1
Amino-acids

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1 Introduction

The usual steady increase in the number of papers eligible for citation in this Chapter continues to press the question whether to be more selective in the material reported or to restrict the description of the work reported. Even more papers are cited this year, and this represents a certain amount of extra selectivity. The main areas of literature expansion, in the biological and metabolic studies of amino-acids, continue to be largely excluded from this Chapter.

Textbooks and Reviews.—An unusually large number of reviews have appeared recently covering the occurrence and biosynthesis of D-amino-acids,[1a] the chemistry of β-amino-acids [1b] and of cyclic α-imino-acids,[1c] free radicals formed in condensation reactions of sugars with amino-acids,[2a] αβ-unsaturated and related amino-acids in peptides,[2b] and cross-linking residues in proteins.[2c] Amino-acids present in potato,[3] unusual amino-acids in fungi,[4] and the biological significance of *N*-methylated lysine and arginine derivatives,[5] are topics in recent reviews.

Reviews of amino-acid chemistry [6] and of the distribution of non-protein amino-acids [7] provide material which is largely complementary to that in this Report.

Other reviews and textbooks are cited in the various sections of this Chapter.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—The variations found in the proportions of amino-acids in fossils of different age are probably due to variable rates of decomposition of the protein amino-acids, but may indicate stages in molecular evolution.[8] There are several other papers in the earth sciences literature dealing

[1] 'Chemistry and Biochemistry of Amino-acids, Peptides, and Proteins', ed. B. Weinstein, Dekker, New York, 1977, Vol. 4, (*a*) J. S. Davies, p. 1; (*b*) C. N. C. Drey, p. 241; (*c*) A. B. Mauger, p. 179.

[2] (*a*) M. Namiki, T. Hayashi, and Y. Ohta, *Adv. Exp. Med. Biol.*, 1977, **86B**, 471; (*b*) E. Gross, *ibid.*, p. 131; (*c*) M. Friedman, *ibid.*, p. 1; N. P. Stimler and M. L. Tanzer, *ibid.*, p. 675; R. A. Anwar, G. E. Gerber, and K. M. Baig, *ibid.*, p. 709.

[3] R. L. M. Synge, *Potato Res.*, 1977, **20**, 1; A. M. C. Davies, *ibid.*, p. 9.

[4] J. L. C. Wright and L. C. Vining, *Filamentous Fungi*, 1976, **2**, 475.

[5] E. Tyihak, B. Szende, and K. Lapis, *Life Sciences*, 1977, **20**, 385.

[6] E. A. Bell and D. I. John, in 'MTP International Review of Science, Series Two', p. 1. vol. 6, ed. H. N. Rydon, Butterworths, London, 1976.

[7] A. Kjaer and P. O. Larsen, in 'Biosynthesis', A Specialist Periodical Report, ed. J. D. Bu'Lock, The Chemical Society, 1977, Vol. 5, p. 120.

[8] C. Ivanov and R. Stoyanova, *Doklady Bolg. Akad. Nauk*, 1977, **30**, 1129.

with the natural occurrence of amino-acids, but excepting those inferring fossil age from racemization (see Section 5), they are mostly of a routine analytical nature.

Further examples of the existence of D-amino-acids in pea seedlings (see Vol. 9, p. 2) are of aspartic and glutamic acids.[9] Other simple amino-acids (leucine, tyrosine, and phenylalanine) exist as their amides, together with 5-hydroxylysine and canavanine, in *Ladino* clover seeds.[10] Simple derivatives of familiar amino-acids which have been found in new locations are *O*-acetylserine (in *Nicotiana tabacum*),[11] 3-*N*-oxalyl-2,3-diaminopropionic acid in seeds of *Crotalaria*,[12] *Acacia*,[13] and *Lathyrus sativus*,[14] accompanied by the 2-*N*-oxalyl isomer in *Lathyrus*,[14] and 2-amino-3-ureidopropionic acid (*alias* albizzine) in *Dialium*.[15] The organic component which envelopes the silicaceous cell walls of diatoms includes 4-hydroxy- and 3,4-dihydroxy-L-proline.[16]

Less-common amino-acids in plants, reported in the recent literature, include cyclopentenylglycine in seeds of *Hydnocarpus anthelminthica*,[17] N^G-methylarginine and the N^GN^G-dimethyl analogue in seeds of broad bean,[18] octopine, octopinic acid, lysopine, and histopine in *Agrobacterium tumefaciens*-induced sunflower crown gall tumours,[19] and *S*-methylmethionine (vitamin U).[20] A useful review of unusual amino-acids in edible mushrooms, including *cis*-3-amino-L-proline in *Morchella esculenta*, L-2-aminohex-4-ynoic acid and its *threo*- and *erythro*-3-hydroxy- analogues with L-3-(3-carboxy-4-furyl)alanine in *Tricholomopsis rutilans* (see also Vol. 7, p. 3), and γ-propylidene-L-glutamic acid in *Mycena pura*, has appeared.[21] 2*S*,3*R*-2-Amino-3-hydroxypent-4-ynoic acid is a toxic amino-acid present in the fungus *Sclerotium rolfsii*.[22]

Although no attempt can be made to cover the full literature on microbial production of amino-acids, room is found for representative papers.

Auxotrophic mutants of *Pseudomonas aeruginosa* accumulate L,L-2,6-diaminopimelic acid,[23] and α-methylene-γ-aminobutyric acid, the enzymic decarboxylation product of γ-methylene-L-glutamic acid, occurs in *Mycena pura*[24] (*cf.* Vol. 8, p. 3). The purer realms of the biosynthesis literature include the conversion of L-lysine into ε-hydroxy-lysine by cell-free extracts of *Aerobacter aerogenes*,[25] and

[9] T. Ogawa, M. Kimoto, and K. Sasaoka, *Agric. Biol. Chem.*, 1977, **41**, 1811.
[10] T. Kasai, K. Furukawa, and S. Sakamura, *Agric. Biol. Chem.*, 1976, **40**, 2489.
[11] I. K. Smith, *Phytochemistry*, 1977, **16**, 1293.
[12] M. Y. Qureshi, D. J. Pilbeam, C. S. Evans, and E. A. Bell, *Phytochemistry*, 1977, **16**, 477.
[13] C. S. Evans, M. Y. Qureshi, and E. A. Bell, *Phytochemistry*, 1977, **16**, 565.
[14] F. L. Harrison, P. B. Nunn, and R. R. Hill, *Phytochemistry*, 1977, **16**, 1211.
[15] P. S. Peiris and A. Sirimawathie Seneviratne, *Phytochemistry*, 1977, **16**, 1821.
[16] D. Sadava and B. E. Volcani, *Planta*, 1977, **135**, 7.
[17] U. Cramer and F. Spener, *European J. Biochem.*, 1977, **74**, 495.
[18] T. Kasai, M. Sano, and S. Sakamura, *Agric. Biol. Chem.*, 1976, **40**, 2449.
[19] J. D. Kemp, *Biochim. Biophys. Res. Comm.*, 1977, **74**, 862; E. Hack and J. D. Kemp, *ibid.*, 1977, **78**, 785.
[20] A. A. Bezzubov and N. N. Gessler, *Priklady Biokhim. Mikrobiol.*, 1977, **13**, 301.
[21] S. Hatanaka, Y. Niimura, K. Taniguchi, F. Kinoshita, and H. Katayama, *Mushroom Science*, 1976, **9** (Part I), 809 (*Chem. Abs.*, 1977, **86**, 103 099).
[22] H. C. Potgieter, M. M. J. Vermeulen, D. J. J. Potgieter, and H. F. Strauss, *Phytochemistry*, 1977, **16**, 1757.
[23] F. Saleh and P. J. White, *J. Gen. Microbiol.*, 1976, **96**, 253.
[24] S. Hatanaka and K. Takishima, *Phytochemistry*, 1977, **16**, 1820.
[25] G. J. Murray, G. E. D. Clark, M. A. Parniak, and T. Viswanatha, *Canad. J. Biochem.*, 1977, **55**, 625.

L-ornithine into L-Δ^1-pyrroline-5-carboxylic acid by ornithine aminotransferase.[26] Since rumen ciliate protozoa can convert proline, ornithine, or arginine into δ-aminovaleric acid, the α-amino-acids must be on the biosynthetic pathway to the δ-amino-acid.[27] The controversy (see Vol. 9, p. 2) continues concerning the significance of the existence of saccharopine and 2-amino-adipic acid in higher plants to the lysine biosynthetic pathway.[28] The production of amino-acids by immobilized enzymes has been reviewed.[29]

Higher organisms are represented in papers reporting the presence of more than ten quaternary amines, including δ-valerobetaine, γ-butyrobetaine, and the betaines of glycine, valine, and homoserine, in the ovary of the shellfish *Callista brevishiphonata*,[30] and a similar mixture in the adductor muscle of the fan mussel *Atrina pectinata*.[31] The spruce budworm *Choristoneura fumiferana* contains *N*-phosphorylarginine.[32]

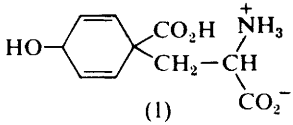
Methylated amino-acids identified as constituents of proteins provide new material for structure–function hypotheses. Ribosomal proteins of *Escherichia coli* carry *N*-terminal *N*-methylalanine, *N*-methylmethionine,[33, 34] and *N*-trimethylalanine [34] residues, and include a γ-methylglutamyl residue.[35] Cytochromes from *Crithidia oncopelti* and *Candida krusei* contain *NN*-dimethylproline [36] and N^{ϵ}-trimethyllysine [37] residues, respectively. N^{G}-Dimethylarginine occurs in sizeable amounts in non-histone nuclear proteins from rat-liver nuclei.[38] Majusculamides A and B contain *N*-methyl-*O*-methyl-D-tyrosine and *N*-methyl-L-valinamide residues.[39]

New Natural Free Amino-acids.—Sunflower plants infected with *Agrobacterium tumefaciens* develop crown gall tumours from which novel acidic amino-acids histopine [N^{α}-(1-carboxyethyl)-L-histidine] [19] and N^{α}-(1,3-dicarboxypropyl)-L-ornithine [40] have been isolated. Plant sources for other new amino-acids are *Gymnocladus dioicus*, a legume whose seeds have already proved to contain several uncommon amino-acids and from which L-*cis*-5-hydroxypipecolic acid has been isolated.[41] Related species *Morus alba* and *Lathyrus japonias* also contain this amino-acid.[42] The *trans*-configuration has been assigned to 4-

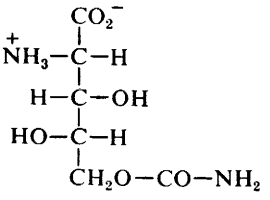
[26] R. J. Smih, S. J. Downing, and J. M. Phang, *Anal. Biochem.*, 1977, **82**, 170.
[27] R. Onodera, W. Tsutsumi, and M. Kandatsu, *Agric. Biol. Chem.*, 1977, **41**, 2169.
[28] R. Nawaz and H. Soerensen, *Phytochemistry*, 1977, **16**, 599.
[29] 'Methods in Enzymology', Vol. 44 (1976).
[30] T. Yasumoto and N. Shimizu, *Nippon Suisan Gakkaishi*, 1977, **43**, 201 (*Chem. Abs.*, 1977, **86**, 117 886).
[31] T. Hayashi and S. Konosu, *Nippon Suisan Gakkaishi*, 1977, **43**, 343.
[32] D. J. Durzan and J. A. Pitel, *Insect Biochem.*, 1977, **7**, 11.
[33] R. Chen, J. Brosius, B. Wittmann-Liebold, and W. Schaefer, *J. Mol. Biol.*, 1977, **111**, 173; R. Chen and U. Chen-Schmiesser, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 4905.
[34] F. Lederer, J. H. Alix, and D. Hayes, *Biochem. Biophys. Res. Comm.*, 1977, **77**, 470.
[35] S. J. Keene, M. L. Toews, and J. Adler, *J. Biol. Chem.*, 1977, **252**, 3214.
[36] G. W. Pettigrew and G. M. Smith, *Nature*, 1977, **265**, 661.
[37] D. J. Wilbur and A. Allerhand, *F.E.B.S. Letters*, 1977, **74**, 272.
[38] L. C. Boffa, J. Karn, G. Vidali, and V. G. Allfrey, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 969.
[39] F. J. Marner, R. E. Moore, K. Hirotsu, and J. Clardy, *J. Org. Chem.*, 1977, **42**, 2815.
[40] J. L. Firmin and R. G. Fenwick, *Phytochemistry*, 1977, **16**, 761.
[41] J. Despontin, M. Marlier, and G. Dardenne, *Phytochemistry*, 1977, **16**, 387.
[42] S. Hatanaka and S. Kaneko, *Phytochemistry*, 1977, **16**, 1041.

carboxy-L-proline, isolated from *Chondria coerulesceus*.[43] Lentinic acid, the *N*-(γ-L-glutamyl) derivative of $MeSO_2CH_2(SOCH_2)_3CH(NH_2)CO_2H$, has been isolated from *Lentinus edodes*.[44]

New amino-acids of interest in biosynthetic studies are β-hydroxy-N^{ϵ}-trimethyl-lysine, identified as an intermediate in the biosynthesis in *Neurospora crassa* of carnitine,[45] and 'pretyrosine' (1), on the pathway to L-tyrosine in blue-green algae *Pseudomonas aeruginosa*.[46] Aromatic amino-acids in *Cortinarius brunneus*



(1)



(2)

and in *Pachymatisma johnstoni* include 4-hydroxy-3-methoxy-L-phenylalanine[47] and 6-bromohypaphorine[48] (6-bromo-L-tryptophan N^{α}-trimethyl betaine), respectively.

