex-4-enoic acid	21
DL-α-(3-hydroxyphenyl)glycine	23
DL-α-(3,5-dihydroxyphenyl)glycine	23
β-(cyclohexa-1,4-dienyl)alanine (*i.e.* 3,6-dihydrophenylalanine: L and DL)	49
β-(cyclohex-1-enyl)-DL-alanine (*i.e.* 3,4,5,6-tetrahydrophenylalanine)	49
β-(1-hydroxycyclohexyl)-DL-alanine	49
β-(cyclopent-2-enyl)-DL-alanine	99
β-(cyclopent-3-enyl)-DL-alanine	99
β-(cyclohex-2-enyl)-DL-alanine	99
β-(cycloheptl-1-enyl)-DL-alanine	99
DOPA dimer (12)	52
cycloDOPA (15)	53
4-bromoacetyl-DL-phenylalanine	100
4-bromoacetamido-DL-phenylalanine	100
3-chloroacetamido-DL-phenylalanine	100
4-fluoro-3-chloroacetamido-DL-phenylalanine	100
3,4,5-tri-iodo-DL-phenylalanine	101
3,5-di-isopropyl-3′-iodo-DL-thyronine	102
β-(3-chloro-4-methoxy-1-naphthyl)-DL-alanine	103
β-)3-chloro-4-hydroxy-1-naphthyl)-DL-alanine	103
β-(4-methoxy-1-naphthyl)-DL-alanine	103
β-(4-hydroxy-1-naphthyl)-DL-alanine	103
DL-β-(4-methoxy-1-naphthyl)-α-methylalanine	103
DL-β-(4-hydroxy-1-naphthyl)-α-methylalanine	103
DL-α-(2-indanyl)glycine	104
β-trimethylsilyl-DL-alanine	104

[96] M. Akhtar and P. M. Jordan, *Chem. Comm.*, 1968, 1691.
[97] S. C. Smith and N. H. Sloane, *Biochim. Biophys. Acta*, 1967, **148**, 414.
[98] Y. Murooka, T. Harada, and Y. Izumi, *Bull. Chem. Soc. Japan*, 1968, **41**, 633.
[99] T. H. Porter, R. M. Gipson, and W. Shive, *J. Medicin. Chem.*, 1968, **11**, 263.
[100] J. I. DeGraw, M. Cory, W. A. Skinner, M. C. Theisen, and C. Mitoma, *J. Medicin. Chem.*, 1968, **11**, 225.
[101] V. B. Schatz, B. C. O'Brien, and W. R. Sandusky, *J. Medicin. Chem.*, 1968, **11**, 140.
[102] T. Matsuura, T. Nagamachi, K. Matsuo, and A. Nichinaga, *J. Medicin. Chem.*, 1968, **11**, 899.
[103] A. J. Ablewhite and K. R. H. Wooldridge, *J. Chem. Soc.* (*C*), 1967, 2488.
[104] T. H. Porter and W. Shive, *J. Medicin. Chem.*, 1968, **11**, 402.

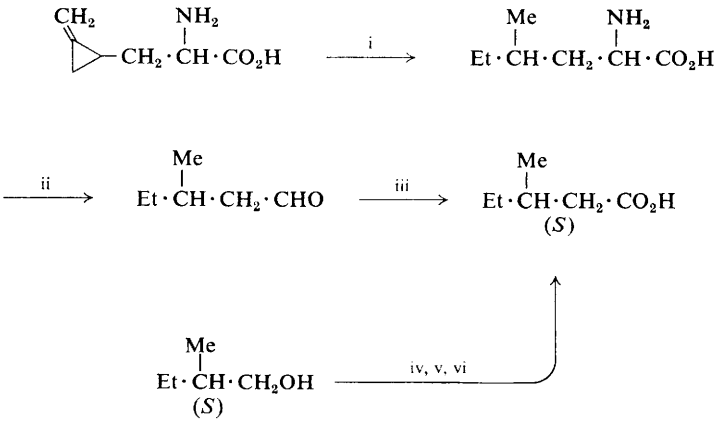
Compound	*Ref.*
DL-2-amino-3,3-dimethylhex-5-enoic acid	50[a]
DL-2-aminohexa-4,5-dienoic acid	50[a], 50[b]
DL-2-amino-3,3-dimethylhexa-4,5-dienoic acid	50[a]
DL-2-aminohepta-4,5-dienoic acid	50[a], 50[b]
DL-2-amino-3,3-dimethylhepta-4,5-dienoic acid	50[a]
DL-2-amino-3,3-dimethylnona-4,5-dienoic acid	50[a]
DL-2-aminohepta-5,6-dienoic acid	50[b]
DL-2-amino-3-methylhepta-5,6-dienoic acid	50[b]
DL-2-amino-5-*t*-butyl-6,6-dimethylhepta-3,4-dienoic acid	50[b]
DL-2-amino-5-methylhepta-3,4-dienoic acid	50[b]
DL-2-aminohept-4-en-6-ynoic acid	50[b]
ε-hydroxy-β-carboxy-DL-norleucine (diastereoisomers separated but not distinguished)	105[a]
β-carboxy-DL-lysine (diastereoisomers separated but not distinguished)	105[b]
β-(3,4-dihydroxyphenyl)-α-methyl-DL-serine (*threo* and *erythro*)	58
S-benzyl-β,γ-dimethyl-DL-homocysteine	106
S-benzyl-α,γ,γ-trimethyl-DL-homocysteine	106
Se-benzyl-β,γ-dimethyl-DL-selenohomocysteine	106
Se-benzyl-α,γ,γ-trimethyl-DL-selenohomocysteine	106
β-methyl-DL-methionine	107
α-methyl-DL-selenomethionine	107
β-methyl-DL-selenomethionine	107
γ-methyl-DL-selenomethionine	107
Se-benzylselenohomocysteine (L and D)	108
Selenomethionine (L and D)	108
Se-benzylselenocysteine (L and D)	108
Selenolanthionine (L, D, and *meso*)	108
DL-5,5,5-trifluoro-4-trifluoromethyl-2-aminovaleric acid	109
γ,γ′-difluoro-DL-valine	110
δ,δ′-difluoro-DL-leucine	110
γ-fluoro-DL-allothreonine	110
β-(5-nitro-3,4,-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-DL-alanine (5-nitro-DL-Willardiine)	111
β-(5-bis[2-chloroethyl]amino-3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-DL-alanine (DL-Willardiine mustard)	111
DL-α-amino-α-methyl-γ-(1-oxo-2(2*H*)-isoquinolyl)butyric acid	112
DL-α-amino-α-methyl-γ-(2-oxo-1(1*H*)-quinolyl)butyric acid	112
β-hydroxy-L-asparagine (*threo* and *erythro*)	113
β-hydroxy-DL-isoleucine (*threo* and *erythro*)	66
β-methoxy-DL-isoleucine (*threo* and *erythro*)	66
DL-α-amino-γ-(methylamino)butyric acid (5-azanorleucine)	114
DL-α-amino-β-(ethylamino)propionic acid (4-azanorleucine)	114

[105a] E. Kuss, *Z. physiol. Chem.*, 1967, 348, 1589. [b] E. Kuss, *Z. physiol. Chem.*, 1967, 348, 1596.
[106] G. Zdansky, *Arkiv Kemi*, 1968, **29**, 47.
[107] G. Zdansky, *Arkiv Kemi*, 1967, **27**, 447.
[108] G. Zdansky, *Arkiv Kemi*, 1968, **29**, 437 and 443.
[109] J. Lazar and W. A. Sheppard, *J. Medicin. Chem.*, 1968, **11**, 138.
[110] H. Lettré and U. Wölcke, *Annalen*, 1967, **708**, 75.
[111] A. P. Martinez, W. W. Lee, and L. Goodman, *J. Medicin. Chem.*, 1968, **11**, 60.
[112] S. Kamiya and K. Koshinuma, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1985.
[113] A. Singerman and Y. Liwschitz, *Tetrahedron Letters*, 1968, 4733.
[114] T. J. McCord, L. D. Booth, and A. L. Davies, *J. Medicin. Chem.*, 1968, **11**, 1077.

