1
Amino-acids

BY G. C. BARRETT

1 Introduction

This chapter continues to offer detailed coverage of the chemical and biochemical literature on the amino-acids, but with only superficial treatment of biological aspects (distribution of the common amino-acids, metabolism, and biosynthesis).

Textbooks and Reviews.—Several sources of up-to-date information have become available, dealing with biosynthesis,[1] stereochemical studies of metabolism,[2] toxic and other amino-acids with plant-defensive roles,[3] and a broader review of non-protein amino-acids.[4] Electrochemical synthesis of amino-acids has been surveyed.[5]

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—Identification of four previously undetected leucine isomers (2-amino-2-ethylbutyric acid, both diastereoisomers of 2-methylnorvaline, *C*-t-butylglycine, and 2-amino-2,3-dimethylbutyric acid) in the Murchison meteorite[6] contributes further support to the hypothesis that a single one-carbon precursor can account for all amino-acids so far found in this sample.

A review of amino-acids present in marine algae has appeared.[7] Other α-amino-acids found in new locations are diaminopimelic acid from the cell wall of Legionnaires' disease bacterium,[8] L-2-amino-4,5-hexadienoic acid from *Amanita neooroidea*,[9] cyclopentenylglycine in Flacourtiaceae,[10] and 3-(2-furoyl)alanine from roots of *Rumex obtusifolius*[11] (this compound is now believed to be formed

[1] Biochemistry of Plants, Vol. 4, ed. P. K. Stumpf, Vol. 5, ed. B. J. Miflin, Academic Press, New York, 1980; L. Ninet and J. Renaut, *Bull. Soc. Chim. Fr., Part 2*, 1980, 80.

[2] D. J. Aberhart, *Recent Adv. Phytochem.*, 1979, **13**, 29.

[3] B. Unterhalt, *Dtsch. Apoth.-Ztg.*, 1980, **120**, 1093; 'Herbivores: Their Interaction with Secondary Plant Metabolites', ed. G. A. Rosenthal and D. H. Janzen, Academic Press, New York, 1979.

[4] E. A. Bell, in 'Encyclopaedia of Plant Physiology', Vol. 8 (Secondary Plant Products), New Series, ed. E. A. Bell and B. V. Charlwood, Springer Verlag, Berlin, 1980, p. 403; E. A. Bell, *Rev. Latino-am. Quim.*, 1980, **11**, 16; E. A. Bell, *Endeavour*, 1980, **4**, 102.

[5] I. A. Avrutskaya, in 'Elektrosint. Monomerov', ed. L. G. Feoktistov, Izd. Nauka, Moscow, 1980, p. 124 (*Chem. Abstr.*, 1981, **93**, 122 346).

[6] J. R. Cronin, W. E. Gandy, and S. Pizzarello, in 'Biogeochemistry of Amino-acids', ed. P. E. Hare, T. C. Hoening, and K. King, Wiley, New York, 1980, p. 153.

[7] E. Fattorusso and M. Piattelli, in 'Marine Natural Products: Chemical and Biological Perspectives', ed. P. J. Scheuer, Academic Press, New York, 1980, Vol. 3, p. 95.

[8] G. O. Guerrant, M. S. Lambert, and C. W. Moss, *J. Clin. Microbiol.*, 1979, **10**, 815.

[9] S. Hatanaka and K. Kawakami, *Sci. Pap. Coll. Gen. Educ., Univ. Tokyo*, 1980, **30**, 147 (*Chem. Abstr.*, 1980, **93**, 61 778).

[10] U. Cramer, A. G. Rehfeldt, and F. Spener, *Biochemistry*, 1980, **19**, 3074.

[11] T. Kasai, M. Okuda, and S. Sakamura, *Agric. Biol. Chem.*, 1980, **44**, 2723.