Diastereoisomers of 2-amino-4-keto-3-methylpentanoic acid isolated from *Bacillus cereus* 439 are of particular interest as vitamin B_{12} antimetabolites.[49] The *N*-terminus of each of the nucleoside peptide antibiotics, the polyoxins, is 5-*O*-carbamoyl-2-amino-2-deoxy-L-xylonic acid (2; 'polyoxamic acid').[50] Full details are available of the isolation of 3-(2,5-*SS*-dicysteinyl-3,4-dihydroxyphenyl)-alanine from the tapetum lucidum of alligator eye (see Vol. 8, p. 4).[51]

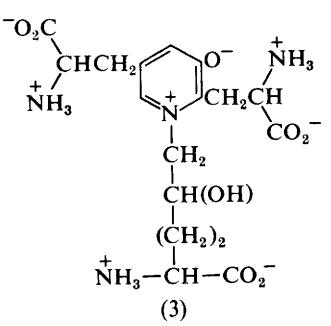
Higher homologues of the amino-acids are represented in *N*-β-alanyldopamine, from wings of *Papilio xuthus*,[52] and 4-acetamido-2-butenoic acid ($MeCONHCH_2CH_2CH{=}CHCO_2H$) from *Fusarium graminearum*.[53]

New Amino-acids from Hydrolysates.—In a previous section, the occurrence of unusual, but known, amino-acids in peptides and proteins has been surveyed, and this section is exclusively concerned this year with residues at cross-link sites in proteins.[2b, 2c]

Analogues of the familiar lysine-based cross-links desmosine and allysine have arisen in protein studies, with the identification of hydroxyallysine as an intermediate in the formation of collagen cross-links,[54] ε-(γ-glutamyl)lysine as a

[43] G. Impellizzeri, M. Piatelli, S. Sciuto, and E. Fattorusso, *Phytochemistry*, 1977, **16**, 1601.
[44] K. Yasumoto, K. Iwami, H. Mizusawa, and H. Mitsuda, *Nippon Nogei Kagaku Kaishi*, 1976, **50**, 563 (*Chem. Abs.*, 1977, **86**, 185 866); G. Höfle, R. Gmelin, H.-H. Luxa, M. N'Galamulume-Treves, and S. I. Hatanaka, *Tetrahedron Letters*, 1976, 3129.
[45] R. A. Kaufman and H. P. Broquist, *J. Biol. Chem.*, 1977, **252**, 7437.
[46] N. Patel, D. L. Pierson, and R. A. Jensen, *J. Biol. Chem.*, 1977, **252**, 5839.
[47] G. Dardenne, M. Marlier, and A. Welter, *Phytochemistry*, 1977, **16**, 1822.
[48] W. D. Raverty, R. H. Thomson, and T. J. King, *J.C.S. Perkin I*, 1977, 1204.
[49] D. Perlman, K. I. Perlman, M. Bodanszky, A. Bodanszky, R. L. Foltz, and H. W. Matthews, *Bio-org. Chem.*, 1977, **6**, 263.
[50] S. Funuyama and K. Isono, *Biochemistry*, 1977, **16**, 3121.
[51] S. Ito and J. A. C. Nicol, *Biochem. J.*, 1977, **161**, 499.
[52] Y. Umebachi and H. Yamashita, *Comp. Biochem. Physiol. B*, 1977, **56**, 5.
[53] R. F. Vesonder, L. W. Tjarks, A. Ciegler, G. F. Spencer, and L. L. Wallen, *Phytochemistry*, 1977, **16**, 1296.
[54] R. C. Siegel, *J. Biol. Chem.*, 1977, **252**, 254.

cross-link in the keratin fraction of human stratum corneum,[55] and the novel desmosine relatives pyridinoline (3) and anabilysine (4), the fluorescent material from bovine Achilles tendon collagen,[56] and the cross-link residue in glutaraldehyde-treated ovalbumin,[57] respectively.



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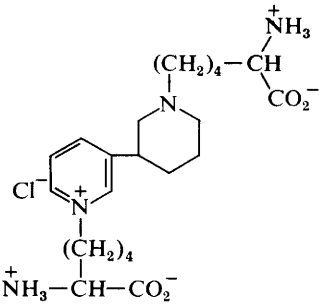
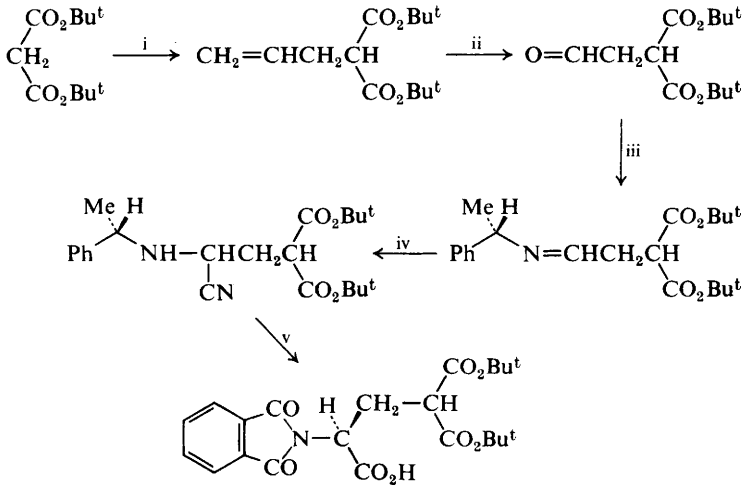


(4)

3 Chemical Synthesis and Resolution of Amino-acids

Asymmetric Synthesis.—The general possibilities for asymmetric synthesis of α-amino-acids illustrated in recent Volumes of this Report are developed further in studies published in 1977. Routes based on chiral Schiff bases give variable asymmetric yields, but



Reagents: i, allyl bromide; ii, O_3; iii, (−)-PhCHMeNH$_2$; iv, HCN; v, separation of diastereoisomers and subsequent steps according to previously-established procedure (Vol. 9, p. 5)

Scheme 1

[55] J. L. Abernethy, R. L. Hill, and L. A. Goldsmith, *J. Biol. Chem.*, 1977, **252**, 1837.
[56] D. Fujimoto, K. Akiba, and N. Nakamura, *Biochem. Biophys. Res. Comm.*, 1977, **76**, 104.
[57] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J.C.S. Chem. Comm.*, 1977, 759.

L-γ-carboxyglutamic acid has been obtained as its *N*-phthaloyl γγ-di-t-butyl ester derivative in 100% optical purity (Scheme 1).[58] The alternative approach, alkylation of the Schiff base formed between a chiral ketone and an α-amino-acid ester, has been studied for the asymmetric synthesis of α-methyl-α-amino-acids from DL-alanine t-butyl ester.[59] A variant of this procedure, alkylation of the cobalt(III) complex of *N*-salicylideneglycine, has been used for the synthesis of L-glutamic acid from methyl acrylate;[60] electrochemical reduction was used in this case[60] and in an extraordinary example, cathodic reduction of *syn*- or *anti*-phenylglyoxylic acid oximes leading to *R*(−)-phenylglycine predominantly at cathodic potentials below 1.4 V, and to enantiomeric excesses of the *S*-isomer at potentials above this value,[61] when strychnine is present.

Prochiral acylaminoacrylates and cinnamates give moderate asymmetric yields of corresponding *N*-acylamino-acids by hydrogenation in the presence of chiral phosphine–rhodium complex catalysts.[62–64] This system is now more a test-bed for new homogeneous catalysts and no additional interest in amino-acid synthesis has emerged from the most recent papers.

Higher homologous amino-acids for which asymmetric syntheses have been reported are 3-aminobutanoic acid (Michael addition of a chiral amine to crotononitrile followed by hydrolysis and catalytic reduction),[65] and *S*-homoproline and *S*-homopipecolic acid *via* the corresponding chiral lactams.[66]

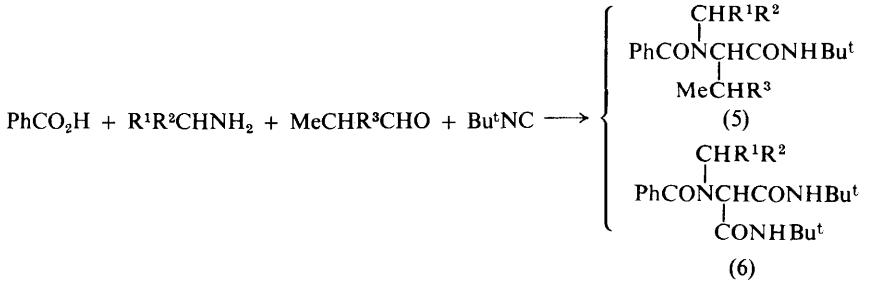
General Methods of Synthesis of α-Amino-acids.—The preceding section has served to preview some standard synthetic methods, but a broad view of synthetic methods, both those of long standing and others undergoing current evaluation, is attempted here.

Direct methods of assembly of α-amino-acids, either by alkylation of glycine derivatives[59,60,67] (including α-hydroxy- and -methoxyglycines[68]) and alanine derivatives[59,67] or by the carboxylation of aliphatic amines[69] are of special interest. Ureidoalkylation of arenes must by now be one of the methods of choice for the synthesis of aryl-substituted phenylglycines[68a] and certain aliphatic amino-acids,[68c] while carbanion alkylation involving glycine-derived Schiff bases[59,60] shows signs of conforming to the requirements of reliable high-yield procedures so that these routes, too, may more credibly enter the standard repertoire (but unwanted di-alkylation can be troublesome[59]). The most interesting paper in this area[69] describes γ-radiolytic carboxylation of amines in aqueous

[58] M. Oppliger and R. Schwyzer, *Helv. Chim. Acta*, 1977, **60**, 43.
[59] T. Oguri, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.*, 1977, **25**, 2287.
[60] Y. N. Belokon, T. F. Saveleva, and M. B. Saporovskaya, *Izvest. Akad. Nauk. S.S.S.R., Ser. khim.*, 1977, 428.
[61] M. Jubault, E. Raoult, J. Armand, and L. Boulares, *J.C.S. Chem. Comm.*, 1977, 250.
[62] M. D. Fryzuk and B. Bosnich, *J. Amer. Chem. Soc.*, 1977, **99**, 6262.
[63] K. Achiwa, *Chem. Letters*, 1977, 777.
[64] R. Glaser and J. Blumenfeld, *Tetrahedron Letters*, 1977, 2525; R. Glaser and S. Geresh, *ibid.*, p. 2527; R. Glaser, M. Twaik, S. Geresh, and J. Blumenfeld, *ibid.*, p. 4635; R. Glaser, J. Blumenfeld, and M. Twaik, *ibid.*, p. 4639.
[65] M. Furukawa, T. Okawara, and Y. Terawaki, *Chem. Pharm. Bull.*, 1977, **25**, 1319.
[66] T. Wakabayashi, K. Watanabe, and Y. Kato, *Synth. Comm.*, 1977, **7**, 239.
[67] J. J. Fitt and H. W. Gschwend, *J. Org. Chem.*, 1977, **42**, 2639.
[68] (*a*) D. Ben-Ishai and Z. Bernstein, *Tetrahedron*, 1977, **33**, 3261; (*b*) D. Ben-Ishai, J. Altman, and N. Peled, *ibid.*, p. 2715; (*c*) D. Ben-Ishai, R. Moshenberg, and J. Altman, *ibid.*, p. 1533.
[69] A. Davison, N. T. Barker, and D. F. Sangster, *Austral. J. Chem.*, 1977, **30**, 807.

formate buffers; $EtNH_2$ gives a mixture of alanine and β-alanine, isomeric aminopropanes give all possible mono-carboxylation products, while proline is formed from either 1,4-diaminobutane or pyrrolidine (accompanied by ornithine or 3-carboxyproline, respectively).

Use of isocyanides is illustrated in a study of the Ugi synthesis, exploring the influence of reactant concentrations on the proportions of the four-component condensation product (5) and of the side-product [6; an interesting puzzle is provided by the fact that (6) is not formed when the aldehyde component is omitted] when used for the synthesis of amino-malonic acid derivatives (Scheme 2).[70] β-Branched amino-acid derivatives result from the Michael addition of carbanions to isocyanoacrylate esters.[71]



Scheme 2

Reduction of α-keto-acid oximes,[61, 72] phenylhydrazones,[73] or Schiff bases [60, 74] gives corresponding α-amino-acid derivatives. Other standard general methods which have been used are alkylation of diethyl acetamidomalonate,[49, 114, 130, 147, 151, 159, 160a, 172] the hydantoin synthesis,[57, 75] including the synthesis of hydantoins in moderate yields from anodic oxidation of an alcohol with ammonium carbonate and KCN,[75] substitution reactions of α-halogenopropionates [76] and γ-bromobutyrates,[95] and alkylation of glycines,[125] α-isocyano- and -nitro-propionates,[99] and azlactones.[148]

Prebiotic Synthesis; Model Reactions.—A still larger number of papers has appeared on this topic, partly due to studies of the scope for organic synthesis in models of the present environments on other planets, as opposed to primordial Earth (*e.g.* Mars, Jupiter). Apart from the chemistry of hydrogen cyanide polymers, there are few significant new additions to synthetic methods arising from these studies. The pioneers in this field have reviewed the origin of organic compounds on Earth and in meteorites.[77]

Studies of gas-phase reactions of a familiar type but in a novel context – the ammonia-rich atmosphere of Jupiter – have shown that HCN and higher alkanes

[70] A. Gieren, B. Dederer, G. George, D. Marquarding, and I. Ugi, *Tetrahedron Letters*, 1977, 1503.
[71] U. Schöllkopf and R. Meyer, *Annalen*, 1977, 1174.
[72] J. Pospisek and K. Blaha, *Coll. Czech. Chem. Comm.*, 1977, **42**, 1069.
[73] I. Tabakovic, M. Trkovnik, and M. Dzepina, *Croat. Chem. Acta*, 1977, **49**, 497.
[74] K. Nakamura, A. Ohno, and S. Oka, *Tetrahedron Letters*, 1977, 4593.
[75] E. P. Krysin, V. V. Tsodikov, and V. A. Grinberg, *Elektrokhimiya*, 1976, **12**, 1590.
[76] Y. Nakajima, R. Kinishi, J. Oda, and Y. Inouye, *Bull. Chem. Soc. Japan*, 1977, **50**, 2025.
[77] S. L. Miller, H. C. Urey, and J. Oro, *J. Mol. Evol.*, 1976, **9**, 59.