Compound	*Ref.*
DL-α-amino-β-(methylamino)propionic acid (4-azanorvaline)	114
L- and D-α-amino-β-(methylamino)propionic acid (4-azanorvaline)	47
$N^{\epsilon}N^{\epsilon}$-bis(2-cyanoethyl)-L-lysine	115
DL-Capreomycidine (16)	54
DL-α,γ-dimethylnorleucine	116
DL-α-methyl-$N^{\delta}N^{\delta}$-diethylornithine	116
DL-α-(1-methylcyclopropyl)alanine	116
α-ethyl-3,4-dimethoxy-DL-phenylalanine	116
α-methyl-4-morpholino-DL-phenylalanine	116
β-(2-amino-4-pyrimidinyl)alanine. (Laterine: DL and L)	117
DL-1-methyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid	24
β-(2-amino-4-pyrimidinyl)alanine. (Laterine: DL and L)	117
2,3-*trans*-3,4-*cis*-3,4-dihydroxy-DL-proline	74
N^{α},N^{α}-dimethyl-L-histidine	70

3 Physical and Stereochemical Studies of Amino-acids

A. Determination of Absolute Configuration.—Hypoglycin A (11) had been predicted [51] to have the configuration (2*S* : 4*S*). Degradation to 3-methylpentanoic acid and comparison with material of known (*S*) configuration (Scheme 6) established the configuration at C-4 as (*S*) and confirmed the



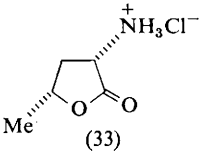
Reagents: PtO_2—H_2; ii, NBS; iii, Ag_2O; iv, HBr; v, Mg; vi, CO_2

Scheme 6

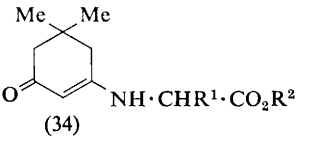
prediction, since the configuration at C-2 was known to be (*S*) from enzymic studies. Chemical correlation with (*R*)-isovaline has shown that both (+)-α-methylphenylglycine [118] and (+)-α-methylserine [119] belong to the

[115] J. F. Cavins and M. Friedman, *Biochemistry*, 1967, **6**, 3766.
[116] C. J. Abshire, *Experientia*, 1968, **24**, 778.
[117] Yu. P. Shvachkin, G. A. Korshunova, N. A. Bashkirova, and M. A. Prokof'ev, *Doklady Akad. Nauk S.S.S.R.*, 1968, **179**, 1127.
[118] H. Mizuno, S. Terashima, K. Achiwa, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1749.
[119] N. Takamura, S. Terashima, K. Achiwa, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1776.

(*S*) series. N.m.r. studies on the corresponding lactone hydrochlorides have enabled the absolute configurations at C-4 of some γ-hydroxy-L-amino-acids obtained from toxins of *Amanita phalloides* to be determined.[120] Similarly, n.m.r. was used to assign the (2*S*:4*R*) configuration to the lactone hydrochloride (33), which was obtained by photochlorination of L-norvaline in concentrated hydrochloric acid, followed by lactonisation and separation from the resulting mixture of diastereoisomers. Comparison of the chromatographic mobility of the (2*S*:4*R*)-γ-hydroxynorleucine obtained by hydrolysis of (33) with that of γ-hydroxy-L-norleucine isolated[121] in 1966 from *Lathyrus odoratus* indicates that the absolute configuration of the latter is (2*S*:4*S*).



(33)



(34)

The signs of the Cotton effects shown by cobalt complexes of amino-acids,[122] *N*-acryloyl derivatives of amino-acids,[123] osmate esters of *N*-acryloyl derivatives of amino-acids,[125] *N*-(*N*-oxido-2-pyridyl)-amino-acids,[125] and dimedonyl derivatives of amino-acids and their esters (34)[126] have been correlated with absolute configuration. The compounds (34) obtained by condensing dimedone (the analogous derivatives of dihydroresorcinol were also examined) with amino-acid alkyl esters were investigated particularly thoroughly and offer some promise as convenient chromophoric derivatives for the assignment of absolute configuration from o.r.d. measurements.[126]

B. Crystal Structures of Amino-acids.—The crystal structures of L-cysteine,[127] L-cysteic acid,[128] L-ornithine hydrochloride,[129] sarcosine hydrochloride,[130] diiodo-L-tyrosine dihydrate,[131] and 1-aminocyclopentane carboxylic acid[132] have been described, and further refinements of the structure of L-cysteine hydrochloride[133] have been published. The mono-

[120] T. Wieland, M. Hasan, and P. Pfaender, *Annalen*, 1968, **717**, 205.
[121] L. Fowden, *Nature*, 1966, **209**, 807.
[122] C. J. Hawkins and P. J. Lawson, *Chem. Commun.*, 1968, 177.
[123] N. Sakota, *J. Chem. Soc. Japan*, 1968, **89**, 425.
[124] N. Sakota and N. Koine, *J. Chem. Soc. Japan*, 1967, **88**, 1087.
[125] V. Tortorella and G. Bettoni, *Gazzetta*, 1968, **98**, 316.
[126] P. Crabbe, B. Halpern, and E. Santos, *Tetrahedron*, 1968, **24**, 4315.
[127] M. M. Harding and H. A. Long, *Acta Cryst.*, 1968, **24**, 1096.
[128] H. Konishi, T. Ashida, and M. Kakudo, *Bull. Chem. Soc. Japan*, 1968, **41**, 2305.
[129] N. N. Saha, S. K. Mazumdar, and S. Guha, *Science and Culture*, 1968, **34**, 72.
[130] N. N. Saha, S. K. Mazumdar, and S. C. Bhattacharyya, *Science and Culture*, 1968, **34**, 47.
[131] J. A. Hamilton and L. K. Steinrauf, *Acta Cryst.*, 1967, **23**, 817.
[132] R. Chandrasekharan, M. Mallikarjunan, G. Chandrasekharan, and R. Zand, *Current Sci.*, 1968, **37**, 91.
[133] R. Ramachandra Ayyar, *Z. Krist.*, 1968, **126**, 227.

clinic crystalline modification of DL-α-amino-*n*-butyric acid contains molecules in three different conformations, but only one conformer is found in the tetragonal form.[134]

C. Optical Rotatory Dispersion (O.r.d.) and Circular Dichroism (C.d.).—Details of an improved dichrograph for use with amino-acids and proteins have been reported.[135] The improved technique uses an average transient computer to achieve a better signal : noise ratio, and the c.d. spectrum of L-tryptophan in water in the range 310–190 nm was determined in this way. C.d. measurements on a number of L-amino-acids have revealed a negative dichroic band near 250 nm in addition to the strong band previously found at shorter wavelengths.[136]

There is considerable current interest in the o.r.d. of L-cystine and related compounds,[137–139] but as these studies are concerned with contributions from disulphide bonds to the o.r.d. of proteins, they will not be discussed here. Changes in the optical rotatory properties of amino-acids are induced by 6M-urea: these effects cannot be ascribed to changes in the ionisation state or to refractive index changes.[140] Papers on the assignment of configuration to amino-acids by examination of the o.r.d. of suitable derivatives have been listed above (see p. 15).