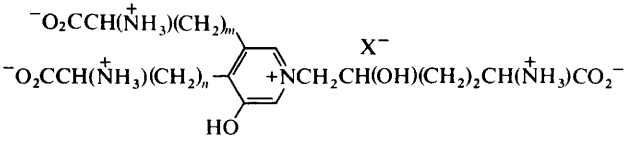
from ascorbalamic acid during isolation from the plant [11]). An improved isolation procedure (3-hydroxyproline from seeds) gives an excellent account of modern methodology which is generally applicable.[12]

γ-Carboxyglutamic acid is a constituent of ovocalcin (hen eggshell),[13] and bovine teeth phosphoprotein contains α-aminoadipic acid,[14] probably derived from a lysine residue *via* the corresponding aldehyde ('allysine'). Several papers dwell on the possibility that crosslinking amino-acids previously located in proteins may be artifacts of the isolation procedures; although pyridinoline (see Vol. 11, p. 3), now structurally revised to (l; probably $n = 1$, $m = 2$),[15] has been established to be an *in vivo* component of collagen,[16] this has been disputed.[17] The tetrafunctional collagen crosslink, dehydrohistidinohydroxymerodesmosine, has also been shown not to be an artifact.[18]

Simple derivatives of the common protein amino-acids continue to be found, either in an uncombined form [*N*-methyl-L-alanine and *N*-methyl-L-serine in high concentrations in *Dichapetalum cymosum*;[19] *N*-(γ-L-glutamyl)ethanolamine in mushrooms;[20] *N*-*p*-coumarylglutamic acid in black tea;[21] and H·Leu·NHNMeP(O)(OH)OMe, as antibiotic FR-900137 from *Streptomyces unzenensis*[22]] or as protein constituents (*NNN*-trimethyl-L-alanine and $N^{\varepsilon}N^{\varepsilon}N^{\varepsilon}$-trimethyl-L-lysine in ribosomal protein L11 from *E. coli*,[23] and *NN*-dimethylproline at the *N*-terminus of a cytochrome[24]).

New Natural Free Amino-acids.—Plant sources and new free amino-acids are: *Caylusea abyssinica* (2 diastereoisomers of 4-carboxy-4-hydroxy-2-amino-adipic acid, with the (*S*)-configuration at C-2 assumed, as well as two diastereoisomers of 4-hydroxy-4-methylglutamic acid);[25] further information on mugineic acid (see Vol. 12, p. 3) from root-washings of Gramineae;[26] *Avena sativa* root washings as source of avenic acid A, (2), a new amino-acid with iron-chelating ability;[27,28] seeds of *Ateleia herbert smithii Pittier* are the source of the remarkable new cyclobutanes 2,4-methanoproline and 2,4-methanoglutamic acids [(3) and (4) respectively;[29] antibiotic SF-1836 (17) is a homologue of the former[167]]; and

[12] A. G. Szymanowicz, G. Poulin, N. Fontaine, J. P. Werquin, and J. P. Borel, *J. Chromatogr.*, 1980, **190**, 457.
[13] G. Krampitz, H. Meisel, and W. Witt-Krause, *Naturwissenschaften*, 1980, **67**, 38.
[14] B. Y. Hiraoka, K. Fukasawa, K. M. Fukasawa, and M. Harada, *J. Biochem.* (*Tokyo*), 1980, **88**, 373.
[15] Z. Deyl, K. Macek, M. Adam, and T. Vancskova, *Biochim. Biophys. Acta*, 1980, **625**, 248.
[16] D. Fujimoto, *Biochem. Biophys. Res. Commun.*, 1980, **93**, 948.
[17] D. F. Elsden, N. D. Light, and A. J. Bailey, *Biochem. J.*, 1980, **185**, 531.
[18] P. H. Bernstein and G. L. Mechanic, *J. Biol. Chem.*, 1980, **255**, 10414.
[19] J. N. Eloff, *Z. Pflanzenphysiol.*, 1980, **98**, 403.
[20] Y. Oka, T. Ogawa, and K. Sasaoka, *Agric. Biol. Chem.*, 1980, **44**, 1959.
[21] F. Imperato, *Chem. Ind.* (*London*), 1980, 388.
[22] Y. Kuroda, H. Tanaka, M. Okamoto, T. Goto, M. Kosaka, H. Aoki, and H. Imanaka, *J. Antibiot.*, 1980, **33**, 280.
[23] M. J. Dognin and B. Wittmann-Liebold, *Hoppe-Seyler's Z. Physiol. Chem.*, 1980, **361**, 1697.
[24] G. M. Smith and G. W. Pettigrew, *Eur. J. Biochem.*, 1980, **110**, 123.
[25] O. Olsen and H. Soerensen, *Phytochemistry*, 1980, **19**, 1717.
[26] K. Nomoto, H. Yoshioka, T. Takemoto, S. Fushiya, S. Nozoe, and S. Takagi, *Koen Yoshishu-Tennen Yuki Kagobutsu Toronkai, 22nd*, 1979, 619 (*Chem. Abstr.*, 1981, **93**, 47161).
[27] S. Fushiya, Y. Sato, S. Nozoe, K. Nomoto, T. Takemoto, and S. Takagi, *Tetrahedron Lett.*, 1980, **21**, 3071.
[28] S. Fushiya, Y. Sato, and S. Nozoe, *Chem. Lett.*, 1980, 1215.
[29] E. A. Bell, M. Y. Qureshi, R. J. Pryce, D. H. Janzen, P. Lemke, and J. Clardy, *J. Am. Chem. Soc.*, 1980, **102**, 1409; synthesis M. C. Purring, *Tetrahedron Lett.*, 1980, **21**, 4577; P. Hughes, M. Martin, and J. Clardy, *ibid.*, p. 4579.