can be formed by photolysis of $NH_3 : H_2 : He : CH_4$ (1 : 15 : 2 : 3)[77] and that electric discharge in a similar mixture containing water can lead to amino-acids when cyanide ions are also present.[78] An unexpected result,[79] the formation of porphyrin-like pigments in such systems, has been reported, and the continuing investigations of another group of workers have demonstrated further the formation of amino-acids and urea from glow-discharge electrolysis of aqueous ammonia in the presence of elemental carbon,[81] or of bicarbonate, or formate ions.[82]

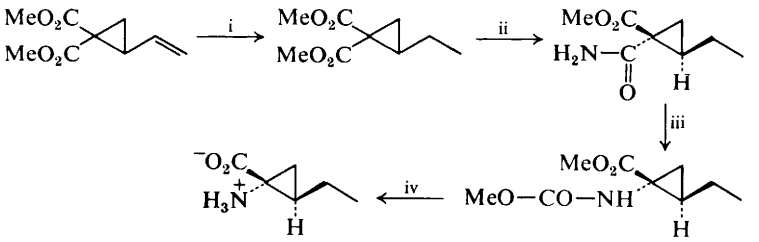
Hydroxylamine–formaldehyde mixtures have been shown [82–85] to be capable of yielding about 40 amino-acids in aqueous solution at pH 5.5, at 105 °C with [82] or without kaolin.[83] Transition metal molybdates are important in influencing the relative amounts of alanine, aspartic acid, β-alanine, and particularly proline, at the expense of glycine and serine.[84] These studies are relatively unusual in this area in not involving some external energy supply (electromagnetic or acoustic) but the production of glycine and alanine from hydroxylamine-formaldehyde in high-intensity ultrasound [85] was reported a little earlier. If simple monosaccharides are regarded as oligomers of formaldehyde, the reported formation of amino-acids in aqueous solutions of sugars in the presence of nitrates under N_2,O_2, or CO_2, in u.v. light,[86] at first sight a refreshing new approach, becomes more easily related to conventional studies in this area. A more extraordinary detail from this study, however, is that exclusion of nitrate does not bring amino-acid synthesis to a halt, amino-groups in glutamic acid and lysine formed under these conditions originating from atmospheric nitrogen.[86]

Aqueous solutions of HCN exposed to ^{60}Co γ-radiation form polymers from which glycine, alanine, valine, serine, threonine, aspartic and glutamic acids, amongst other compounds, are formed by hydrolysis.[87] ^2H-Labelling studies show that poly(aminomalononitriles) formed from HCN–water mixtures under u.v.-irradiation are the major sources of α-amino-acids formed by hydrolysis of the reaction product.[88] The same intermediate may be involved in the pathway from NH_3–CH_4–H_2O electric discharge reaction mixtures to α-amino acids,[88] and Matthews, Minard, and co-workers argue convincingly that the lower energy of the reaction pathway on which this intermediate lies gives the hypothesis still more support.[88]

Protein and Other Naturally Occurring Amino-acids.—Several examples of the use of standard general methods of synthesis of α-amino-acids, as well as unusual

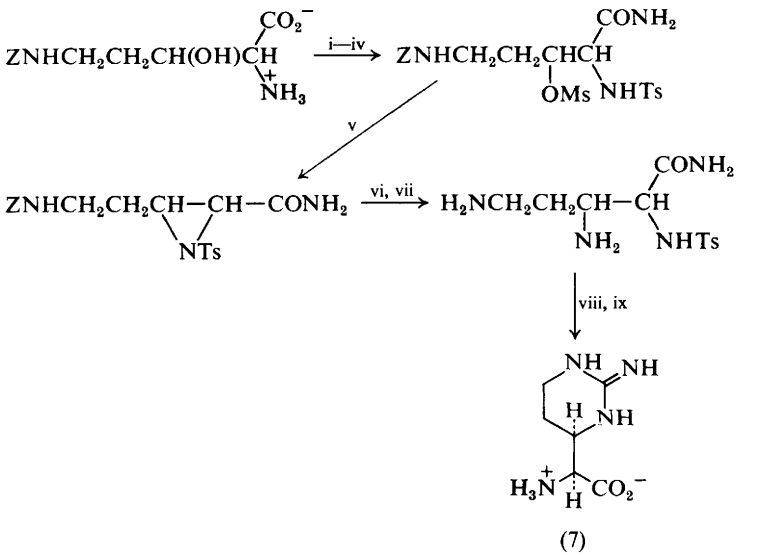
[78] J. P. Ferris, C. Nakagawa, and C. T. Chen, *Life Sci. Space Res.*, 1977, **15**, 95.
[79] V. I. Kalinichenko, V. B. Bondarev, M. V. Gerasimov, L. M. Mukhin, and E. N. Safonova, *Doklady Akad. Nauk. S.S.S.R.*, 1977, **236**, 245.
[80] C. I. Simionescu, B. C. Simionescu, R. Mora, M. Leanca, and E. Ioanid, *Compt. rend.*, 1977, **284**, 743.
[81] (*a*) K. Harada and S. Suzuki, *Naturwiss.*, 1977, **64**, 484; (*b*) *Nature*, 1977, **266**, 275.
[82] H. Hatanaka and F. Egami, *Bull. Chem. Soc. Japan*, 1977, **50**, 1147.
[83] M. Ventilla and F. Egami, *J. Mol. Evol.*, 1977, **9**, 105.
[84] H. Hatanaka and F. Egami, *J. Biochem.*, 1977, **82**, 499.
[85] A. Sokolskaya, *Origins Life*, 1976, **7**, 183.
[86] M. A. Khenokh and M. V. Nikolaeva, *Zhur. Evol. Biokhim. Fiziol.*, 1977, **13**, 105 (*Chem. Abs.*, 1977, **86**, 184 776); *Studia Biophys.*, 1977, **63**, 1.
[87] M. A. Sweeney, A. P. Toste, and C. Ponnamperuma, *Origins Life*, 1976, **7**, 187.
[88] C. Matthews, J. Nelson, P. Varma, and R. Minard, *Science*, 1977, **198**, 622.

methods, are illustrated in this section. A simple synthesis of DL-proline from pyrrolidine, giving an overall 45% yield, involves successive *N*-chlorination, dehydrochlorination, and addition of HCN to the resulting 1-pyrroline followed by hydrolysis.[89] A one-pot synthesis of 4-hydroxyproline from glyoxal and oxaloacetic acid with NH_4OH under physiological conditions, followed by reduction with sodium borohydride, gives a 40% yield.[90] Dieckmann cyclization of an *N*-(2-methoxycarbonylethyl)glycine ester represents another approach to the same ring system, and has been used for the synthesis of the stereoisomer of 3-hydroxy-5-methylproline recently shown to be a constituent of Actinomycin Z_1 (see Vol. 8, p. 5).[91]



Reagents: i, tosyl hydrazide, diglyme; ii, NH_3 in MeOH; iii, Br_2–NaOH/MeOH; iv, H_2O

Scheme 3



(7)

Reagents: i, TsCl; ii, CH_2N_2; iii, NH_3; iv, MsCl; v, Et_2NH; vi, NH_3; vii, H_2–Pd; viii, BrCN; ix, HBr

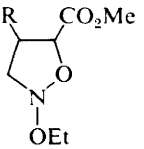
Scheme 4

[89] U. Schmidt and H. Poisel, *Angew. Chem., Internat. Edn.*, 1977, **16**, 777.
[90] S. G. Ramaswamy and E. Adams, *J. Org. Chem.*, 1977, **42**, 3440.
[91] A. B. Mauger, O. A. Stuart, E. Katz, and K. T. Mason, *J. Org. Chem.*, 1977, **42**, 1000.

Out-of-the-way methods are mandatory for 1-aminocycloalkanecarboxylic acids, as illustrated in Scheme 3 for the synthesis of coronamic acid.[92] More complex ring systems are present in L-capreomycidine (7 in Scheme 4) and discadenine -[6-(3-methyl-2-butenylamino)purin-3-yl]butyrine (Vol. 9, p. 4),[93] and a new synthesis of the former amino-acid has been reported (Scheme 4),[94] as well as a first synthesis of the latter from the purine and ethyl α-phthalimido-β-bromobutyrate.[95]

A reliable procedure has been worked out[96] for the preparation of N^4-ethyl-L-asparagine (see also Vol. 8, p. 14). Serine is obtained[74a] by 18-crown-6-catalysed reaction of azide ion with methyl 3-hydroxy-2-bromopropionate followed by reduction, but isoserine is also formed when the same reaction is used, but without catalysis.[74a]

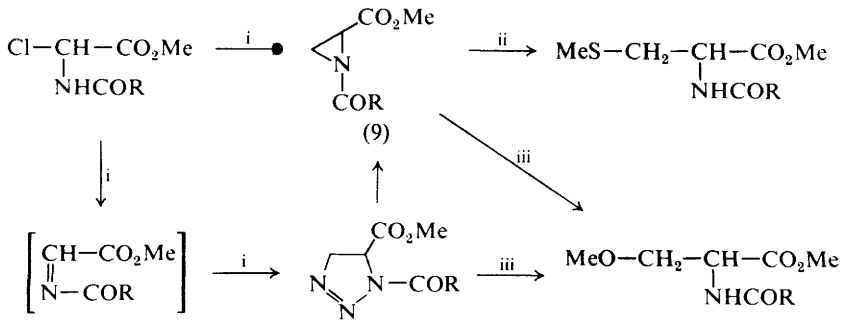
Among higher homologous amino-acids which occur in peptide antibiotics,4-amino-2-hydroxybutanoic acid and its 3-methyl derivative have been synthesized



(8)

from the isoxazolidones (8; R = H and R = Me, respectively), readily obtained from the nitrone CH_2=NO(OEt) and acrylates RCH=$CHCO_2$Me.[97]

Derivatives of *O*-methylserine and *S*-methyl cysteine may be obtained from glycine *via* *N*-benzyloxycarbonylaziridine-2-carboxylates (9 in Scheme 5).[98]



Reagents: i, CH_2N_2; ii, MeSH; iii, MeOH

Scheme 5

[92] A. Ichihara, K. Shiraishi, and S. Sakamura, *Tetrahedron Letters*, 1977, 269.
[93] T. Nomura, Y, Tanaka, H. Abe, and M. Uchiyama, *Phytochemistry*, 1977, **16**, 1819,
[94] T. Shiba, T. Ukita, K. Mizuno, T. Teshima, and T. Wakamiya, *Tetrahedron Letters*, 1977, 2681.
[95] M. Uchiyama and H. Abe, *Agric. Biol. Chem.*, 1977, **41**, 1549.
[96] R. W. Dineen and D. O. Gray, *Org. Prep. Proced. Internat.*, 1977, **9**, 39.
[97] H. Sato, T. Kusumi, K. Imaye, and H. Kakisawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 2815.
[98] Z. Bernstein and D. Ben-Ishai, *Tetrahedron*, 1977, **33**, 881.

α-Alkyl Analogues of the Protein Amino-acids.—The property of powerful reversible inhibition of amino-acid decarboxylases by α-methyl analogues of some α-amino-acids stimulated the search for convenient synthetic methods. Alkylation of methyl α-isocyanopropionate or the α-nitro analogue with acetoxymethylimidazole has been employed for the synthesis of α-methylhistidine,[99] while similar alkylation of an alanine Schiff base [100, 101] has been developed into a satisfactory new synthesis of α-methylornithine (see also Vol. 7, p. 10). Full experimental details have been published [102] of the synthesis of α-methyl-α-amino-acids by cathodic reduction of an alanine Schiff base in the presence of an alkyl halide, followed by hydrogenolysis.[102]

A novel example of the oxo-Wittig rearrangement, resulting in the conversion of *N*-benzyloxycarbonyl-L-proline into α-benzylproline,[103] may be applicable to other amino-acid derivatives; it involves successive treatment with $LiPr^{i}_{2}N$ and benzyl chloride.

α-Alkoxy-, α-Alkylamino-, and α-Alkylthio-analogues of the Protein Amino-acids.—A novel oxidative alkoxylation procedure, in which an *N*-acylamino-acid is treated with dicyclohexylcarbodi-imide in an alcohol, involves the corresponding oxazol-5(4*H*)-one as intermediate, but has yet to be shown to be applicable other than in the favourable case of *N*-phenylacetyl phenylglycine.[104] Another novel synthesis involving the reaction of the αβ-dehydro-amino-acid with thallium(III) acetate gives a mixture of corresponding αβ-dimethoxy-α-*N*-acylamino-acid ester diastereoisomers.[105]

Further development of methods discussed in recent Volumes of this series deals with the formation of 2-acetoxy-2-acylamino-acids from corresponding acylaminomalonic acid mono-esters by anodic oxidation [106] and a surprising synthesis under these conditions of 3-acetoxy-2-acylamino-alkanoic acids from corresponding β-alkylaspartates,[106] also full details of the synthesis of α-hetero-atom-substituted α-amino-acid derivatives from *o*-chloranil–oxazol-5(4*H*)-one adducts.[107] A review of α-mercapto-α-amino-acids has appeared.[108]

Side-chain Halogenated Analogues of the Protein Amino-acids.—While fluorine-substituted protein amino-acids in particular are important as potential or actual enzyme inhibitors, halogenoalkyl amino-acids more generally provide useful intermediates for the synthesis of other compounds.