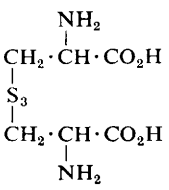
D. Nuclear Magnetic Resonance (N.m.r.) Spectra.—The interpretation of the ^1H n.m.r. spectra of DL-threonine and DL-valine in terms of 'rotational isomers' by Aruldas[141] has been under fire from several quarters.[142, 143a] It was pointed out that the barriers to rotation about the C(α)—C(β) bonds were unlikely to be great enough to enable observation of distinct rotamers at room temperature,[142] and it was also shown that the DL-threonine examined by Aruldas must have been grossly contaminated with DL-allothreonine.[142, 143a] It is, of course, unnecessary to postulate restricted rotation about the C(α)—C(β) bond in order to account for the non-equivalence of the methyl groups which is always observed in the n.m.r. spectra of valine and its derivatives—the methyl groups would be non-equivalent even if completely free rotation were possible, because of the adjacent asymmetric centre. Further studies on the 'anomalous' vicinal coupling constants of the aliphatic protons in the phenylalanine anion in aqueous solution are consistent with the suggestion that the two less

[134] T. Ichikawa, Y. Ittaka, and M. Tsuboi, *Bull. Chem. Soc. Japan*, 1968, **41**, 1027.
[135] Y. P. Myer and L. H. MacDonald, *J. Amer. Chem. Soc.*, 1967, **89**, 7142.
[136] R. D. Anand and M. K. Hargreaves, *Chem. and Ind.*, 1968, 880.
[137] D. L. Coleman and E. R. Blout, *J. Amer. Chem. Soc.*, 1968, **90**, 2405.
[138] M. Carmack and L. A. Neubert, *J. Amer. Chem. Soc.*, 1967, **89**, 7134.
[139] P. C. Kahn and S. Beychok, *J. Amer. Chem. Soc.*, 1968, **90**, 4168.
[140] L. I. Katzin and G. C. Kresheck, *Arch. Biochem. Biophys.*, 1968, **126**, 418.
[141] G. Aruldas, *Spectrochim. Acta*, 1967, **23**, *A*, 1345.
[142] J. F. Newmark and R. A. Newmark, *Spectrochim. Acta*, 1968, **24**, *A*, 952.
[143a] B. Bak and F. Nicolaisen, *Acta Chem. Scand.*, 1967, **21**, 1980. [b] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 1968, **90**, 4533. [c] B. Bak, C. Dambmann, F. Nicolaisen, E. J. Pedersen, and N. S. Bhacca, *J. Mol. Spectroscopy*, 1968, **26**, 78. [d] B. Bak, C. Dambmann, and F. Nicolaisen, *Acta Chem. Scand.*, 1967, **21**, 1674.

favourable staggered conformations have enhanced stability at low temperatures and concentrations, possibly due to specific solute–solvent interactions.[143b]

The ^1H n.m.r. spectra of all the protein amino-acids have been determined in trifluoroacetic acid and deuteriotrifluoroacetic acid at 220 MHz.[143c] The spectrum of tryptophan in deuteriotrifluoroacetic acid indicated exchange of hydrogen for deuterium in both rings of the indole system.[143c, 143d] Since this exchange only proceeds at a significant rate under conditions of very high acidity, it was suggested that it may be possible to use acid-catalysed exchange for selective isotopic labelling of tryptophan residues.[143d] The protons at the 2- and 6-positions of the indole ring undergo exchange most rapidly, and it is of interest in this connection to note that the dissociation constant for the protonation of the side chain of tryptophan in strong sulphuric acid solutions has recently been determined.[144]

^1H N.m.r. data at 60 MHz for 4-oxoprolines and *cis*- and *trans*-4-substituted prolines have been published, and correlations permitting assignment of configuration at the 4-position from the spectra have been deduced.[145] The ^1H n.m.r. spectra of a number of sulphur-containing amino-acids (including L-cystine, L-cysteine, L-cysteic acid, DL-lanthionine, and L-djenkolic acid) have been determined at 60 MHz in deuterium oxide as part of an n.m.r. study of bis(2-amino-2-carboxyethyl)-trisulphide (35),



(35)

which is found in acid hydrolysates of wool.[146] N.m.r. is now being used routinely for the characterisation of new amino-acids when sufficient material is available and the majority of references in the appendix to the section devoted to naturally occurring amino-acids report n.m.r. data. A particularly important application is in differentiation between diastereoisomers of amino-acids with two asymmetric centres—see, *e.g.*, refs. 3, 66, and 145.

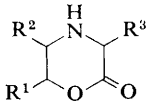
The ^{13}C n.m.r. spectra of glycine and alanine have been examined using a double-resonance technique as a preliminary part of a programme to investigate the potential of ^{13}C n.m.r. spectrometry as a tool for conformational work with macromolecules.[147]

[144] R. C. Armstrong, *Biochem. Biophys. Acta*, 1968, **158**, 174.
[145] R. H. Andreatta, V. Nair, and A. V. Robertson, *Austral. J. Chem.*, 1967, **20**, 2701.
[146] K. D. Bartle, J. C. Fletcher, D. W. Jones, and R. L'Amie, *Biochim. Biophys. Acta*, 1968, **160**, 106.
[147] W. J. Horsley and H. Sternlicht, *J. Amer. Chem. Soc.*, 1968, **90**, 3738.

4 Chemical Studies of Amino-acids

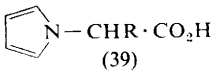
A. General Reactions.—The reactions of fluoronitropyridines with amino-acids have been described,[148, 149] and the *N*-(5-nitro-6-methyl-2-pyridyl) derivatives of most of the common α-amino-acids have been characterised.[149] The reaction of glycine (as its sodium salt or ethyl ester) with epoxides gives *N*-(2-hydroxyalkyl) derivatives (36) from which lactones (37) can be

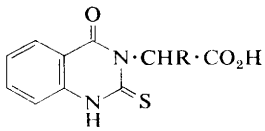
$$HOCHR^1 \cdot CHR^2 \cdot NH \cdot CHR^3 \cdot CO_2R^4$$
(36)