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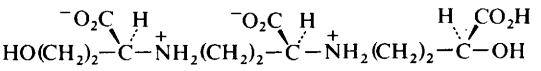

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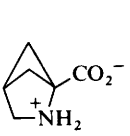

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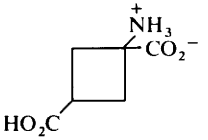

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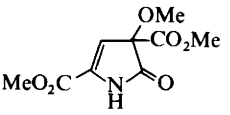
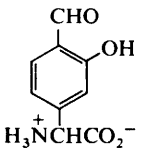


(5)

sargassumlactam, (5), a new $\beta\gamma$-unsaturated γ-lactam from the marine alga *Sargassum kjellmanianum*.[30] Shinorine,[31] claimed as a new amino-acid (from the red alga *Chondrus yendoi*), is identical with mytilin A (see Vol. 12, p. 4), a member of the palythine family (Vol. 11, p. 3).

Fungal and bacterial sources of new amino-acids are: *Streptomyces catenulae* (antibiotic FR-900130 is L-2-amino-3-butynoic acid);[32] unspecified *Actinomyces* [source of forphenicine, (6)];[33] *Streptomyces filamentosus* [antibiotic SF-1961, (7)];[34] 2-(3-alanyl)clavam, (8), from *Streptomyces clavuligerus*;[35] arogenic acid, (9), a biosynthetic precursor of phenylalanine and tyrosine (from a *Neurospora crassa* mutant).[36]



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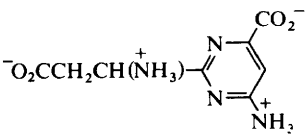

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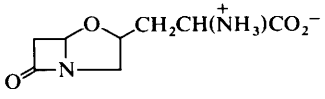

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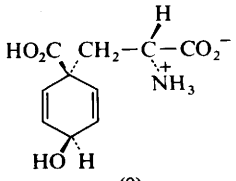

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[31] I. Tsujino, K. Yabe, and I. Sekikawa, *Bot. Mar.*, 1980, **23**, 65.
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[33] T. Yamamoto, K. Kojiri, H. Morishima, H. Naganawa, T. Aoyagi, and H. Umezawa, *J. Antibiot.*, 1978, **31**, 483.
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[35] M. Kellett, D. Pruess, and J. P. Scannell, *U.S.P.* 4 202 819 (*Chem. Abstr.*, 1980, **93**, 130 567).
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New Amino-acids from Hydrolysates.—One of the four possible stereoisomers of 3,4-dihydroxy-L-proline, the 2,3-*trans*-3,4-*trans* isomer, is a component of the virotoxins, toxic peptides of *Amanita virosa*.[37] Additional information on the chlorotyrosine derivatives from vancomycin (see Vol. 12, p. 5) has been published.[38]