Conversion of hydroxyalkyl amino-acids into halogenoalkyl analogues has been achieved using PCl_5 [109] or Ph_3P–CBr_4 [110] respectively for the preparation of *erythro*-

[99] M. Miyoshi, K. Matsumoto, and T. Miyahara, Japan Kokai, 76/115 474 (*Chem. Abs.*, 1977, **86**, 155 651).
[100] P. Bey and J. P. Vevert, *Tetrahedron Letters*, 1977, 1455; 1978, 1215.
[101] G. Stork, A. Y. W. Leong, and A. Tonzin, *J. Org. Chem.*, 1976, **41**, 3491.
[102] T. Iwasaki and K. Harada, *J.C.S. Perkin I*, 1977, 1730.
[103] P. A. Crooks, R. H. B. Galt, and Z. S. Matusiak, *Chem. and Ind.*, 1976, 693.
[104] K. Tajima, *Chem. Letters*, 1977, 279; *Noguchi Kenkyusho Jiho*, 1977, **20**, 24 (*Chem. Abs.*, 1978, **88**, 62 318); Japan Kokai, 77/53 832 (*Chem. Abs.*, 1977, **87**, 117 674).
[105] M. P. Paradisi and G. P. Zecchini, *Tetrahedron*, 1977, **33**, 1729.
[106] T. Iwasaki, H. Horikawa, K. Matsumoto, and M. Miyoshi, *J. Org. Chem.*, 1977, **42**, 2419.
[107] J. M. Riordan, M. Sato, and C. H. Stammer, *J. Org. Chem.*, 1977, **42**, 236.
[108] U. Schmidt, *Pure Appl. Chem.*, 1977, **49**, 163.
[109] A. Srinivasan, R. W. Stephenson, and R. K. Olsen, *J. Org. Chem.*, 1977, **42**, 2256.
[110] T. Wieland, D. Schermer, G. Rohr, and H. Faulstich, *Annalen*, 1977, 806.

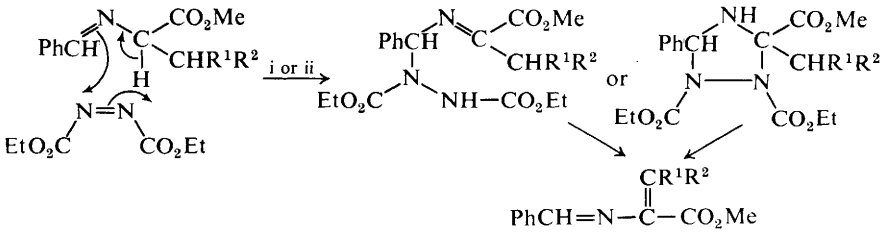
β-chloro- and -bromo-α-aminobutyric acid derivatives from threonine. Similar substitution reactions have been accomplished for hydroxyproline.[110] Less direct methods are involved for certain fluoro-substituted amino-acids; 4,4-difluoro-L-proline has been prepared from hydroxy-L-proline *via* 4-oxoproline-dioxopiperazine using SF_4–HF as reagent,[111] while a synthesis of 2-^{2}H-3-fluoro-D-alanine uses fluoropyruvic acid as starting material.[112] ββ-Difluoroaspartic acid, and the correspondingly substituted asparagine, have been prepared by fluorination of di-t-butyl oxaloacetate and conventional elaboration of the oxime of the product.[113] Long routes to 5,5-difluorolysine [114] (starting from 2-acetylaminomalonic acid ester) and to trifluoro-DL-alanine (starting from ethyl 2-iodo-2-trifluoromethylpropanoate formed by radical addition of CF_3I to $CH_2{=}CHCO_2Et$) [115] have been announced.

Aliphatic Amino-acids Carrying Hydroxy-groups in Side-chains.—Copper complexes of glycine Schiff bases have been used for the synthesis of β-hydroxyalkyl-α-amino-acids by alkylation by aldehydes;[116, 117] α-hydroxymethylserine,[116] and threonine, phenylserine, and β-hydroxytryptophan [117] have been obtained in recent studies of this well established route. An interesting outcome of one of these studies [117] is the formation of 3-methoxycarbonylproline from the salicylylglycine ethyl ester–copper(II) complex and methyl acrylate.

A straightforward route to DL-*cis*- and -*trans*-3-hydroxyprolines,[118] and a stereoselective synthesis of *threo*-3-hydroxy-4-amino-acids *via* pyrrolidin-2-ones,[119] have been reported.

Amino-acids with Unsaturated Side-chains.—Two main areas of interest, the propensity of 1-alkenyl- and -alkynyl-homologues of the protein amino-acids to act as powerful irreversible inhibitors of amino-acid decarboxylases, and the existence of dehydro-amino-acids (particularly αβ-unsaturated α-amino-acids) in certain naturally-occurring peptides,[108] have stimulated increased efforts towards efficient synthesis of the amino-acids concerned. Pride of place in this section should go to the novel route to dehydro-amino-acid imines involving an ene reaction between *N*-benzylidene amino-acid esters (of valine, phenylalanine, or isoleucine) and diethyl azodicarboxylate (Scheme 6).[120] Triazolidines are also formed. Other routes to dehydro-analogues of protein amino-acids which are represented in recent papers are already well-established (dehydrochlorination of an *N*-chloro-*N*-acylamino-acid ester,[121, 122] thermolysis of β-alkylsulphinyl-

[111] F. N. Shirota, H. T. Nagasawa, and J. A. Elberling, *J. Medicin. Chem.*, 1977, **20**, 1176.
[112] G. Gal, J. M. Chemerda, D. F. Reinhold, and R. M. Purick, *J. Org. Chem.*, 1977, **42**, 142.
[113] J. J. M. Hageman, M. J. Wanner, G. J. Koomen, and U. K. Pandit, *J. Medicin. Chem.*, 1977, **20**, 1677.
[114] F. N. Shirota, H. T. Nagasawa, and J. A. Elberling, *J. Medicin. Chem.*, 1977, **20**, 1623.
[115] Y. Maki and K. Inukai, *Yuki Gosei Kagaku Kyokai Shi*, 1976, **34**, 722 (*Chem. Abs.*, 1977, **87**, 68 600).
[116] M. J. O'Connor, J. R. Brush, and S.-B. Teo, *Austral. J. Chem.*, 1977, **30**, 683.
[117] Y. N. Belokon, N. G. Faleev, V. M. Belikov, V. A. Maksakov, P. V. Petrovskii, and V. A. Tsyryapkin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 890.
[118] R. P. Philp and A. V. Robertson, *Austral. J. Chem.*, 1977, **30**, 123.
[119] T. Katsuki and M. Yamaguchi, *Bull. Chem. Soc. Japan*, 1976, **49**, 3287.
[120] R. Grigg and J. Kemp, *J.C.S. Chem. Comm.*, 1977, 125; R. Grigg, J. Kemp, G. Sheldrick and J. Trotter, *ibid.*, 1978, 109.
[121] A. J. Kolar and R. K. Olsen, *Synthesis*, 1977, 457.
[122] H. Poisel, *Chem. Ber.*, 1977, **110**, 942, 948.



Reagents: i, 130 °C, 48 h; ii, boiling benzene or toluene, 0.5—24 h

Scheme 6

amino-acids in the presence of a phosphine or phosphite as sulphenic acid acceptor,[123] base-catalysed elimination of β-chloroalkyl amino-acid derivatives,[109] and rearrangement of acylimines formed by treatment of *o*-chloranil–oxazol-5(4*H*)-one adducts with base [107]).

Side-chain dehydrogenation of *N*-benzyloxycarbonyl-L-tryptophan by *Chromobacterium violaceum* [124] involves *syn*-elimination leading to the *Z*-isomer.

βγ-Unsaturated amino-acids may be synthesized by successive alkylation and carboxylation of the silylated propargylamine Schiff base $PhCH{=}NCH_2C{\equiv}CSiMe_3$;[125] α-ethynyl- and α-vinyl-dopas have been prepared in this way.[125] Vinylglycine has been synthesized previously, but only in modest yield, and a reliable alternative synthesis from acrolein cyanohydrin *via* 2-bromobut-3-enoic acid has been established.[126] Homologues, *e.g.* isodehydrovaline $CH_2{=}CMeCH(\overset{+}{N}H_3)\text{-}CO_2^-$, are obtainable from corresponding α-nitroacrylates [$Me_2C{=}CHCO_2Me + HNO_3 \rightarrow Me_2C{=}C(NO_2)CO_2Me$] by base isomerization.[126]

Aromatic and Heterocyclic Amino-acids.—A number of specifically interesting syntheses can be cited here; simple new amino-acids prepared by standard methods are listed later. Phenylalanine yields a mixture of *o*, *m*, and *p*-tyrosines and dihydroxyphenylalanines by reaction in acetate buffer (pH 6.0) with ascorbic acid in the presence of Cu^{2+} ions.[127] Tyrosine is converted into dopa by horseradish or mushroom peroxidase.[128] An alternative synthesis of cyclodopa from dopa methyl ester using potassium iodate for effecting the cyclization involves an iodoquinonimine intermediate.[129]

3-(5-Hydroxy-6-oxo-1,6-dihydro-2-pyridyl)-DL-alanine, a 2(1*H*)-pyridone isomer of mimosine, and its 1-hydroxy-2-oxo-1,2-dihydro-4-pyridyl isomer have been synthesized.[130]

***N*-Substituted Amino-acids.**—Studies of a conventional type are represented in the synthesis of side-chain mono-, di-, and tri-methyl arginines from ornithine and

[123] D. H. Rich and J. P. Tam, *J. Org. Chem.*, 1977, **42**, 3815.
[124] M. E. Gustafson, D. Miller, P. J. Davis, J. P. Rosazza, C. Chang, and H. G. Floss, *J.C.S. Chem. Comm.*, 1977, 842.
[125] D. Taub and A. A. Patchett, *Tetrahedron Letters*, 1977, 2745; B. W. Metcalf and K. Jund, *ibid.*, 1977, 3689.
[126] J. E. Baldwin, S. B. Haber, C. Hoskins, and L. I. Kruse, *J. Org. Chem.*, 1977, **42**, 1239.
[127] S. Ishimitsu, S. Fujimoto, and A. Ohara, *Chem. Pharm. Bull.*, 1977, **25**, 471.
[128] R. P. Patel and M. R. Okun, *Physiol. Chem. Phys.*, 1977, **9**, 85.
[129] G. Büchi and T. Kamikawa, *J. Org. Chem.*, 1977, **42**, 4153.
[130] R. L. N. Harris and T. Teitei, *Austral. J. Chem.*, 1977, **30**, 649.

correspondingly *N*-methylated *S*-methylisothiouronium iodide,[131] and of N^{β}-alkyl-β-amino-alanines.[132]

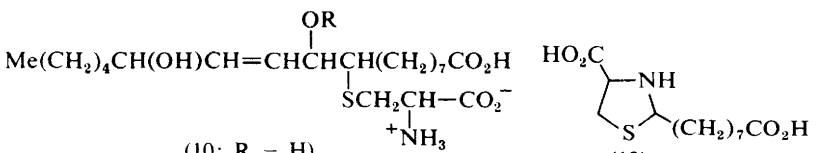
Secondary amines formally derived from two α-amino-acids have been known for many years, and have become of renewed interest recently because of their occurrence as metabolites of crown gall tumours.[19, 40] Diastereoisomer mixtures formed from α-ketoglutaric acid by condensation with L-arginine followed by borohydride reduction[133, 134] have been separated into the natural product nopaline, and its isomer isonopaline; all four isomers of octopine, formed similarly from D- or L-arginine and pyruvic acid, have been obtained.[135] Lysinoalanine, a structurally-similar secondary amine, is in equilibrium with lysine and dehydroalanine in aqueous solution.[136, 137]

Modification of the amino-acid amine function can be brought about directly in certain cases, *e.g.* the preparation of 1-nitro-proline, -pipecolic acid, and -sarcosine,[138] by peroxytrifluoroacetic acid oxidation of the *N*-nitroso-imino-acids,[138] but *N*-hydroxy-amino-acids are best prepared from an α-keto-acid and hydroxylamine followed by sodium cyanoborohydride reduction.[139]

α-Aza-amino-acids.—New results on analogues in which the α-CH group of the protein amino-acids is replaced by a nitrogen atom are the synthesis[140] of N^{α}-ethoxycarbonyl-α-aza-ornithine phenyl ester, and the unusually high tendency for *N*-acyl derivatives to cyclize to oxadiazolones.[141]

α-Amino-acids containing Sulphur or Selenium.—Optically-active cysteine derivatives are available in optical yields up to 54% by addition of a thiol to methyl α-phthalimidoacrylate or to a 4-methyleneoxazolone in the presence of a cinchona alkaloid.[142] A stereospecific synthesis of (2*S*,3*R*)-2-amino-3-mercaptobutyric acid employs Boc-D-allothreonine methyl ester as starting material.[143]

The preparation of *S*-substituted cysteines generally involves routine methods, but the reaction of cysteine with linoleic acid hydroperoxide in ethanol to give (10)—(12)[144] is of particular interest. L-Cysteine gives 2*S*,5*S*-, and 2,5-*SS*-di-



(10; R = H)
(11; R = Et)

(12)

[131] A. Patthy, S. Bajusz, and L. Patthy, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1977, **12**, 191.
[132] R. S. Asquith, K. W. Yeung, and M. S. Otterburn, *Tetrahedron*, 1977, **33**, 1633.
[133] D. Cooper and J. L. Firmin, *Org. Prep. Proced. Internat.*, 1977, **9**, 99.
[134] R. E. Jensen, W. T. Zydbak, K. Yasuda, and W. S. Chilton, *Biochem. Biophys. Res. Comm.*, 1977, **75**, 1066.
[135] J. F. Biellmann, G. Braulaut, and L. Wallen, *Bio-org. Chem.*, 1977, **6**, 89.
[136] E. Gross, *Adv. Chem. Ser.*, 1977, no. 160.
[137] J. W. Finley and J. T. Snow, *J. Agric. Food Chem.*, 1977, **25**, 1421.
[138] H. T. Nagasawa, W. P. Muldoon, and F. N. Shirota, *J. Medicin. Chem.*, 1977, **20**, 1588.
[139] B. L. Moeller, I. J. McFarlane, and E. E. Conn, *Acta Chem. Scand. B*, 1977, **31**, 343.
[140] C. J. Gray, K. Al-Dulaimi, A. M. Khoujah, and R. C. Parker, *Tetrahedron*, 1977, **33**, 837.
[141] C. J. Gray, J. C. Ireson, and R. C. Parker, *Tetrahedron*, 1977, **33**, 739.
[142] H. Pracejus, F. Wilke, and K. Haulmann, *J. prakt. Chem.*, 1977, **319**, 219.
[143] J. L. Morell, P. Fleckenstein, and E. Gross, *J. Org. Chem.*, 1977, **42**, 355.
[144] H. W. Gardner, R. Kleiman, D. Weisleder, and G. E. Inglett, *Lipids*, 1977, **12**, 655.

cysteinyldopa and a small (1%) yield of the hitherto unknown 6-*S*-cysteinyldopa through mushroom tyrosinase co-oxidation with dopa.[145]

Selenocystine continues to find use for the synthesis of selenium analogues of the well-known sulphur amino-acids, this time in combination with formaldehyde to give DL-selenaproline.[146]

A List of Amino-acids which have been Synthesized for the First Time.—New amino-acids not mentioned elsewhere in this Chapter are collected here.