(37)

$$HOCH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CHR \cdot CO_2H$$
(38)

obtained: 2,5-dioxopiperazine and the products of its reaction with the epoxide are also formed.[150] β-Propiolactone reacts rapidly with all the protein amino-acids at pH 9 and room temperature.[151] It was inferred from earlier work on the reaction of β-propiolactone with simple amines that acylation of the α-amino group giving (38) occurs in the absence of other nucleophilic functionalities, but this was not confirmed by isolation of the products. At low pH values only sulphur-containing amino-acids react, and the reaction is specific at pH 3·0 and 0° for methionine, which presumably gives an acylsulphonium derivative.[151] α-Amino-acids react with 2,5-diethoxytetrahydrofuran in acetic acid in the presence of sodium acetate giving α-pyrrolo-acids (39), which are of interest as amino-acid analogues.[152] Reaction of *o*-(methoxycarbonyl)phenyl isothiocyanate with α-amino-acids in alkaline solution gives hydroquinazolones (40) which may


(39)

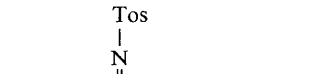

(40)

undergo further cyclisation if functional groups are present in the side chain.[153] A detailed study of the acylation of α,ω-diamino-acids using phenyl esters has been reported:[154] *p*-nitrophenyl acetate acylates the unprotected diamino-acids homolysine, lysine, and ornithine exclusively at the ω-amino group at pH 11, but the selectivity is less at lower pH values. Selective ω-acylation is not possible at any alkaline pH in the cases of

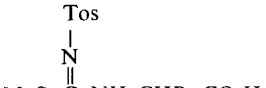
[148] T. Talik and Z. Talik, *Bull. Acad. polon. Sci., Ser. Sci. chim.*, 1968, **16**, 13.
[149] Z. Talik and B. Brekiesz-Lewandowska, *Roczniki Chem.*, 1967, **41**, 2095.
[150] K. Jankowski and C. Berse, *Canad. J. Chem.*, 1967, **45**, 2865.
[151] M. A. Taubman and M. Z. Atassi, *Biochem. J.*, 1968, **106**, 829.
[152] J. Gloede, K. Poduska, H. Gross, and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1968, **33**, 1307.
[153] E. Cherbuliez, O. Espejo, B. Willhalm, and J. Rabinowitz, *Helv. Chim. Acta*, 1968, **51**, 241.
[154] J. Leclerc and L. Benoiton, *Canad. J. Chem.*, 1968, **46**, 1047.

α,γ-diaminobutyric acid and α,β-diaminopropionic acid, however. Treatment of amino-acid salts with *S*-methylisothiosemicarbazide hydroiodide in boiling water or ethanol gives *N*-(aminoguanyl)-amino-acids (41), which are rather bizarre dipeptide analogues.[155] The related derivatives (42) have also been obtained, by hydrazinolysis of the products (43) of the reaction of amino-acid salts with (44).[155]

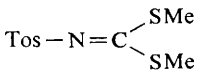
(41)



(42)

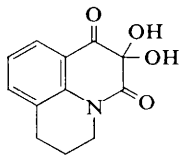


(43)



(44)

Thermal decarboxylation of α-amino-acids in the presence of ketones gives, after hydrolysis of the intermediate decarboxylated Schiff base, either the normal decarboxylation product or the amine corresponding to the ketone (*i.e.* the transamination product) or both, depending on the structures of the reactants.[156] Studies [157, 158] of the reaction of the ninhydrin analogue (45) with α-amino-acids have provided, by analogy, further corroboration of McCaldin's mechanism [159] for the ninhydrin reaction.



(45)

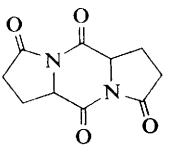
B. Other Reactions.—The volatile products obtained by pyrolysis of phenylalanine, tyrosine, tryptophan and histidine have been examined by gas–liquid chromatography (g.l.c.) and mass spectrometry.[160] Among the volatile degradation products obtained from phenylalanine were toluene, styrene, benzene, ethylbenzene, benzonitrile, phenylethylamine, water and carbon dioxide. The complexity of the pyrolytic pathway and the multiplicity of products obtained from this one amino-acid alone must undermine

[155] J. Gante, *Chem. Ber.*, 1968, **101**, 1195.
[156] A. F. Al-Sayyab and A. Lawson, *J. Chem. Soc.* (*C*), 1968, 406.
[157] H. Wittmann, W. Dreveny, and E. Ziegler, *Monatsh.*, 1968, **99**, 1205.
[158] H. Wittmann, W. Dreveny, and E. Ziegler, *Monatsh.*, 1968, **99**, 1543.
[159] D. J. McCaldin, *Chem. Rev.*, 1960, **60**, 39.
[160] G. P. Shulman and P. G. Simmonds, *Chem. Comm.*, 1968, 1040.

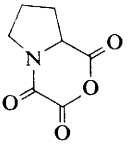
any hopes [161] that identification of characteristic pyrolysis products might be useful for qualitative amino-acid analysis.

Amino-acids with thioether side chains are oxidised to the corresponding sulphoxides in high yield by means of diethyl azodicarboxylate.[162] This reagent converts cysteine to cystine (cysteic acid if a very large excess of oxidant is used) and also reacts with tyrosine, but in this case the nature of the products has not yet been determined.

Dehydration of glutamic acid with acetic anhydride–pyridine gives a high melting substance to which the structure (46) was originally assigned.[163] This structure was recently criticised [164] and a different structure proposed, but the original work [163] has now been repeated: [165] the formulation (46) was confirmed and it was shown that the criticisms of it are invalid because they are based on an erroneous interpretation of the i.r. data. A convenient method for the dehydration of asparagine and glutamine (discovered during attempted *N*-carboxyanhydride preparations) uses phosgene in dioxan:[166] yields of the benzyloxycarbonyl derivatives of β-cyano-L-alanine and γ-cyano-L-α-aminobutyric acid prepared in this way were excellent. This alternative to the usual dehydration using dicyclohexylcarbodi-imide [167] seems to be a great improvement.



(46)



(47)

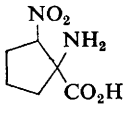
When α-amino-acids with primary amino-groups are treated with oxalyl chloride, intractable tarry substances are produced under all conditions.[168] These tarry materials probably arise *via* oxazolones, and this suggestion is borne out by the fact that proline, which cannot form an oxazolone, gives a crystalline *N*-oxalic anhydride (47) when heated with oxalyl chloride in dioxan.[168] The cyclisation of *N*-oxalyl-α-dialkyl-α-amino-acids has also been investigated.[169]

α-Sulpho-β-alanine, previously not easily available in quantity, can be obtained from the reaction of acrylonitrile with oleum in poor yield,[170] but in almost quantitative yield by treatment of β-alanine with a large

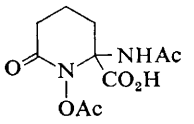
[161] C. Merritt and D. H. Robertson, *J. Gas Chromatog.*, 1967, **5**, 96.
[162] R. Axen, M. Chaykovsky, and B. Witkop, *J. Org. Chem.*, 1967, **32**, 4117.
[163] J. A. King and F. H. McMillan, *J. Amer. Chem. Soc.*, 1952, **74**, 2859.
[164] S. El-Zanfally, M. Khalifa, and Y. M. Abou-Zeid, *Tetrahedron*, 1966, **22**, 2307.
[165] M. R. Harnden, *J. Heterocyclic Chem.*, 1968, **5**, 307.
[166] M. Wilchek, S. Ariely, and A. Patchornik, *J. Org. Chem.*, 1968, **33**, 1258.
[167] C. Ressler and H. Ratzkin, *J. Org. Chem.*, 1961, **26**, 3356.
[168] W. R. Hearn and R. E. Worthington, *J. Org. Chem.*, 1967, **32**, 4072.
[169] W. R. Hearn and J. Medina-Castro, *J. Org. Chem.*, 1968, **33**, 3980.
[170] A. Zilkha, I. Barzilay, J. Naiman, and B.-A. Feit, *J. Org. Chem.*, 1968, **33**, 1686.

excess of oleum at room temperature.[171] Preliminary experiments indicate that this reaction may be general for β-amino-acids.