3 Chemical Synthesis and Resolution of Amino-acids

General Methods of Synthesis of Amino-acids.—Standard syntheses of amino-acids have been applied to the synthesis of analogues of ibotenic acid,[39] including alkylation of diethyl acetamidomalonate (used in other laboratories;[40] see also refs. 75, 78, and 117). Alkylation of the potassium enolate of the Schiff base $(RS)_2C{=}NCH_2CO_2Et$ with alkyl halides illustrates a general synthesis of α-amino-acids from glycine derivatives which is of increasing importance.[41] As in other examples of this approach,[84] di-alkylation is feasible. The Bucherer-Bergs hydantoin synthesis (see refs. 120 and 121) and Strecker synthesis (see ref. 94) have been useful general procedures.

Yields of 21—84% have been claimed for the conversion of a primary amide into an α-acylamino-acid ($R^1CHO + CO + R^2CONH_2 \rightarrow R^2CONHCHR^1CO_2H$), catalysed by $Co_2(CO)_8$.[42] Effects of electron or radical scavengers on the amination of carboxylic acids induced by γ-irradiation have been studied.[43] Hydrogenolysis of 1-aryl-3-azido-azetidinones has been explored as a route to β-amino-acid amides.[44]

Examples of the applications of standard synthetic approaches to β- and higher homologous amino-acids are included later in this chapter.

Asymmetric Synthesis of Amino-acids.—Further development of previously established methods is illustrated in a synthesis of 2-t-butylglycine ('t-leucine') based on the asymmetric addition of HCN to the Schiff base derived from pivalic aldehyde and (*S*)-1-phenylethylamine, followed by hydrolysis and hydrogenolysis (see also Scheme 1);[45] asymmetric addition of $PhCH_2SH$ to α-phthalimidoacrylate catalysed by acrylonitrile–cinchona alkaloid co-polymers [to give an enantiomeric excess of the (*S*)-isomer of *N*-phthaloyl-*S*-benzylcysteine when quinine or cinchonidine are used];[46] asymmetric hydroformylation and hydrocarboxylation of enamides catalysed by hydridorhodium(II)carbonyl–chiral phosphine complexes[47] (use of a chiral aldehyde in the distantly related α-acylamino-acid synthesis[42] described in the preceding section led to no enantiomeric excess); and asymmetric hydrogenation processes of various types {alkylidene-oxazolinones

[37] A. Buku, H. Faulstich, T. Wieland, and J. Dabrowski, *Proc. Natl. Acad. Sci. U.S.A.*, 1980, **77**, 2370.
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[43] K. Ema and T. Masuda, *Technol. Rep. Osaka Univ.*, 1980, **30**, 313 (*Chem. Abstr.*, 1980, **93**, 168 566).
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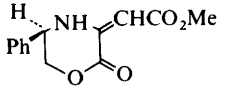
[47] Y. Becker, A. Eisenstadt, and J. K. Stille, *J. Org. Chem.*, 1980, **45**, 2145.

with rhodium–chiral phosphine complexes[48] or with common hydrogenation catalysts in the presence of (*S*)-l-phenylethylamine[49] or Al–Hg,[50,51] or H_2–Raney Ni[50] hydrogenation of chiral 6-phenyl-2-alkylidene-oxazinones [(10) gives L-aspartic acid in 14—17% optical yield][50] and chiral dioxazepinones[51]}. The latter is an example of hydrogenation of a chiral Schiff base, related to the asymmetric synthesis of β-amino-acids by hydrogenation of (*Z*)-3-[(*R*)-1-phenylethylamino]-αβ-unsaturated esters.[52]