Compound	*Ref.*
3(2′, 3′, or 4′-Fluorophenyl)-DL-alanine	147
3-(2′, 3′, or 4′-Trifluoromethylphenyl)-DL-alanine	147
3-(2′-Chloro-5′-trifluoromethylphenyl)-DL-alanine	147
3-(4′-Chloro-5′-trifluoromethylphenyl)-DL-alanine	147
3-(2′,5′-Difluorophenyl)-DL-alanine	147
3-(3′-Carboxy-4′-hydroxyphenyl)-DL-alanine	148
3-(3′-Carboxy-4′-aminophenyl)-DL-alanine	148
2-(3′-Aminophenyl)glycine	148
2-(3′-Hydroxymethylphenyl)glycine	148
2-(3′-Aminomethylphenyl)glycine	148
2-(3′-Carboxyphenyl)glycine	148
2-(3′-Carboxy-4′-hydroxyphenyl)glycine	148
3-(1′-Tetralyl)alanine	149
3-[5′-(5,6,7,8-Tetrahydroquinolinyl)]alanine	149
2-(1′-Tetralyl)glycine	150
2-(5,6,7,8-Tetrahydroquinolin-5-yl)glycine	150
3-Methyl-DL-histidine	151
3-Ethyl-DL-histidine	151
3-n-Hexyl-DL-histidine	151
3-(3-*p*-Hydroxyphenyl-1,2,4-oxadiazolyl)-DL-alanine	152
4-(Tetrazol-5′-yl)-2-aminobutyric acid	153
S-(Uridin-5-yl)cysteine	154
N-Phthalazinyl-DL-lysines	154*a*

Labelled Amino-acids.—Syntheses have been recorded of (*R*)-and (*S*)-[2-^{2}H]-glycine derivatives, and the following labelled protein amino-acids: (2*S*,3*R*)- and (2*S*,3*S*)-[3-^{3}H]serine,[156] (*methyl-R*) and (*methyl-S*)-[*methyl*-^{2}H,^{3}H]methionine,[157] selectively deuteriated histidine, tyrosine, phenylalanine, and tryptophan,[158]

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(2*S*,3*R*)-[3-^{2}H]tyrosine,[156] [5,7-$^3H_2$] and [4,6-$^3H_2$]tryptophan,[159] and (2*S*,3*S*)- and (2*S*,3*R*)-[3-^{14}C,3-^{3}H]analogues,[124] phenyl-deuteriated or tritiated phenylalanines,[160a] and [*methyl*-$^2H_3$]-DL-threonine.[160b] A general method for the preparation of [2-^{2}H]amino-acids from the ^{1}H-analogues [161] employs Ac_2O and ^2H_2O, based on the well-known lability of the ring hydrogen atom of the 2-methyloxazol-5(4*H*)-one formed in this reaction. The list of ^{3}H-labelled amino-acids is lengthened by reports of [$^3H_2$]ruthenium oxide–alumina treatment of taurine,[162] and of similar exchange processes with proline.[163] However, a particularly interesting study of direct exchange with valine and isoleucine, involving microwave discharge activation of $^3H_2$, has appeared;[164] ^{3}H atoms formed in this way cause general, but not random, exchange. The α-position is least readily exchanged in solid L-valine (to the extent of 7.1%), but with net retention of configuration; 32.7% exchange occurs at the β-position, and 60.2% at the γ-carbon atom, and β-exchange involves inversion of configuration.[164b] Small amounts of tritiated glycine were formed by side-chain cleavage in these experiments.[164] Addition as well as ^{3}H–^{1}H exchange is observed in ^{3}H-atom attack on 3,4-dehydroproline and L-2-amino-4-(2′-aminoethoxy)-*trans*-3-butenoic acid.[164]

(2*R*,3*S*)-[*U*-^{14}C,3-$^3H_1$]- and (2*R*,3*R*)-[*U*-^{14}C,2,3-$^3H_2$]cysteine, together with (2*R*)-[*U*-^{14}C,3,3,3′,3′-$^3H_4$]cystine, have been employed in studies of penicillin G biosynthesis.[165] ^{14}C-Labelled *O*-succinyl-L-homoserine has been synthesized.[166]

Several papers have appeared describing the synthesis of ^{11}C-carboxyl-labelled amino-acids.[167] Other amino-acids labelled with short-lived isotope (^{13}N-labelled alanine [168] and asparagine)[169] and ^{15}N-labelled alanine [170] and other protein amino-acids [171] have been reported, while the synthesis of DL-[2-^{13}C,3′-^{15}N,2′,5′-$^2H_2$]histidine [172] represents something of a jamboree of labelling approaches.

Cysteine-[^{35}S]sulphonic acid [173] and *p*-[^{128}I]iodophenylalanine [174] have been prepared.

Resolution of Amino-acids.—Detailed studies of the preferential adsorption of the

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D-enantiomer from solutions of DL-amino-acid derivatives by (−)-quartz [175] show that a protonated amino-group favours adsorption and enhances the enantioselectivity. While the objective of this study is related to possible mechanisms for the predominance of L-amino-acids in life processes, other adsorbents are far more effective for routine resolution of amino-acids. Amino-acids bound to polystyrene [176] or polyacrylamide [177] provide a stationary phase for the resolution of DL-amino-acids, when copper(II) or nickel(II) ions are present. Further details have been published [178] (see Vol. 9, p. 17) of the resolution of an acidic amino-acid (DL-aspartic and glutamic acids) by preferential complexation of one enantiomer with copper(II) perchlorate and an enantiomer of a basic amino-acid (arginine, lysine, or ornithine); the reverse process, in which a racemic basic amino-acid is resolved using an enantiomer of an acidic amino-acid, has also been established,[178] and the procedure has been extended to the resolution of DL-histidine with copper(II) perchlorate and L-asparagine.[179] A related study [180] using cobalt(III) complexes of amino-acid Schiff bases describes the moderate enrichment of the relative amount of one enantiomer in a solution of a DL-amino-acid.

Chromatographic separation of diastereoisomers formed between a chiral reagent and a DL-amino-acid is illustrated for *N*-(*d*-camphor-10-sulphonyl)-amino-acid *p*-nitrobenzyl esters [181] and (−)-α-methoxy-α-methyl-1-naphthaleneacetyl-amino-acid methyl esters [182] using h.p.l.c. The analytical use of g.l.c. for the same purpose, using either the diastereoisomer separation principle or the use of chiral stationary phases, is discussed in Section 6 of this Chapter.

Resolution of *N*-acetyl-*p*-methoxyphenylglycine as its ammonium salt provides another example of the preferential crystallization procedure,[183] while time-honoured diastereoisomeric salt separation procedures have been used for the resolution of *N*-benzyloxycarbonyl γγ-di-t-butyl γ-carboxy-DL-glutamate,[184] and in several other studies.[92, 112, 113]

Novel approaches employing enzyme systems are involved in the asymmetric hydrolysis of DL-5-indolylmethylhydantoin to L-tryptophan,[185] and the formation of L-lysine from DL-α-amino-ε-caprolactam.[186] Both these procedures are bacterial syntheses, while a more conventional application, the preferential hydrolysis of the 2*S*-diastereoisomer of methyl (2*RS*,4*S*)-2-acetylamino-4-methylhexanoate, involves α-chymotrypsin catalysis.[187]

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4 Physical and Stereochemical Studies of Amino-acids

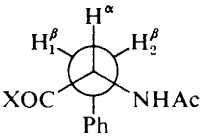
Crystal Structures of Amino-acids and their Derivatives.—Scope still exists for new *X*-ray studies with simple amino-acid derivatives, and *N*-formyl-L-methionine,[188] 2′-hydroxy-DL-phenylalanine,[189] 1-methyl-3-carbamoylpyridinium *N*-acetyl-L-tryptophanate,[190] DL-homocysteic acid,[191] *N*-pivalyl-*N*′-methyl-L-glutaminyl methylamide,[192] the L-valine aza-homologue AcNHCHPr^{i}NHCONHMe,[193] L-histidine hydrochloride,[194] zinc(II) and cadmium(II) complexes of *S*-methyl-L-cysteine,[195] and DL-aspartic acid hydrochloride [194a] have come under scrutiny. More unusual compounds subjected to *X*-ray study are (2*S*,3*R*)-2-amino-3-hydroxypent-4-ynoic acid, a toxic α-amino acid from the fungus *Sclerotium rolfsii*,[22] and coronatine, an acyl derivative of the aminocyclopropane carboxylic acid in Scheme 3.[194b]

Assignment of configuration at sulphur to diastereoisomers of *S*-adenosyl-L-methionine and of *S*-carboxymethyl-L-methionine has been reported.[196]

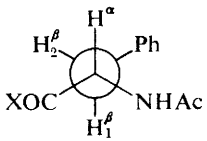
The knowledge of the crystal structure of an amino-acid leads to speculation about its conformation revealed in this way, especially any differences compared with conformations it adopts in proteins, and an example of a continuing trickle of papers of this type deals with isoleucine and alloisoleucine salts.[197] Neutron diffraction analysis permits the placing of hydrogen atoms, and L-histidine monohydrochloride monohydrate [195a] is the latest of the protein amino-acids to be studied in this way.

N.M.R. Spectroscopy.—A review [198] includes coverage of the conformational behaviour of amino-acids in solution as revealed by n.m.r. studies.

Continuing studies of side-chain conformational behaviour concern several of the protein amino-acids. Selective deuteriation assists the interpretation of ^{1}H-n.m.r. data in this area, with [γ-^{2}H]leucine being shown to adopt preferentially the conformer with side-chain gauche to the amino-group, and *trans* to the



(13)



(14)

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carboxy group.[199] A similar study of L-[β-^{2}H]phenylalanine reveals the importance of solvent in determining conformation, with the most crowded conformer (13) actually predominating in a non-polar solvent, while the proportion of (14), which would be presumed to be the preferred conformation, increases with increasing solvent polarity.[200] Long-chain *O*-alkyltyrosines also provide an unexpected result, with the most crowded conformer being the second most abundant of the three possible staggered forms.[201] Aggregation of these derivatives favours their adoption of the least crowded conformation.[201] ^{1}H-N.m.r. and c.d.–pH titration studies of histidine and its derivatives show that the side-chain conformation of this amino-acid is determined by neighbouring charged groupings;[202] the ratio of the two imidazole tautomers of histidine varies with pH, and this fact, as shown by three-bond ^{13}C–^{1}H coupling constants [203] and ^{15}N-n.m.r. of ^{15}N-enriched histidine derivatives,[204] needs to be taken into account in interpretation of pH titration data for histidine. Conformational information derived from n.m.r. data has been reported for 1-aminocyclohexanecarboxylic acid derivatives,[205] and *cis–trans* ratios for the tertiary amide bond in *N*-acetyl-L-proline methylamides as a function of solvent (the *cis*-form is favoured in polar solvents) have been determined.[206]

More specialized n.m.r. studies dealing with amino-acids have been reported, in some cases developing instrumental techniques (*e.g.* wide-line n.m.r. lineshape analysis [207]), but relaxation time data for proline in water–glycerol mixtures [208] and for solid amino-acids [209] provide information on dynamic behaviour. Double nuclear resonance of ^{14}N,^{2}H-labelled glycines in various crystalline modifications has been studied.[210] Other less sophisticated physical studies provide acid dissociation constants for di-amino-acids [211] and exchange rates of the tryptophan N^{indole} proton with water as a function of pH and temperature.[212] A particularly interesting study [213] employs ^{35}Cl-n.m.r. for studying the interaction of Cl^- ions with arginine, histidine, or lysine as a function of pH.

Interaction of D- or L-tryptophan with human serum albumin has been deduced [214] to involve the benzo moiety and the amino-group as 'binding' sites.

O.R.D. and C.D. Spectra.—Advances in instrumentation, particularly the

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penetration to shorter wavelengths which is possible in some prototypes, continue to provide new data on amino-acids, and the o.r.d. and c.d. of different conformations of L-proline to 160 nm have been calculated, to assist the interpretation of experimental data.[215] Vibrational c.d. spectra of D- and L-alanine in ^2H_2O have been measured, illustrating the potential of the technique for the study of solution behaviour.[216]

α-Trimethylammonio-acid amides appear to show more complex c.d. behaviour in the wavelength region 200—260 nm than would be expected for the amide chromophore.[217] Routine studies with chromophorically-substituted amino-acids continue, recent papers describing attempts to establish correlations between sign of Cotton effect and absolute configuration for *N*-acetoacetyl-,[218] *N*-2,4-dinitrophenyl-,[219] and *N*-salicylidene-amino-acids.[220]

Mass Spectrometry.—A larger number of papers than usual has come under scrutiny for inclusion in this Section, due partly to the efforts of newcomers who have adopted techniques established by pioneer specialists, but more because of the possibilities in structure determination using newer, milder, ionization techniques (chemical ionization and field ionization).