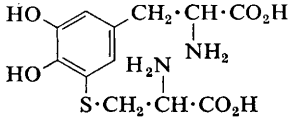
Unless the acetylation of 1-amino-2-nitrocyclopentane carboxylic acid (48) is performed under mild conditions, further reaction occurs with rearrangement, giving an *N*-acetoxypiperidone (49).[172] The mechanism was not examined in very great detail, but is presumably related to similar rearrangements of 2-nitrocyclopentanones, which yield *N*-acetoxy-imides when heated with acetic anhydride.[173]



(48)



(49)



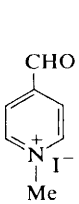
(50)

C. Non-enzymic Models of Biochemical Processes Involving Amino-acids.— The reactions of cysteine with *o*-quinones have been studied [174, 175] in order to obtain background information on the reactions which are probably involved in the biosynthesis of phaeomelanins. Thus cysteine and *N*-acetylDOPAquinone ethyl ester give, after removal of the protecting groups, 5-*S*-cysteinyl-DOPA (50). Since (50) gives on oxidation two pigments which are spectroscopically identical to pigments obtained from chicken feathers, it may be implicated in the biogenesis of phaeomelanins.[175]

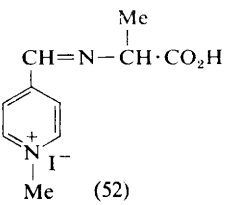
In non-enzymic transamination reactions catalysed by pyridine-4-aldehydes in the absence of metal ions, a 3-hydroxy group is generally required to (*a*) catalyse aldimine formation and (*b*) assist the aldimine-ketimine tautomeric change. If the pyridine nitrogen carries a positive charge, however, this requirement lapses because the positive charge provides an inductive effect strong enough to bring about the necessary changes without additional assistance. Thus *N*-methyl-4-formyl-pyridinium iodide (51) rapidly forms an aldimine (52) with alanine, and a slower subsequent prototropic shift gives the ketimine (53) and finally transamination products after hydrolysis. The conversion of (52) to (53) was shown to be a general base catalysed process with the dihydropyridine species (54) as a sufficiently long-lived intermediate to be detected spectroscopically.[176]

The oxidative coupling of 4-hydroxy-3,5-di-iodophenylpyruvic acid (55) and 3,5-di-iodophenylalanine gives fair yields of thyroxine (56), and is therefore an attractive model for its biosynthesis.[177, 178] Evidence has

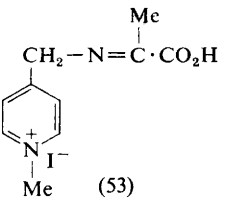
[171] D. Wagner, D. Gertner, and A. Zilkha, *Tetrahedron Letters*, 1968, 4479.
[172] W. B. Turner, *J. Chem. Soc.* (*C*), 1967, 2225.
[173] A. Hassner and J. Larkin, *J. Amer. Chem. Soc.*, 1963, **85**, 2181.
[174] G. Prota, G. Scherillo, F. Napolano, and R. A. Nicolaus, *Gazzetta*, 1967, **97**, 1451
[175] G. Prota, G. Scherillo, and R. A. Nicolaus, *Gazzetta*, 1968, **98**, 495.
[176] J. R. Maley and T. C. Bruice, *J. Amer. Chem. Soc.*, 1968, **90**, 2843.
[177] G. Hillman, *Z. Naturforsch.*, 1956, **11b**, 424.
[178] R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, 1961, **26**, 1977.



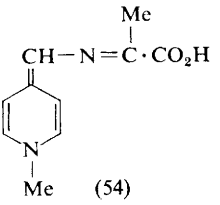
(51)



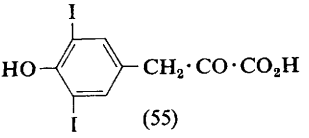
(52)



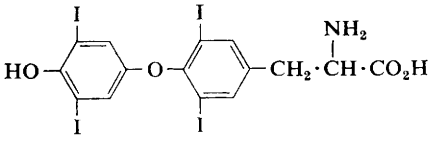
(53)



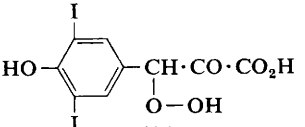
(54)



(55)



(56)



(57)

recently been presented to show that hydroperoxide (57) is first formed, followed by an anaerobic reaction with 3,5-di-iodotyrosine, but the mechanism of the final coupling has not yet been clarified.[179, 180]

The non-enzymic hydroxylating system of Udenfriend (ferrous ion, ascorbic acid, ethylenediamine tetra-acetic acid and oxygen) causes hydroxylation of proline in a manner which does not involve the production of hydrogen peroxide or hydroxyl radicals:[181] the mechanism probably conforms to the general mechanism which has been proposed[182] for oxidations by this system.

The oxidative decarboxylation reactions of primary, secondary, and tertiary amino-acids which are induced by hypohalite have been studied in very great detail as models for some stages in alkaloid biosynthesis.[183]

D. Effects of Electromagnetic Radiation on Amino-acids.—There continues to be much activity in research on the effects of ionising radiation on amino-acids. Several electron spin resonance (e.s.r.) studies of irradiated

[179] A. Nishinaga, H. J. Cahnmann, H. Kon, and T. Matsuura, *Biochemistry*, 1968, **7**, 388.
[180] F. Blasi, F. Fragomele, and I. Covelli, *European J. Biochem.*, 1968, **5**, 215.
[181] M. Bade and B. S. Gould, *Biochim. Biophys. Acta*, 1968, **156**, 425.
[182] G. A. Hamilton, *J. Amer. Chem. Soc.*, 1964, **86**, 3391.
[183] E. E. van Tamelen, V. B. Haarstad, and R. L. Orvis, *Tetrahedron*, 1968, **24**, 687.

amino-acids (in crystals or glasses) have been reported.[184-189] The radicals formed by irradiation of amino-acid crystals are normally lost on dissolution by reaction with the solvent, but if the irradiated crystals are dissolved in an aqueous solution of t-nitrosobutane the radicals (R·) which were formerly held in the crystalline matrix are trapped as relatively stable nitroxide radicals (Scheme 7), and these can then be examined by e.s.r.[190] There have

$$R^{\bullet} + Bu^{t}-N=O \longrightarrow Bu^{t}-\underset{\underset{O^{\bullet}}{|}}{N}-R$$

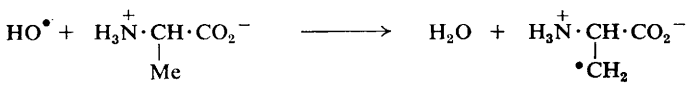
Scheme 7

also been a number of papers dealing with the radiolysis of amino-acids in aqueous solution: interest has been concentrated on aromatic[191-193] and sulphur-containing[194-197] amino-acids, but the effects of γ-radiation on proline and hydroxyproline have also been briefly described.[198]