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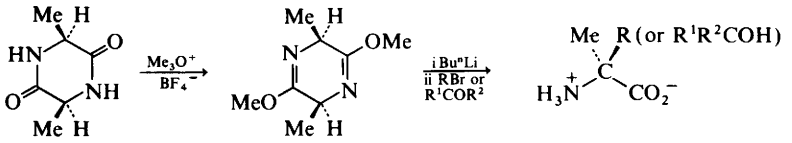
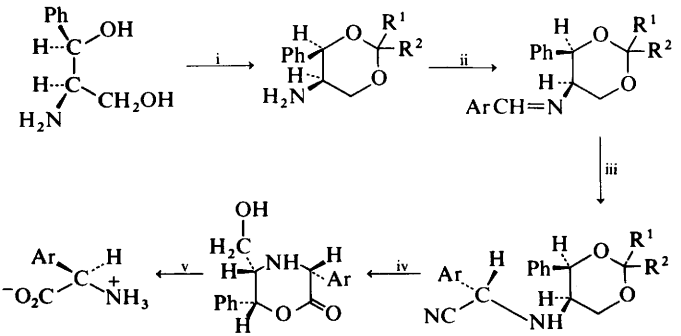


(11)

Enantioselective alkylation of the mono-anion of the L-alanine dioxopiperazine derivative (11) provides a route to α-methyl-α-amino-acids involving moderately high (41—74%) asymmetric induction.[53] The advantage of enclosing a chiral signal-centre in a ring in this area of asymmetric synthesis is further illustrated in a use of chiral 4-phenyl-5-alkylamino-1,3-dioxans (Scheme 1) leading to *C*-arylglycines.[54]



Scheme 1

Reagents: i, R^1R^2CO; ii, ArCHO; iii, HCN; iv, conc. HCl; v, $NaIO_3$ (aq)

[48] J. Koettner and G. Greber, *Chem. Ber.*, 1980, **113**, 2323.
[49] E. I. Karpeiskaya, G. V. Chel'tsova, E. I. Klabunovskii, and A. P. Kharchevnikov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1980, 1082.
[50] M. Tamura and K. Harada, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 561.
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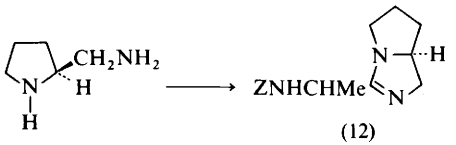
[54] K. Weinges, K. P. Klotz, and H. Droste, *Chem. Ber.*, 1980, **113**, 710; K. Weinges, G. Brune, and H. Droste, *Liebigs Ann. Chem.*, 1980, 212.

Useful asymmetric transformations are illustrated by the conversion (71.7%) of the (*R*)-1-phenylethylammonium salt of (*R,S*)-*N*-benzoyl-*C*-phenylglycine into the corresponding salt of the (*S*)-acid (overall 77% yield) by boiling in toluene solution,[55] and a related use of optically active cobalt(III)tetrammine-*N*-methyl-L-alanine complexes [56] (and see ref. 234). (*R*)-Alanine results from the hydrolysis of the imidazoline (12) formed from either (*R*)- or (*S*)-*N*-benzyloxycarbonylalanine imidate and (*S*)-2-(aminoethyl)pyrrolidine, as a result of auto-epimerization.[57]



(12)

Prebiotic Synthesis; Model Reactions.—A general review [58] and specific survey of results from studies of the formation of amino-acids from sugars and NH_3 in a model sea medium [59] indicate the broad scope of this topic. Most of the recent papers continue the themes established in earlier years [^{60}Co-γ-irradiation of O_2-free aqueous NH_4CN;[60] photolysis of NH_3 in propionic acid gives α- and β-alanines through $NH(^1\Delta)$ insertion of C—H bonds,[61] whereas atomic nitrogen attacks acetic or succinic acids in aqueous media, leading to glycine, aspartic acid, glutamic acid, serine, and threonine;[62] 254 nm irradiation of simple hydrocarbons, water, and NH_3 in the presence or absence of H_2S;[63] carboxylation of primary amines in aqueous solutions at various pH values;[64] and conversions of β-amino-acids into α-amino-acids[240] under contact glow discharge electrolysis conditions [64]]. The increasing emphasis on the involvement of hydrogen cyanide in putative mechanisms for abiogenic synthesis of amino-acids is further justified by the demonstration that this compound is the principal product of i.r.-laser photolysis of a methane–ammonia mixture.[65] Amino-acids are formed in aqueous KCN in the presence of montmorillonite or graphite oxide at 70 °C.[66]