After recent success (see Vol. 9, p. 21) in obtaining data on zwitterionic amino-acids, new results have been reported on the in-beam electron-impact mass spectra (e.i.m.s.) of amino-acids.[221] Chemical ionization mass spectrometry (c.i.m.s.) of α-amino-acids, using NH_4^+ for ionization, appears particularly promising, with M + 1 peaks obtained in each of 19 cases, these being base peaks in the spectra of all but two of the compounds.[222] Peaks at *m/e* 101 and 116 seen in the mass spectra of methionine, ionized either by electron impact or by pyrolysis followed by electron impact, are formed by different pathways.[223] The base peak in the mass spectrum of lysine methyl ester at *m/e* 84 is generated by sequential loss of the methoxycarbonyl radical from the parent ion, followed by elimination of NH_3.[224]

For routine analysis, an amino-acid is converted into one of a range of derivatives of sufficient volatility that thermal fragmentation is avoided in the mass spectrometer, and *N*-trifluoroacetylamino-acid n-butyl esters,[225–227] *N*-penta-

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fluoropropionyl,[228] *N*-succinyl,[229] *N*-benzoyl,[225] *N*-trifluoroacetyl-L-prolyl,[225] and *N*-pentafluorobenzoyl analogues,[225] with (−)-menthyl esters in place of n-butyl esters in some cases,[225] have been used for ultramicrodetermination of amino-acids. Amino-acid phenylthiohydantoins can be identified at levels down to 3 nmoles,[230] and derivatives formed between amino-acids and fluorescamine can be identified by c.i.m.s.[231] Uses in the analysis of amino-acids in physiological samples have been found for c.i.m.s. in the identification of L-dopa, α-methyl-L-dopa, and their metabolites,[228] and in the quantitative analysis of amino-acids in blood specimens.[232]

Field ionization mass spectra can be obtained with 50 nmole samples of ^{15}N-labelled amino-acids.[233]

Other Physical and Theoretical Studies.—Results of spectroscopic studies not covered in a preceding section are discussed here, also miscellaneous physico-chemical studies often providing data of value in accounting for the biological roles of amino-acids.

Raman spectroscopic studies of a familiar type with *N*-acetylamino-acid methyl amides [234] deal with the conformational behaviour of the compounds in solution, compared with their structures in the solid state. Polarized Raman and far i.r. spectra of glycine crystalline modifications have been measured [235] and dielectric relaxation spectra of α- and β-alanine.[236] E.s.r. and ENDOR studies of *X*-irradiated single crystals of amino-acids [237] and of *N*-acetyl-L-cysteine [238] are reported by several research groups. The e.s.r. spectra of 1,4-disubstituted pyrazine cation radicals formed in reaction mixtures containing amino-acids and sugars have been interpreted.[2a, 239]

Under the heading 'miscellaneous physico-chemical studies', papers deal with the thermodynamics of dissolution of two crystalline polymorphs of DL-2-aminobutanoic acid, enthalpies of formation of glycine and L-alanine,[241] enthalpies of interaction of sodium chloride with amino-acids in aqueous solution,[242] activity coefficients of γ-amino-butyric acid and glycylglycine in aqueous sucrose

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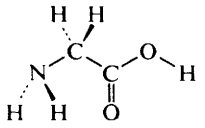
[232] J. M. L. Mee, J. Korth, B. Halpern, and L. B. James, *Biomed. Mass Spectrum.*, 1977, **4**, 178.
[233] J. H. McReynolds and M. Anbar, *Analyt. Chem.*, 1977, **49**, 1832.
[234] Y. Koyama, H. Uchida, S. Oyama, T. Iwaki, and K. Harada, *Biopolymers*, 1977, **16**, 1795.
[235] K. Machida, A. Kagayama, Y. Saito, Y. Kurida, and T. Uno, *Spectrochim. Acta*, 1977, **33A**, 569.
[236] J. L. Salefran, G. Delbos, C. Marzat, and A. M. Bottreau, *Adv. Mol. Relaxation Interact. Processes*, 1977, **10**, 35 (*Chem. Abs.*, 1977, **87**, 23 707).
[237] H. Muto, M. Iwasaki, and Y. Takahashi, *J. Chem. Phys.*, 1977, **66**, 1943; H. Muto, M. Iwasaki, and K. Ohkuma, *J. Magn. Res.*, 1977, **25**, 327; D. M. Close, G. W. Fouse, and W. A. Bernhard, *J. Chem. Phys.*, 1977, **66**, 1534; A. Menegishi, *J. Phys. Chem.*, 1977, **81**, 1688.
[238] J. H. Hadley and W. Gordy, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 216.
[239] T. Hayashi, Y. Ohta, and M. Namiki, *J. Agric. Food Chem.*, 1977, **25**, 1282.
[240] M. H. Abraham, E. Ah-Sing, R. E. Marks, R. A. Schulz, and B. C. Stace, *J.C.S. Faraday Trans. I*, 1977, **73**, 181.
[241] N. N. Song, R. Sabbah, and M. Lafitte, *Thermochim. Acta*, 1977, **20**, 371.
[242] J. W. Larson, W. J. Plymale, and A. F. Joseph, *J. Phys. Chem.*, 1977, **81**, 2074.

solutions,[243] apparent molal heat capacities of amino-acids, interpreted in terms of interactions between neutral or charged amino and carboxy groups,[244] and the viscosity of solutions of glycine or DL-alanine in dimethylformamide–water mixtures.[245] Studies of possible relevance to primordial events have identified the site of adsorption of amino-dicarboxylic acids (aspartic acid, glutamic, α-amino-pimelic, and α-aminoadipic acids) to hydroxylapatite as the α-carboxy group; L-arginine is adsorbed if the solid is pre-treated with phosphate buffer.[246] Activated charcoal scarcely adsorbs amino-acids from aqueous solutions, with the notable exceptions of tryptophan, phenylalanine, and methionine.[247]

Molecular orbital computation studies include important areas of amino-acid behaviour. Glycine adopts the structure (15) with bifurcated hydrogen bonds,



(15)

when achieving the lowest energy conformation of its neutral form;[248] structural formulae for amino-acids depicted with localized positive charges may not be realistic,[249] and this is a matter of importance in deducing the electrical structures of binding sites of neurotransmitters, including γ-aminobutyric acid and acetylcholine.[249] Correlation between molecular mechanics calculations and *X*-ray and n.m.r. data is included in deducing the ranking of conformations available to *N*-acetylproline methyl ester.[250] Interaction energies involved in the formation of amino-acid–water complexes have been calculated.[251]

5 Chemical Studies of Amino-acids

Racemization.—Applications of racemization kinetics for the estimation of the age of fossils and relatively much younger mammal teeth and bones, as well as ancient wood samples, have been reviewed in recent Volumes of this Report. Knowledge of the age of a sample from ^{14}C data, together with the temperature-dependence of the racemization rate constant for a given amino-acid, allows an estimate to be made of the average temperature to which a sample has been subjected from the time it was laid down to the present time, and an average temperature for the last *ca.* 2200 years of 279 ± ~6 K has been estimated from the degree of racemization found for aspartic acid, glutamic acid, proline, and phenylalanine in sequoia heartwood.[252] A rather smaller racemization rate

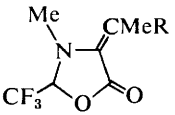
[243] H. Uedaira, *Bull. Chem. Soc. Japan*, 1977, **50**, 1298.
[244] S. Cabani, G. Conti, E. Matteoli, and A. Tani, *J.C.S. Faraday Trans. I*, 1977, **73**, 476.
[245] S. Phang, *Austral. J. Chem.*, 1977, **30**, 1605.
[246] M. Kresak, E. C. Moreno, R. T. Zahradnik, and D. I. Hay, *J. Colloid Interface Sci.*, 1977, **59**, 283.
[247] S. Sato, M. Tadenuma, T. Oba, and K. Takahashi, *Nippon Jozo Kyokai Zasshi*, 1977, **72**, 231 (*Chem. Abs.*, 1977, **86**, 169 289).
[248] S. Vishveshwara and J. A. Pople, *J. Amer. Chem. Soc.*, 1977, **99**, 2422.
[249] W. G. Richards and J. Wallis, *Proc. Roy. Soc. B.*, 1977, **199**, 291.
[250] D. F. De Tar and N. P. Luthra, *J. Amer. Chem. Soc.*, 1977, **99**, 1232.
[251] E. Clementi, F. Cavallone, and R. Scordamaglia, *J. Amer. Chem. Soc.*, 1977, **99**, 5531.
[252] M. H. Engel, J. E. Zumberge, and B. Nagy, *Analyt. Biochem.*, 1977, **82**, 415.

constant (2.1×10^{-5} y^{-1}) for aspartic acid in this source, in comparison with that of the same amino-acid in mammalian samples (see Vol. 8, p. 20), should be noted. Bada's group have shown that the D : L-ratio for aspartic acid in human lens protein is directly related to age,[253] and have reviewed the role of aspartic acid racemization in the ageing process.[254] A correction[255] to an earlier conclusion based on L : D-ratios of proline and hydroxyproline in a wood sample (see Vol. 9, p. 23) is required because of an incorrect assignment of a ^{14}C-calibration sample, and not because of any shortcomings in the basis of the racemization dating procedure.

The racemization of threonine reaches an equilibrium position at about 20% epimerization at the α-position, and the threonine–allothreonine ratio determined for fossil foraminifera cannot be used in geochronology.[256]

Further application of amino-acid racemization data in areas such as those described above is likely to lead to less confident conclusions until the influence of the many parameters involved in amino-acid racemization is better understood. In a review of the applications which have been made, more caution is advocated.[257] The factors which influence the racemization rates of amino-acids in aqueous solution have been listed as ionic strength, pH, nature of buffer, and buffer concentration.[258]

General Reactions.—A number of improvements to standard methods of substitution or modification of amino- and carboxy-groups of amino-acids have been published. *N*-Protected amino-acids can be esterified under mild neutral conditions by treating their caesium salts with alkyl halides.[259] While *N*-trifluoroacetyltyrosine can be prepared conveniently using 1,1,1-trifluoro-3,3,3-trichloroacetone



(16)

in DMSO,[260] *N*-methylvaline or *N*-methylisoleucine gives the product (16; R = Me, Et respectively) of oxidative cyclization of the intermediate *N*-trifluoroacetyl derivatives on treatment with trifluoroacetic anhydride.[261] Formation of 5-(*N*-trifluoroacetamido)thiazoles from *N*-thiobenzoylamino-acid amides and trifluoroacetic anhydride has been reported.[262] Cyclization of *N*-benzyloxycarbonyl-L-α-amino-acids with PCl_5 gives 2-benzyloxyoxazol-5(4*H*)-ones,[263] not

[253] P. M. Masters, J. L. Bada, and J. S. Zigler, *Nature*, 1977, **268**, 71.
[254] P. M. Helfman, J. L. Bada, and M.-Y. Shou, *Gerontology*, 1977, **23**, 419.
[255] M. R. Kleindienst, J. D. Clark, C. Lee, and J. L. Bada, *Nature*, 1977, **267**, 468.
[256] R. A. Schroeder and J. L. Bada, *Geochim. Cosmochim. Acta*, 1977, **41**, 1087.
[257] K. M. Williams and G. G. Smith, *Origins Life*, 1977, **8**, 91.
[258] G. G. Smith, K. M. Williams, and D. M. Wonnacott, *J. Org. Chem.*, 1978, **43**, 1.
[259] S.-S. Wang, B. F. Gisin, D. P. Winter, R. Makofske, I. D. Kulesha, C. Tzougraki, and J. Meienhofer, *J. Org. Chem.*, 1977, **42**, 1286.
[260] C. A. Panetta, *Org. Synth.*, 1977, **56**, 122.
[261] W. A. Koenig and U. Hess, *Annalen*, 1977, 1087.
[262] G. C. Barrett, J. Hume, and A. A. Usmani, in 'Solid Phase Methods in Protein Sequence Analysis', ed. A. Previero and M.-A. Coletti-Previero, Elsevier – North-Holland Biomedical Press, 1977, p. 57.
[263] J. H. Jones and M. J. Witty, *J.C.S. Chem. Comm.*, 1977, 281.

the isomeric benzyloxycarbonylaziridinones as claimed earlier (see Vol. 6, p. 29).

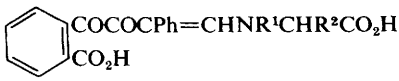
Formic anhydride, formed *in situ* by the reaction of formic acid with dicyclohexylcarbodi-imide in pyridine, is an effective reagent for the formylation of α-amino-acid t-butyl esters,[264] readily converted into α-isocyano-esters by reaction with phosgene,[265] for use in the Ugi four-component condensation reaction leading to N^{α}-acyl-N^{α}-alkylamino-acid amides.

While normal Dakin–West ketonic products AcNHCHRCOMe are formed from aliphatic amino-acids and acetic anhydride at 100 °C, higher temperatures (140—150 °C) lead to β-acetoxyenamine derivatives $Ac_2NCR{=}CMeOAc$.[266]

The nitrosation of imino-acid derivatives has been reviewed.[267] The reaction of L-histidine with nitrous acid in hydrochloric acid gives the corresponding 2-chloro-3-(4′-imidazolyl)propionic acid with retention of configuration.[268] Also reported in this paper[268] is the synthesis of N^{π}-methylhistidine by reaction of protected N^{1m}-benzylhistidine with trimethyloxonium tetrafluoroborate followed by de-protection.