In aerated aqueous solutions, most of the radiation damage to amino-acids is caused by the oxidising species (HO·), as most of the reducing species (solvated electrons and hydrogen atoms) are scavenged by oxygen giving hydroperoxyl radicals, which are not very reactive towards most amino-acids. The transient primary free radicals formed by radiolysis of amino-acids in aerated solutions cannot be observed directly by e.s.r., as it would require inconveniently vigorous irradiation to achieve a sufficient concentration. Chemically produced hydroxyl radicals have therefore been used to simulate the effects of radiation. The generation of hydroxyl radicals (by reaction of titanium trichloride with hydrogen peroxide) in the presence of amino-acids thus leads to the same free radicals as are formed by radiolysis, and these have been studied by e.s.r.[199-201] Except in the case of cysteine and cystine, when sulphur radicals were

[184] P. B. Ayscough and A. K. Roy, *Trans. Faraday Soc.*, 1968, **64**, 582.
[185] P. B. Ayscough, K. Mach, J. P. Oversby, and A. K. Roy, *Chem. Comm.*, 1967, 1084.
[186] M. Fujimoto, W. A. Seddon, and D. R. Smith, *J. Chem. Phys.*, 1968, **48**, 3345.
[187] D. G. Cadena and J. R. Rowlands, *J. Chem. Soc.* (*B*), 1968, 488.
[188] F. G. Liming jun. and W. Gordy, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **60**, 794.
[189] A. Hedberg and A. Ehrenberg, *J. Chem. Phys.*, 1968, **48**, 4822.
[190] C. Lagercrantz and S. Forshult, *Nature*, 1968, **218**, 1247.
[191] G. A. Brodskaya and V. A. Sharpati, *Khim. vysok. Energii*, 1968, **2**, 254.
[192] G. A. Brodskaya and V. A. Sharpati, *Zhur. fiz. Khim.*, 1967, **41**, 2850.
[193] J. Chrysochoos, *Radiation Res.*, 1968, **33**, 465.
[194] J. E. Packer and R. V. Winchester, *Chem. Comm.*, 1968, 826.
[195] T. C. Owen, M. Rodriguez, B. G. Johnson, and J. A. G. Roach, *J. Amer. Chem. Soc.*, 1968, **90**, 196.
[196] V. G. Wilkening, M. Lal, M. Arends, and D. A. Armstrong, *J. Phys. Chem.*, 1968, **72**, 185.
[197] A. Al-Thannon, R. M. Peterson, and C. N. Trumbore, *J. Phys. Chem.*, 1968, **72**, 2395.
[198] K. S. Korgaonkar, S. V. Marathe, and K. A. Chaubal, *Science and Culture*, 1968, **34**, 100.
[199] W. A. Armstrong and W. G. Humphreys, *Canad. J. Chem.*, 1967, **45**, 2589.
[200] H. Taniguchi, K. Fukui, S. Ohnishi, H. Hatano, H. Hasegawa, and T. Maruyama, *J. Phys. Chem.*, 1968, **72**, 1926.
[201] R. Poupko, B. L. Silver, and A. Lowenstein, *Chem. Comm.*, 1968, 453.

produced, the radicals observed were mostly formed by hydrogen abstraction from the β-carbon atom (*e.g.* Scheme 8) because hydrogen on the



Scheme 8

α-carbon atom is deactivated towards the electrophilic hydroxyl radical by the protonated amino-group. Some amino-acids also suffered hydrogen abstraction from other positions in the side-chain, but only glycine (which has little alternative) gave a radical formed by loss of a hydrogen radical from the α-carbon atom. Amino-acid analysis of the ninhydrin-positive products of these reactions showed that the products were identical with those produced by γ-irradiation, confirming the validity of the model. These results suggest that ideas about the course of radiolysis of aerated aqueous amino-acid solutions may have to be revised, as earlier work (based largely on product studies) gave rise to the conclusion [202] that abstraction of hydrogen from the α-carbon atom (*e.g.* Scheme 9) was the first step in the main pathway to products.

Scheme 9

The rates of reaction of a series of amino-acids with radiolytically produced hydrogen atoms in degassed aqueous solution have been determined.[203]

An e.s.r. study (covering most of the protein amino-acids) of the free radicals produced by irradiation of polycrystalline amino-acid samples with light of wavelength 253·7 nm has been published.[204] The signals which were observed were frequently different from those obtained after exposure to ionising radiation: most amino-acids gave radicals formed by loss of a hydrogen atom, but the spectrum obtained from phenylalanine showed evidence of the addition of a hydrogen atom giving a cyclohexadienyl radical. Radicals produced by the addition of hydrogen atoms to the aromatic rings of phenylalanine, tyrosine, and tryptophan have also been detected after γ-irradition or bombardment with thermal hydrogen atoms.[188]

It is now generally accepted that aromatic amino-acid residues, particularly tryptophan and to a lesser extent tyrosine and the other aromatic amino-acids, play a very important role in the photochemistry of proteins, and

[202] B. M. Weeks, S. A. Cole, and W. M. Garrison, *J. Phys. Chem.*, 1965, **69**, 4131.
[203] W. A. Volkert and R. R. Kuntz, *J. Phys. Chem.*, 1968, **72**, 3394.
[204] W. F. Forbes and P. D. Sullivan, *Canad. J. Biochem.*, 1967, **45**, 1831.

this has stimulated considerable interest in the photochemical behaviour of tyrosine and tryptophan.[205-212] It has recently been shown that the photochemical degradation of cystine is sensitised by both tryptophan and tyrosine in aqueous solution, providing a model for the inactivation of cystine-containing enzymes by u.v. light,[213] since there is evidence that quanta primarily absorbed by neighbouring aromatic chromophores contribute to the destruction of cystine residues.

5 Analytical Methods

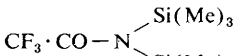
At the present time, roughly a quarter of the papers on amino-acids which appear are devoted to analytical methods. A proportional amount of space will not be allocated to these studies here, however, as most of them are either concerned with situations which are unlikely to interest many readers (*e.g.* amino-acids in biological fluids) or describe minor modifications of established techniques. In any case, the essence of a new or improved analytical procedure is embodied in the practical detail, and it would be inappropriate to present this here. In the ensuing outline, therefore, the majority of publications in this area will be cited without discussion, and only a few advances of general interest will be dealt with more fully.

A book on analytical methods in amino-acid chemistry has been published:[214] the theory and practice of most of the important quantitative methods are covered.

A. Gas–Liquid Chromatography.—The preparation of suitable derivatives of amino-acids and their analysis by g.l.c. has been reviewed recently.[215] Further studies on the use of *N*-trifluoroacetylamino-esters have been described[216-218] and a g.l.c. system (using *N*-trifluoroacetylamino-acid n-butyl esters) which permits quantitative analysis (in 55 min.) of mixtures containing all the protein amino-acids has been developed.[218] This promising new method was tested by analysis of a ribonuclease hydrolysate, which gave results in excellent agreement with those obtained by ion-exchange chromatography.