The common feature of these model reactions is the involvement of an energy source to drive thermodynamically unfavourable processes. Matatov has shown that iron(III)-catalysed decomposition of H_2O_2 can facilitate the production of glycine, serine, threonine, and proline from formaldehyde and hydroxylamine hydrochloride in aqueous solutions.[67]

[55] K. Suzuki, S. Kiyooka, T. Miyagawa, and A. Kawai, *Nippon Kagaku Kaishi*, 1980, 287 (*Chem. Abstr.*, 1980, **93**, 95604).
[56] M. Yamagushi, S. Yano, M. Saburi, and S. Yoshikawa, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 691.
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[61] S. Sato, T. Kitamura, and S. Tsunashima, *Chem. Lett.*, 1980, 687.
[62] M. A. Margulis, L. M. Grundel, and E. L. Girina, *Dokl. Akad. Nauk SSSR*, 1980, **251**, 639.
[63] E. Miyoshi, H. Ebisawa, T. Shirai, and S. Yanagisawa, *Nippon Kagaku Kaishi*, 1980, 1120.
[64] J. Terasawa and K. Harada, *Chem. Lett.*, 1980, 73.
[65] D. O. Davis, G. R. Smith, and W. A. Guillory, *Origins Life*, 1980, **10**, 237.
[66] F. Aragon de la Cruz and C. Viton Barbolla, *An. Quim.*, 1979, **75**, 820.
[67] Yu. I. Matatov, *Zh. Evol. Biokhim. Fiziol.*, 1980, **16**, 189 (*Chem. Abstr.*, 1981, **94**, 42850).

Protein Amino-acids and Other Naturally Occurring Amino-acids.—Little scope exists for thorough coverage of biosynthetic production of amino-acids, important though this topic has become in both commercial and mechanistic terms. The general field can be represented by selected references (reviews of enzymic synthesis;[68] fermentative production of L-glutamine by a *Flavobacterium rigense* mutant;[69] microbial conversion of glycine into L-serine,[70] and accumulation of *O*-methyl-L-homoserine in culture media of methanol-utilizing bacteria;[71] and conversion of *trans*-4-hydroxy-L-proline into L-proline *via* the 4,5-dehydro-analogue[72]).

A synthesis of L-α-aminoadipic acid from L-lysine involves treatment of the N^{α}-benzyloxycarbonyl derivative with NaOCl, elimination with DABCO, and hydrolysis of the resulting nitrile in refluxing 4M-HCl.[73] Cyclization of ornithine, lysine, or 5-hydroxylysine with nitrosylpentacyanoiron(II) gives proline, pipecolic acid, and 5-hydroxypipecolic acid, respectively.[74] Further new syntheses of γ-carboxy-L-glutamic acid involve either alkylation of diethyl benzyloxycarbonylamino-malonate with the Mannich reaction product of di-t-butyl malonate,[75] or carboxylation of *N*-trityl dibenzyl L-glutamate with benzyl chloroformate after carbanion formation with $LiNPr^{i}_{2}$, followed by de-protection with H_2–Pd.[76] Full details have been published[77] of the novel synthesis of kainic acid reported in Vol. 11 (p. 10). γ-Oxo-DL-homotyrosine has been prepared from *p*-methoxyphenacyl bromide and diethyl acetamidomalonate.[78]

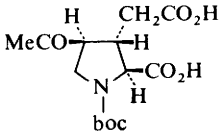
Syntheses of β-amino-acids reported in 1980 include (2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoic acid (present in amastatin), prepared from *N*-benzyloxycarbonyl-D-leucine methyl ester *via* $LiAlHBu^{i}_{2}$ reduction into the aldehyde, thence into the cyanohydrin,[79] and an alternative route to the same series of compounds from chiral oxiranes.[80] *threo*-γ-Hydroxy-L-β-lysine has been prepared by Arndt-Eistert extension of the corresponding lysine derivative.[81] A useful synthetic route to δ-amino-acids has been illustrated with a synthesis of δ-aminolaevulinic acid.[82]