The reactions of amino-acids with aldehydes include processes of importance in metabolism, food science, and analysis. The Maillard reaction of methionine or tryptophan with glucose in aqueous solution proceeds at maximum rate at pH 11, suggesting catalysis by base.[269] Pyrroles and furans are formed in reactions of fructose or rhamnose with alanine or γ-aminobutyric acid at pH 3.5.[270] Simpler reaction products are formed between amino-acids and phenylglyoxal, glyoxal, and methylglyoxal at pH 7 at 15 °C, arginine reacting faster than other amino-acids, especially as the pH is raised.[271]

A series of papers dealing with *o*-phthalaldehyde–amino-acid–thiol condensation products has appeared. The 1-alkylthio-2-alkylisoindole structure assigned to the fluorescent product (see Vol. 9, p. 24) has been confirmed by synthesis,[272a] and the stability of the fluorescence of the 1-alkylthio-compound suggests that the analytical use of this system would be improved by replacing the currently-used mercaptoethanol by ethanethiol.[272b] Lower relative fluorescence results from this reaction when non-protein amino-acids lacking an α-proton are involved.[273] Further studies of another increasingly widely used procedure for fluorescence detection of amino-acids, reaction with fluorescamine, have been described (see Vol. 8, p. 19); imino-acids give products (17) showing maximum long-wavelength



(17)

[264] M. Waki and J. Meienhofer, *J. Org. Chem.*, 1977, **42**, 2019.
[265] R. Urban, D. Marquarding, P. Seidel, I. Ugi, and A. Weinelt, *Chem. Ber.*, 1977, **110**, 2012.
[266] S. I. Zavyalov and G. I. Ezhova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 219.
[267] R. Bonnett and P. Nicolaidou, *Heterocycles*, 1977, **7**, 637.
[268] H. C. Beyerman, L. Maat, A. Noordan, and A. van Zon, *Rec. Trav. chim.*, 1977, **96**, 222.
[269] E. Dworschak and F. Orsi, *Acta Aliment. Acad. Sci. Hung.*, 1977, **6**, 59.
[270] P. E. Shaw and R. E. Berry, *J. Agric. Food Chem.*, 1977, **25**, 641.
[271] K. Takahashi, *J. Biochem.*, 1977, **81**, 395.
[272] S. S. Simons and D. F. Johnson, (*a*) *J.C.S. Chem. Comm.*, 1977, 374; (*b*) *Analyt. Biochem.*, 1977, **82**, 250.
[273] J. R. Cronin and P. E. Hare, *Analyt. Biochem.*, 1977, **81**, 151.

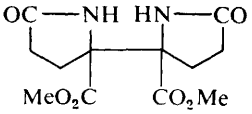
absorption in the range 300—320 nm.[274] Several papers have appeared[275] advocating the use of 4-*NN*-dimethylaminonaphthylazobenzene-4′-isothiocyanate as a colour reagent for amino-acids, giving purple arylthiohydantoins.

Oxidation and reduction of amino-acids are represented in a number of analytical applications (see Section 6) and in reports of the oxidation of amino-acids to CO_2 and nitriles (unsuitable for analytical use, since results are not reproducible within ± 5%),[276] and the electrochemical[277] and hydride reduction[278] of amino-acids. The 2-amino-alkanol formed by the treatment of an L-α-amino-acid with any one of a number of familiar reducing agents is essentially optically-pure,[278] even though wide variations in α_D are observed in the product, depending on the route used. It is concluded[278] that these variations are due to impurities, but since these are stated to be present in amounts smaller than 2%, they must possess extraordinarily large optical rotations.

Alkylamines formed by heating glycine at 240 °C with alumina (simulated primitive earth conditions) include methyl, ethyl, n-propyl, n-butyl, dimethyl, and diethylamines,[279a] while alanine, γ-aminobutyric acid, norvaline, norleucine, sarcosine, and small amounts of *N*-methylamino-acids are formed when the reaction mixture also contains basic manganous carbonate.[279b]

Specific Reactions of Individual Protein Amino-acids.—While some of the reactions covered here are relevant to biological roles of amino-acids (see following section), they mostly reflect the chemistry of side-chain functional groups.

Permanganic acid oxidation of *N*-acylprolines gives corresponding pyroglutamates and side-chain protected ornithines give glutamates;[280] these are unusual products since amino-acids are generally oxidized to aldehydes and ammonia in this reaction. Photo-oxidation of methyl-DL-pyroglutamate in benzene gives a mixture of *meso*- and (±)-oxidative dimerization products (18).[281] Iodine–DMSO oxidation of L-cystine is not possible owing to poor solubility,[282] but with the addition of 12M-HCl as catalyst, stoicheiometric oxidation to cysteic acid, isolated as a 1 : 1-molecular compound with DMSO, is achieved in this system. H_2O_2-Oxidation of L-cystine, lanthionine, or L-homocystine in the presence of hydrochloric acid gives a mixture of sulphonic acids, sulphoxides, and sulphones.[283] Electrochemical reduction of cystine and oxidation of cysteine at a



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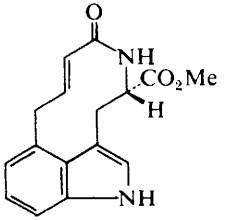
[274] V. Toome, B. Wegrzynski, and J. Dell, *Biochem. Biophys. Res. Comm.*, 1976, **71**, 598.
[275] J. Y. Chang and E. H. Creaser, *J. Chromatog.*, 1977, **132**, 303; J. Y. Chang, *Biochem. J.*, 1977, **163**, 517.
[276] D. S. Mahadeveppa and N. M. M. Gade, *J. Indian Chem. Soc.*, 1977, **54**, 534.
[277] R. Saxena and M. C. Saxena, *Monatsh.*, 1977, **108**, 829.
[278] G. S. Poindexter and A. I. Meyers, *Tetrahedron Letters*, 1977, 3527.
[279] C. Ivanov and N. Slavcheva, (*a*) *Doklady Bolg. Akad. Nauk.*, 1977, **30**, 727; (*b*) *Origins Life*, 1977, **8**, 13.
[280] I. Muramatsu, Y. Motoki, K. Yabuuchi, and H. Komachi, *Chem. Letters*, 1977, 1253.
[281] N. Obata and K. Niimura, *J.C.S. Chem. Comm.*, 1977, 238.
[282] O. G. Lowe, *J. Org. Chem.*, 1977, **42**, 2524.
[283] S. H. Lipton, C. E. Bodwell, and A. H. Coleman, *J. Agric. Food Chem.*, 1977, **25**, 624.

hanging-mercury-drop electrode at several pH values has been investigated by cyclic voltammetry.[284] Selective *S*-methylation of cysteine by aqueous trimethyl phosphate is an unusual result since the other protein amino-acids are unaffected, except histidine and tryptophan to slight extents.[285] L-Cystine appears to give sulphenyl cations by HBr cleavage,[286] since the 3′-(*S*-cysteinyl) derivative is formed with L-tyrosine. In spite of a precedent for the formation of sulphenyl cations, the sulphenyl bromide appears to be a more likely intermediate in this reaction. Cysteinyl-dopa gives highly fluorescent 3,4-dihydroisoquinolines with either formaldehyde or glyoxylic acid,[287] analogous to corresponding products formed by dopa and dopamine. Di-dansylation of tyrosine can be achieved by reaction with dansyl chloride in bicarbonate buffers (pH 9.5), giving remarkably photolabile derivatives,[288] and electrophilic t-butylation of tryptophan gives the 2′,5′,7′-tri-t-butyl derivative (but the N^{im}-t-butyl derivative is the major product);[289] photoalkylation of tyrosine and tryptophan with chloroacetamide,[290] and intramolecular photocyclization[291] of tiglyl-L-tryptophan ethyl ester to give (19),



(19)

mimicking a step in alkaloid biosynthesis, are some of the more interesting papers with more than a little relevance to analytical, synthetic, and biological studies with aromatic amino-acids, as is the finding[292] that aromatic amino-acids, particularly histidine, are degraded to HCN by the action of amino-acid oxidases.

The yellow polymer formed by the reaction of L-lysine with methylglyoxal has been formulated as a series of 3-hydroxypyrrole moieties bridged by vinylene or 1,4-dihydroxy-2-oxobutylene groups.[293]

Treatment of *N*-benzyloxycarbonyl-L-glutamic anhydride with diazomethane gives *N*-benzyloxycarbonyl-α-diazomethyl-γ-methyl-L-glutamate, and not the isomer as previously claimed, which is best prepared from the corresponding α-methyl-L-glutamate.[294]

[284] M. T. Stankovitch and A. J. Bard, *J. Electroanalyt. Chem. Interfacial Electrochem.*, 1977, **75**, 487.
[285] K. Yamauchi, T. Sugimae, and M. Kinoshita, *Tetrahedron Letters*, 1977, 1199.
[286] S. Ito and G. Prota, *J.C.S. Chem. Comm.*, 1977, 251.
[287] G. Agrup, A. Bjorklund, B. Falck, S. Jacobsson, O. Lindvall, H. Rorsman, and E. Rosengren, *Histochemistry*, 1977, **52**, 179.
[288] P. L. Felgner and J. E. Wilson, *Analyt. Biochem.*, 1977, **80**, 601.
[289] E. Wünsch, E. Jaeger, L. Kisfaludy, and M. Loew, *Angew. Chem.*, 1977, **89**, 330.
[290] T. Hamada and O. Yonemitsu, *Chem. Pharm. Bull.*, 1977, **25**, 271.
[291] N. G. Anderson and R. G. Lawton, *Tetrahedron Letters*, 1977, 1843.
[292] E. K. Pistorius, H. S. Gewitz, H. Voss, and B. Vennesland, *Biochim. Biophys. Acta*, 1977, **481**, 384.
[293] A. Bonsignore, G. Leoncini, G. Andiso, L. Zetta, and P. Ferrati, *Ital. J. Biochem.*, 1977, **26**, 162.
[294] C. T. Clarke and J. H. Jones, *Tetrahedron Letters*, 1977, 2367.

Specific Reactions of Amino-acids Related to Biochemical Processes.—Some items cited in the preceding section could equally well have found a place here, although binding studies of aliphatic amino-acids with riboflavin,[295] L-cysteine with vitamin B_{12} (effect of micelles),[296] tryptophan and arginine derivatives with nucleosides,[297] and lysine, histidine, and cysteine derivatives with ATP,[298] clearly have a place in this section.

Methylmercury is formed by the photolysis of aliphatic amino-acids in the presence of mercury(II) chloride, pointing to a mechanism for the biogenesis of this pollutant.[299]

The mechanism of Schiff base formation between pyridoxal-5″-phosphate and DL-alanine in aqueous solution involves a carbinolamine intermediate.[300]

Effects of Electromagnetic Radiation on Amino-acids.—This title is used to collect papers concerned with photochemical and radiolytic studies of amino-acids. Under the former heading, continuing studies of tryptophan and substituted phenylalanine derivatives includes flash photolysis of tryptophan in aqueous solution,[301, 302] and of *N*-acetyltryptophanamide,[303] eosin-sensitized photo-oxidation of tyrosine and other substituted phenylalanines, studied by steady-state kinetic and flash photolytic methods,[304] and photolysis of *N*-acetyl-*p*-nitrophenylalanine ethyl ester in aqueous solutions, to give a small (4%) yield of the azoxy-analogue.[305] Fluorescence excitation and excitation polarization spectra of tryptophan at −58 °C in propylene glycol [306] contribute to knowledge of conformational properties of tryptophan residues in peptides and proteins.

γ-Radiolysis studies (^{60}Co radiation) of amino-acids [307] include specific studies of tyrosine (which yields dopa in aqueous solution) [308] and histidine.[309] Amino-acids with alkyl or benzyl-type side-chains are particularly resistant to γ-radiolysis in aqueous solutions.[310] Radiolytically-generated hydrogen atoms degrade methionine in aqueous solution to α-aminobutyric acid, but have no effect on phenylalanine.[311] Hydrated electrons formed by radiolysis of aqueous solutions react with solutes to form radicals, which undergo further transformations; a kinetic study of this initial step has been carried out for tryptophan.[312] Radicals

[295] N. A. Garcia, J. Silber, and C. Previtali, *Tetrahedron Letters*, 1977, 2073.
[296] F. Nome and J. H. Fendler, *J. Amer. Chem. Soc.*, 1977, **99**, 1557.
[297] V. I. Bruskov and V. N. Bushuev, *Biofizika*, 1977, **22**, 26.
[298] I. Ovary, S. Fazekas, V. Szekessy-Hermann, I. Kulovics, and P. Juhasz, *Acta Agron. Acad. Sci. Hung.*, 1977, **26**, 23.
[299] K. Hayashi, S. Kawai, T. Ohno, and Y. Maki, *J.C.S. Chem. Comm.*, 1977, 158.
[300] B. H. Jo, V. Nair, and L. Davis, *J. Amer. Chem. Soc.*, 1977, **99**, 4467.
[301] J. F. Baugher and L. I. Grossweiner, *J. Phys. Chem.*, 1977, **81**, 1349.
[302] B. Finnstrom, *Chem. Scripta*, 1976, **10**, 184.
[303] R. F. Evans, C. A. Ghiron, R. R. Kuntz, and W. A. Volkert, *Chem. Phys. Letters*, 1976, **42**, 415.
[304] F. Rizzuto and J. D. Spikes, *Photochem. Photobiol.*, 1977, **25**, 465.
[305] E. Escher, *Helv. Chim. Acta*, 1977, **60**, 339.
[306] B. Valeur and G. Weber, *Photochem. Photobiol.*, 1977, **25**, 465.
[307] T. Oku, *Nippon Daigaku Nojuigakubu Gakujutsu Kenkyu Hokoku*, 1977, **34**, 81, 93 (*Chem. Abs.*, 1977, **87**, 85 207, 85 208).
[308] K. R. Lynn and J. W. Purdie, *Internat. J. Radiation Phys. Chem.*, 1976, **8**, 685.
[309] J. Kopoldova and S. Hrncir, *Z. Naturforsch.*, 1977, **32C**, 482.
[310] N. A. Duzhenkova and A. V. Savich, *Khim. Vys. Energ.*, 1977, **11**, 168.
[311] L. K. Mee, S. J. Adelstein, C. M. Steinhart, and N. N. Lichtin, *Radiation Res.*, 1977, **71**, 493.
[312] M. Faraggi and A. Bettelheim, *Radiation Res.*, 1977, **72**, 81.

formed by irradiation of crystalline amino-acids yield e.s.r. spectra, referred to in a preceding section; a method for studying the effects of ^{60}Co-γ-radiation on solid amino-acids depends on measurement of the accompanying light emission.[313]

A sizeable crop of papers has appeared following recent reports of enantioselective photodegradation of DL-amino-acids. Walker [314] again casts doubt (see Vol. 8, p. 16) on the possibility that circularly-polarized light associated with polarized β-radiation can account for this phenomenon, and there is agreement [315] that the selective degradation is the result of ionization and not photodegradation, since too small a fraction of the energy of the radiation appears in the form of light. Further experimental proof of the greater degree of destruction of the D-enantiomer of DL-leucine by antiparallel-polarized ('natural') electrons has been obtained.[316, 317]

Right-circularly polarized light of wavelength 212.8 nm preferentially degrades the D-enantiomer of DL-leucine, resulting in a 1.98% enantiomeric excess of L-leucine after 59% of the original solid sample has been destroyed.[318] Similar results have been obtained with alanine, glutamic acid, and tartaric acid.[319]

6 Analytical Methods

Gas–Liquid Chromatography.—The main topics in the literature on g.l.c. analysis of amino-acids, as in earlier Volumes of this Report, are choice of derivative-forming procedure, instrumental aspects, and methods for determination of optical purity. There are several papers dealing with applications of g.l.c. and mass spectrometry; some are cited here, and others in the mass spectrometry section (see Section 4).