[205] Yu. A. Vladimirov and E. E. Fesenko, *Photochem. and Photobiol.*, 1968, **8**, 209.
[206] B. Rabinovitch, *Arch. Biochem. Biophys.*, 1968, **124**, 258.
[207] E. E. Fesenko and D. I. Roshchupkin, *Zhur. priklad. Spectroskopii*, 1968, **8**, 834.
[208] H. Hase, *J. Phys. Soc. Japan*, 1968, **24**, 223.
[209] R. F. Chen, *Analyt. Letters*, 1967, **1**, 35.
[210] I. I. Sapezhinskii, *Biofizika*, 1968, **13**, 517.
[211] R. Santus, C. Helene, and M. Ptak, *Photochem. and Photobiol.*, 1968, **7**, 341.
[212] H. B. Steen, *Photochem. and Photobiol.*, 1968, **8**, 47.
[213] K. Dose, *Photochem. and Photobiol.*, 1968, **8**, 331.
[214] S. Blackburn, 'Amino-acid Determination—Methods and Techniques', Edward Arnold, London, 1968.
[215] C. W. Gehrke and D. L. Stalling, *Separation Sci.*, 1968, **2**, 101; K. Blau, in 'Biomedical Applications of Gas Chromatography', vol. 2, ed. H. A. Szymanski, Plenum Press, 1968, p. 1.
[216] C. Landault and G. Guichon, *Bull. Soc. chim. France*, 1967, 3985.
[217] A. Darbre and A. Islam, *Biochem. J.*, 1968, **106**, 923.
[218] C. W. Gehrke, R. W. Zumwalt, and L. L. Wall, *J. Chromatog.*, 1968, **37**, 398.

Pertrimethylsilyl derivatives have been employed for g.l.c. analysis of iodo-amino-acids,[219-221] sulphur-containing amino-acids,[222, 223] and seleno-amino-acids.[223] The new silylating reagent (58) has been prepared and used



(58)

for the chemical modification of amino-acids for g.l.c. purposes:[224] the co-products which arise from use of this reagent interfere with the g.l.c. analysis less than those from more familiar reagents such as *N,O*-bis(trimethylsilyl)acetamide.

Condensation of the guanidine side-chain of arginine with acetylacetone gives an N^{δ}-(2-pyrimidinyl)ornithine derivative which is suitable for g.l.c. and mass spectrometric work.[225] Other derivatives which have been recommended for g.l.c. work include *N*-acetylamino-acid n-propyl esters,[226] α-isothiocyanato-esters [227] [(59): these are easily obtained from the corresponding α-amino-esters by successive treatment with carbon disulphide and methyl chloroformate] and *N*-alkylamino-esters.[228] Derivatives suitable for the separation of the optical isomers of some protein amino-acids with functional side-chains have been reported.[229]

(59) (60)

The use of phosphorus-containing modifying groups enables very small amounts of amino-acids to be detected by g.l.c., using a modified alkali flame detector.[230] The minimum detectable limit (response : noise ratio of 2 : 1) for the alanine derivative (60) was *ca.* 5×10^{-12} g.

B. Ion-exchange Chromatography.—2,4,6-Trinitrobenzenesulphonic acid can be used with automatic amino-acid analysers for quantitative determinations,[231] and the same reagent can be used for simultaneous identification and determination of amino-acids and hexosamines, using the fact

[219] N. M. Alexander and R. Scheig, *Analyt. Biochem.*, 1968, **22**, 187.
[220] L. B. Hansen, *Analyt. Chem.*, 1968, **40**, 1587.
[221] E. T. Backer and V. G. Pileggi, *J. Chromatog.*, 1968, **36**, 351.
[222] F. Shahrokhi and C. W. Gehrke, *J. Chromatog.*, 1968, **36**, 31.
[223] K. A. Caldwell and A. L. Tappel, *J. Chromatog.*, 1968, **32**, 635.
[224] D. L. Stalling, C. W. Gehrke, and R. W. Zumwalt, *Biochem. Biophys. Res. Comm.*, 1968, **31**, 616.
[225] H. Vetter-Diechtl, W. Vetter, W. Richter, and K. Biemann, *Experientia*, 1968, **24**, 340.
[226] J. R. Coulter and C. S. Hahn, *J. Chromatog.*, 1968, **36**, 42.
[227] B. Halpern, V. A. Close, A. Wegmann, and J. W. Westley, *Tetrahedron Letters*, 1968, 3119.
[228] J. W. Davies jun. and A. Furst, *Analyt. Chem.*, 1968, **40**, 1910.
[229] G. E. Pollock and A. H. Kawauchi, *Analyt. Chem.*, 1968, **40**, 1356.
[230] G. Ertingshausen, C. W. Gehrke, and W. A. Aue, *Separation Sci.*, 1967, **2**, 681.
[231] J. Harmeyer, H.-P. Sallmann, and L. Ayoub, *J. Chromatog.*, 1968, **32**, 258.

that the ratio of the optical density at 355 nm : 475 nm is >2 for trinitrophenylamino-acids but <0·5 for trinitrophenylhexosamines.[232] A system has been developed for the use of Rosen's ninhydrin solution with the Beckman-Spinco amino-acid analyser.[233] When a problem calls for amino-acid analysis and determination of ^{14}C distribution among the amino-acids the effluent containing the ninhydrin reaction products can be counted.[234] A modified procedure for the resolution of mixtures containing an unusual complement of basic amino-acids (*e.g.* elastin hydrolysates) on an amino-acid analyser has been described.[235] Interest in the use of lithium buffers with automatic amino-acid analysers continues, with particular emphasis on the possibilities for obtaining improved separations in analyses of physiological fluids.[236, 237] The effects of the presence of heavy metal cations (Fe^{3+}, Cu^{2+}, Ni^{2+}) on the results of amino-acid analysis by ion-exchange chromatography have been investigated: iron affects the extinction coefficients of the ninhydrin reaction products of several amino-acids, and interferes seriously with the determination of cysteic acid.[238] If methanol-containing buffers are used in ion-exchange chromatography to improve the resolution of threonine and serine, there is a danger of loss of aspartic and glutamic acids by esterification.[239, 240] The substitution of t-butanol for methanol avoids this difficulty and causes no decrease in the resolution of threonine and serine.[240] Preliminary results on the use of an amphoteric modified cellulose for ion-exchange chromatography have been reported.[241]

C. Thin-layer Chromatography.—The t.l.c. of amino-acids has been reviewed by Pataki:[242] the invaluable handbook [243] by the same author on the subject is no doubt well known to most readers.

A new method for the detection of amino-acid derivatives with aromatic groups on thin-layer chromatograms uses the fact that such aromatic groups can act as electron donors and form coloured charge-transfer complexes with acceptors such as chloranil and 2,4,7-trinitrofluorenone. An important point about this method of detection is its non-destructive nature: the components originally applied to the plate can be recovered by extraction with an aqueous solvent in which the charge-transfer acceptor is insoluble.[244] Tyrosine derivatives can be detected on t.l.c. plates by

[232] P. C. Kelleher and C. J. Smith, *J. Chromatog.*, 1968, **34**, 7.
[233] W. S. Knight, *Analyt. Biochem.*, 1968, **22**, 539.
[234] A. C. Olson, L. M. White, and A. T. Noma, *Analyt. Biochem.*, 1968, **24**, 120.
[235] B. C. Starcher, *J. Chromatog.*, 1968, **38**, 293.
[236] J. H. Peters, B. J. Berridge jun., J. G. Cummings, and S. C. Lin, *Analyt. Biochem.*, 1968, **23**, 459.
[237] T. L. Perry, D. Stedman, and S. Hansen, *J. Chromatog.*, 1968, **38**, 460.
[238] A. Rudolph, *J. Chromatog.*, 1967, **31**, 479.
[239] L. W. Nauman and W. Galster, *J. Chromatog.*, 1968, **34**, 102.
[240] L. W. Nauman, *J. Chromatog.*, 1968, **36**, 398.
[241] G. Manecke and P. Gergs, *J. Chromatog.*, 1968, **34**, 125.
[242] G. Patakai, *Chromatog. Rev.*, 1967, **9**, 23.
[243] G. Patakai, 'Dünnschichtchromatographie in der Aminosäure- und Peptid-Chemie', Walter de Gruyter, Berlin, 1966.
[244] Y. Burstein, M. Fridkin, and M. Shinitzky, *Biochim. Biophys. Acta*, 1968, **160**, 141.

irradiation with u.v. light, which causes formation of brown pigments.[245] Other papers on the t.l.c. of amino-acids have described studies on the separation of sulphur-containing amino acids,[246] detection with variamine blue and cupric acetate,[247] applications of circular t.l.c.,[248] quantitative determination of amino-acids based on spot size in two-dimensional t.l.c.,[249] the comparative efficiency of different kinds of cellulose for t.l.c. separations,[250] analysis of amino-acid mixtures containing large amounts of glycerol,[251] and the t.l.c. analysis of amino-acids in physiological fluids.[252-254]

D. Other Methods.—Two procedures for the determination of D-amino-acids using a D-amino-acid acetyltransferase (AAT) from yeast have been described.[255] In one method (which was investigated for amounts of D-amino-acids of the order 1 μmole) the D-amino-acid is incubated with AAT, coenzyme A (CoA), excess acetyl phosphate, and phosphotransacetylase (PTA) at pH 7·6 and 30°, when the reactions shown in Scheme 10

$$\text{CoA} + \text{Acetyl phosphate} \xrightarrow{\text{PTA}} \text{Ac·CoA} + \text{Phosphate}$$

$$\text{Ac·CoA} + \text{D-Amino-acid} \xrightarrow{\text{AAT}} \text{Ac-D-Amino-acid} + \text{CoA}$$

Scheme 10

occur. The extent of acetylation, and hence the amount of D-amino-acid, is determined by colorimetric estimation (hydroxamic acid reaction) of the acetyl phosphate which is consumed. The errors of the method are about $\pm 1\%$. The second procedure involves enzymic acetylation with radioactive acetyl-CoA. AAT is completely stereospecific and has a very broad substrate specificity; it catalysed the acetylation of 44 of the 47 D-amino-acids tested.

Many other topics in the analytical chemistry of amino-acids have been discussed, including: electrophoresis on paper [256, 257] and thin layers,[258, 259] the use of papers impregnated with liquid ion-exchangers,[260] the comparative efficiency of several chromatographic papers for amino-acid separation and determination of basic amino-acids with mercuric salt

[245] A. Ber and L. Wasserman, *Experientia*, 1968, **24**, 224.
[246] V. R. Villaneuva and M. Barbier, *Bull. Soc. chim. France*, 1967, 3992.
[247] B. Duk, Z. Kwapniewski, and J. Sliwiok, *Chem. analit.*, 1968, **13**, 193.
[248] M. M. Hashmi, A. S. Adil, N. A. Chughtai, F. R. Chugtai, and M. A. Shahid, *Mikrochim. Acta*, 1968, 291.
[249] E. Bancher, J. Washüttl, and M. D. Olfat, *Mikrochim. Acta*, 1968, 773.
[250] C. L. de Ligny and E. C. M. Kok, *J. Chromatog.*, 1968, **38**, 224.
[251] E. J. Shellard and G. H. Jolliffe, *J. Chromatog.*, 1967, **31**, 82.
[252] R. B. Meffered jun., R. M. Summers, and J. G. Fernandez, *Analyt. Letters*, 1968, **1**, 279.
[253] H. H. White, *Clinica Chim. Acta*, 1968, **21**, 297.
[254] E. Plöchl, *Clinica Chim. Acta*, 1968, **21**, 271.
[255] J. H. Schmitt and M. H. Zenk, *Analyt. Biochem.*, 1968, **23**, 433.
[256] J. L. Frahn and J. A. Mills, *Analyt. Biochem.*, 1968, **23**, 546.
[257] P. J. Peterson, *J. Chromatog.*, 1968, **38**, 301.
[258] R. L. Munier, C. Thommegay, and G. Sarrazin, *Bull. Soc. chim. France*, 1967, 397.
[259] J. Chudzik and A. Klein, *J. Chromatog.*, 1968, **36**, 262.
[260] E. Soczewinski and M. Rojowska, *J. Chromatog.*, 1968, **32**, 364.

precipitants,[261, 262] fluorimetric determination using butan-2,4-dione,[263] potentiometric determination of sulphur-containing amino-acids in the presence of ascorbic acid,[264] polarographic determination,[265] and titrimetric estimation in non-aqueous media.[266]

E. Determination of Specific Amino-acids.—Papers on the determination of the following amino-acids have appeared: glycine,[267] L-leucine and DL-valine,[268] ornithine,[269] γ-aminobutyric acid,[269, 270] δ-aminolaevulinic acid,[271] available lysine in foods,[272, 273] ε-aminocaproic acid,[274] tryptophan,[275–277] cystine,[278, 279] lanthionine,[280, 281] lysinoalanine,[281] hydroxyproline,[282] phenylalanine,[283, 284] and tyrosine.[284, 285]

[261] F. Kai, *Bull. Chem. Soc. Japan*, 1968, **41**, 875.
[262] F. Kai, *Bull. Chem. Soc. Japan*, 1967, **40**, 2297.
[263] E. Sawicki and R. A. Carnes, *Analyt. Chim. Acta*, 1968, **41**, 178.
[264] N. Santi and E. Peillon, *Ann. pharm. franç.*, 1968, **26**, 177.
[265] B. P. Zhantalai and Ya. I. Tur'yan, *Zhur. analit. Khim.*, 1968, **23**, 282.
[266] G. M. Gal'pern, V. A. Il'ina, L. P. Petrova, and F. D. Sidel'kovskaya, *Zavodskaya Lab.*, 1968, **34**, 416.
[267] A. Masood and O. C. Saxena, *Microchem. J.*, 1968, **13**, 178.
[268] O. C. Saxena, *Microchem. J.*, 1968, **13**, 321.
[269] K. Molitoris, *J. Chromatog.*, 1968, **34**, 399.
[270] N. Seiler and M. Wiechmann, *Z. physiol. Chem.*, 1968, **349**, 588.
[271] J. J. Chisolm jun., *Analyt. Biochem.*, 1968, **22**, 54.
[272] L. Blom, P. Hendricks, and J. Caris, *Analyt. Biochem.*, 1967, **21**, 382.
[273] P. Hocquelet, *Ann. Fals. et Expertise chim.*, 1968, **61**, 155.
[274] S. Simard-Savoie, L. M. Breton, and M. Beaulieu, *J. Chromatog.*, 1968, **38**, 143.
[275] J. Cegarra and J. Gacén, *J. Soc. Dyers and Colourists*, 1968, **84**, 216.
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