Volatile derivatives of amino-acids are formed by masking the amino- and carboxy-groups, and *N*-trifluoroacetyl n-butyl esters,[320–323] *N*-trifluoroacetyl hexafluoropropyl esters,[324] *N*-pentafluoropropionyl hexafluoropropyl esters,[325] *N*-heptafluorobutyryl isobutyl esters,[326, 327] *N*-acetyl propyl esters,[328, 329] and silylated thiohydantoins [330] have been illustrated further. Mixed disulphides can form during the routine derivatization procedure applied to cystine and homo-

[313] D. I. Thwaites, G. Buchan, K. V. Ettinger, J. R. Mallard, and A. Takavar, *Internat. J. Appl. Radiation Isotopes*, 1976, **27**, 663.
[314] D. C. Walker, *Origins Life*, 1976, **7**, 303.
[315] W. A. Bonner, *Nature*, 1976, **264**, 197; L. Keszthelyi, *ibid.*, p. 197.
[316] W. A. Bonner, M. A. Van Dort, M. R. Yearian, H. D. Zeman, and G. C. Li, *Israel J. Chem.*, 1977, **15**, 89.
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[325] J. D. Huizinga, A. W. Teelken, F. A. J. Muskiet, J. van der Meulen, and B. G. Wolthers, *New England J. Med.*, 1977, **296**, 692 (*Chem. Abs.*, 1977, **86**, 152 215).
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[327] S. L. Mackenzie and L. R. Hogge, *J. Chromatog.*, 1977, **132**, 485.
[328] I. Tunblad-Johansson, *Acta Pathol. Microbiol. Scand., Supplement*, 1977, **259**, 17.
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[330] F. E. Dwulet and F. R. N. Gurd, *Analyt. Biochem.*, 1977, **82**, 385.

cystine,[320] and there are stringent demands on the chemical operations used in these procedures if g.l.c.–m.s. procedures on picomole amounts of amino-acids are to give unambiguous results.[321] This sensitivity is required for quantitation of γ-amino-butyric acid in brain tissue or in cerebrospinal fluid,[324, 325] and m.s.-detection is required at these levels,[321, 324, 325, 327] or electron-capture,[324] or nitrogen-sensitive [329] detectors which are up to 200 times more sensitive than flame-ionization detectors.[329]

δ-Aminolaevulinic acid in blood plasma can be determined by conversion into the corresponding pyrrole by reaction with acetylacetone, using 6-amino-5-oxohexanoic acid as internal standard.[331]

The determination of the optical purity of an amino-acid involves either the separation of the enantiomeric pair of volatile derivatives of the type listed above, on an optically active stationary phase, or the separation of diastereoisomeric pairs formed between the *N*-trifluoroacetyl- [332–334] or *N*-pentafluoropropionyl- [335–337] or *N*-(−)-2-chloroisovaleryl-DL-amino-acid and an optically-active alcohol [333, 336, 337] or amine (L-leucine isopropyl ester [332]). These techniques have been used for determining L : D-ratios for amino-acids from fossils, meteorites,[332] and proteins; an extension of this technique for the assignment of absolute configuration to an enantiomer of an amino-acid from a natural source based on retention time data has been illustrated,[334, 337] for example in showing that amino-acids in alamethicins are of the L-configuration.[334]

Ion-exchange Chromatography.—The use of ion-exchange chromatography as the basis for the automated analysis of amino-acids in mixtures continues to stimulate the study of improved techniques, and of modifications designed to overcome problems with certain unusual amino-acids or with interfering species. Papers of a non-routine nature deal with the determination of hydroxylysine in urine,[338] γ-carboxyglutamic acid using an amino-acid analyser equipped with an anion-exchange column,[339] analysis of histidine, ornithine, tryptophan, and lysine in an improved low-salt alkaline buffer,[340] *N*-methylated basic amino-acids,[341] taurine in plasma,[342] and ^{14}C-labelled amino-acids.[343] Neutral amino-acids present in human plasma and cerebrospinal fluid may be analysed without prolonged initial preparation before ion-exchange separation.[344] A 9.5 h two-column separation of 55 ninhydrin-positive compounds present in blood and urine has been reported as

[331] J. MacGee, S. M. B. Roda, S. V. Elias, A. Lington, M. W. Tabor, and P. B. Hammond, *Biochem. Med.*, 1977, **17**, 31.
[332] J. J. Flores, W. A. Bonner, and M. A. Van Dort, *J. Chromatog.*, 1977, **132**, 152.
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[340] K.-T. D. Liu, *J. Chromatog.*, 1977, **132**, 160.
[341] R. Helm, O. Vancikova, K. Macek, and Z. Deyl, *J. Chromatog.*, 1977, **133**, 390.
[342] K. H. Tachiki, H. C. Hendrie, J. Kelams, and M. H. Aprison, *Clinica Chim. Acta*, 1977, **75**, 455.
[343] R. Sylvester-Bradley, *Ann. Appl. Biol.*, 1977, **85**, 313.
[344] S. E. Moeller, *Analyt. Biochem.*, 1977, **79**, 590.

a stringent test of the Hitachi amino-acid analyser,[345] and a similar display of the sophistication of modern instruments in the separation of 145 ninhydrin-positive compounds[346] has been reported. The enantiomeric purity of *N*-methylamino-acids can be established by diastereoisomer formation by coupling *N*-benzyloxycarbonyl derivatives with N^{ϵ}-benzyloxycarbonyl-L-lysine benzyl ester, removal of protecting groups, and ion-exchange separation using the automatic amino-acid analyser;[347] although coupling and de-protection should be free of racemization, fewer steps are needed when g.l.c. is used for the analysis of enantiomer mixtures and greater accuracy is possible.

Modified instrumentation for converting an amino-acid analyser for fluorescence detection based on *o*-phthalaldehyde as reagent has been discussed,[348] and a useful modification allowing the different stages in ninhydrin colour development to be monitored by following absorbance changes at different wavelengths gives more scope for identifying the less common amino-acids tending to 'overlap' the protein amino-acids.[349]

The separation of amino-acids in systems comprising a hydrophobic solid support and water–organic solvent mixtures containing a small amount of anionic detergent is effectively an ion-exchange process, and allows the separation of 19 amino-acids by gradient elution within 30 minutes.[350]

Thin-layer Chromatography.—An important but well-established technique, such as t.l.c., tends to generate an increasing proportion of routine papers, and this is very much the case in amino-acid analysis and accounts for the relatively small proportion of the current literature cited in this section.

Minor improvements in amino-acid analysis are associated with high-performance t.l.c. of dansyl amino-acids[351] and *N*-phenylthiohydantoins;[352] in the latter case, the incorporation of a fluorescent agent in the silica gel leads to a lowering of the detection limit by some 10—20-fold. More conventional studies with *N*-(*p*-phenylazophenyl)thiohydantoins[353] and the separation of N^{α}-2,4-dinitrophenyl-lysine from other DNP-amino-acids[354] have been reported, while the resolution of racemic amino-acids on cellulose films[355] and the determination of the optical purity of D-[^{75}Se]selenomethionine by diastereoisomer formation with (−)-camphorsulphonyl chloride[356] are out of the ordinary, though not new in principle.

Comparison of the various colour reagents for amino-acids continues to favour the *o*-phthalaldehyde–alkanethiol fluorescence method as far as sensitivity is

[345] K. Murayama and N. Shindo, *J. Chromatog.*, 1977, **143**, 137.
[346] P. Adriaens, B. Meesschaert, W. Wuyts, H. Vanderhaege, and H. Eyssen, *J. Chromatog.*, 1977, **140**, 103.
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[349] B. V. Charlwood and E. A. Bell, *J. Chromatog.*, 1977, **135**, 377.
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[351] N. Seiler and B. Knoedgren, *J. Chromatog.*, 1977, **131**, 109.
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[355] K. Bach and H. J. Haas, *J. Chromatog.*, 1977, **136**, 186.
[356] P. P. H. L. Otto and G. F. van Veen-van Staalduinen, *J. Radioanalyt. Chem.*, 1977, **35**, 37.

concerned;[357, 358] it is either ten times more sensitive than ninhydrin,[357] or of similar sensitivity since both reagents permit the identification of 50—200 pmole amounts,[358, 359] but in any case fluorescamine is less sensitive.[358] The fluorescence intensity of these derivatives can be enhanced by using aqueous DMSO as solvent.[360]

Other Separation Methods.—H.p.l.c. methods employing non-polar stationary phases such as octadecylsilica [361] for the separation of amino-acid mixtures results in the separation of components in order of increasing hydrophobicity. Examples of applications are the identification of primary amines in cerebrospinal fluid using the *o*-phthalaldehyde reagent,[362] and the separation of phenylthiohydantoins of all protein amino-acids [363] using conventional adsorbents or using covalently-bonded tripeptides.[364]

A rapid, sensitive procedure for the separation of proline from hydroxyproline is based on high-voltage paper electrophoresis.[365]

Determination of Specific Amino-acids.—The two main topic areas of this section deal with the assay of particular amino-acids by specific enzymes or by methods recognizing side-chain functional groups.

Estimation of L-alanine based on NADH formation with L-alanine dehydrogenase,[366] or by the chemiluminescence produced by the H_2O_2–luminol–ferricyanide system (the peroxide deriving from the L-amino-acid oxidase-catalysed degradation of the amino-acid) [367] has been described. Specific enzyme electrode methods [368] for L-asparagine,[369] L-phenylalanine,[370] and L-glutamic acid [371] follow previously-established methodology, while a variation involving *Bacterium cadaveris* held at the surface of an ammonia-sensing membrane electrode is advocated [372] for the determination of L-aspartic acid based on the L-aspartase activity of the living organism.

Non-enzymic methods for the assay of particular amino-acids involve reactions which have been discussed in the 'Chemical Studies' section of this Chapter in past years, or which are based on textbook amino-acid chemistry. The identification of

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[358] E. Schiltz, K. D. Schnackerz, and R. W. Gracy, *Analyt. Biochem.*, 1977, **79**, 33.
[359] R. Reisfeld and S. Levi, *Analyt. Letters*, 1977, **10**, 483.
[360] P. M. Froehlich and L. D. Murphy, *Analyt. Chem.*, 1977, **49**, 1606.
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[367] S. N. Lowery, P. W. Carr, and W. R. Seitz, *Analyt. Letters*, 1977, **10**, 931.
[368] G. G. Guilbault, in 'Biomedical Applications of Immobilised Enzymes and Proteins', ed. T. M. S. Chang, Plenum Press, New York, 1977, Vol. 2, p. 163.
[369] R. Wawro and G. A. Rechnitz, *J. Membrane Sci.*, 1976, **1**, 143.
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[372] R. K. Kobos and G. A. Rachnitz, *Analyt. Letters*, 1977, **10**, 751.

γ-carboxy-L-glutamic acid in bone, teeth, or prothrombin can be accomplished [373] after its release by alkaline hydrolysis (see also Vol. 8, p. 5). Assay of tryptophan by colorimetry at 2 μg levels [374] or by its fluorescence after h.p.l.c. separation,[375] and determination of cysteine or cystine down to 10^{-12} mole levels by polarography,[376] illustrate well-established techniques in this area; specific assay of di- and tri-iodothyronines and thyroxine is covered in papers too numerous to mention (they are abstracted in the 'Biochemical Methods' section of *Chemical Abstracts*), based on methods cited in this section in earlier Volumes. The inhibitory effect of methionine on the colour reaction between lactic acid and *p*-hydroxybiphenyl in H_2SO_4 has been used [377] for the spectrophotometric assay of this amino-acid; an alternative method [378] depends on g.l.c. analysis of MeSCN formed by cyanogen bromide treatment of plant samples. *S*-Methylmethionine levels in plants can be determined by degradation at pH 9.7 at 97 °C, giving homoserine and Me_2S, analysed by g.l.c.[20] The γ-aminobutyric acid content of human cerebrospinal fluid can be determined at 1 picomole sensitivity by presenting samples to membrane receptors equilibrated with the ^{3}H-labelled amino-acid and measuring the resulting distribution of labelled amino-acid.[379]

[373] P. V. Hauschka, *Analyt. Biochem.*, 1977, **80**, 212.
[374] S. M. M. Basha and R. M. Roberts, *Analyt. Biochem.*, 1977, **77**, 378.
[375] A. M. Krstulovic, P. R. Brown, D. M. Rosie, and P. B. Champlin, *Clinical Chem.*, 1977, **23**, 1984.
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[377] M. Tonkovic and O. Hadzija, *Mikrochim. Acta*, 1977, **2**, 241.
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