Aliphatic Amino-acids.—*C*-t-Butylglycine ('t-leucine') is accessible through addition of MeMgI to 2-phenyl-4-isopropylidene-oxazolinone or to $Me_2C{=}C(CO_2Et)_2$ followed by hydrolysis or Curtius rearrangement, respective-

[68] N. Esaki, K. Soda, H. Kumagai, and H. Yamada, *Biotechnol. Bioeng.*, 1980, **22** (*Suppl. 1*), 127; Y. Hirose and H. Shibai, *ibid.*, p. 111.
[69] K. Nabe, T. Ujimaru, N. Izuo, S. Yamada, and I. Chibata, *Appl. Environ. Microbiol.*, 1980, **40**, 19.
[70] Y. Tanaka, K. Araki, and K. Nakayama, *J. Ferment. Technol.*, 1980, **58**, 417.
[71] Y. Tanaka, K. Araki, and K. Nakayama, *Biotechnol. Lett.*, 1980, **2**, 67.
[72] J. Varner, *Biochem. Biophys. Res. Commun.*, 1980, **96**, 692.
[73] A. I. Scott and T. J. Wilkinson, *Synth. Commun.*, 1980, **10**, 127.
[74] M. T. Beck, A. Katho, and L. Dozsa, *Magy. Kem. Foly.*, 1980, **86**, 337.
[75] A. Juhasz and S. Bajusz, *Int. J. Pept. Protein Res.*, 1980, **15**, 154.
[76] R. K.-Y. Zee-Cheng and R. E. Olsen, *Biochem. Biophys. Res. Commun.*, 1980, **94**, 1128.
[77] W. Oppolzer and H. Andres, *Helv. Chim. Acta*, 1979, **62**, 2282.
[78] W. Keller-Schierlein and B. Joos, *Helv. Chim. Acta*, 1980, **63**, 250.
[79] D. H. Rich, B. J. Moon, and A. S. Boparai, *J. Org. Chem.*, 1980, **45**, 2288.
[80] K. Kato, T. Saino, R. Nishizawa, T. Takita, and H. Umezawa, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1618.
[81] T. Teshima, T. Ando, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 1191.
[82] G. Schulz and W. Steglich, *Chem. Ber.*, 1980, **113**, 787.

ly.[83] Unsaturated analogues of D-α-amino-adipic acid[84] and of 3-halo-4-aminobutanoic acids[85] have been prepared by alkylation of $Ph_2C{=}NCH_2CO_2Et$ with $EtO_2CCH{=}CHCH_2Br$, and from $ClCH_2C{\equiv}CCO_2H$, respectively, followed by straightforward elaboration. Kolbe reactions with mixtures of differently protected glutamic acids lead to 2,4-di-aminosuberates.[86]

Proline derivatives and analogues feature as synthetic objectives for several laboratories. A 90:10 *cis*:*trans*-mixture of 1-benzyl-2-methylazetidine-carboxylates emerges from condensation of methyl 2,4-dibromopentanoate with benzylamine;[87] an improved preparation of (*S*)-3,4-dehydroproline based on H_3PO_2–HI reduction of pyrrole-2-carboxylic acid involves resolution with (+)-tartaric acid,[88] which is not necessary in the apparently easier route from L-hydroxyproline involving protection and Chugaev elimination of the xanthate (formed with CS_2 and $Bu^n{}_4N^+HSO_4^-$);[89] 1,2-dehydroproline gives the 3-phenoxy-analogue through allylic bromination followed by treatment with thallium phenoxide, easily reduced to *cis*:*trans*-3-phenoxyproline.[90] Conversion of kainic acid into the strongly neuro-excitatory proline derivative (13) is achieved by ozonolysis of the *N*-boc-derivative.[91]



(13)

α-Alkyl Analogues of Protein Amino-acids.—Asymmetric synthesis of α-methyl-α-amino-acids has been illustrated earlier in the chapter,[53] and the same general objective, formation of the α-carbanion of a protected amino-acid followed by alkylation, has been used in a synthesis of α-methyltryptophan.[92] Synthesis of α-hydroxymethylserine from the reaction of formaldehyde with cobalt(III), copper(II), or nickel(II) complexed glycine Schiff bases,[93] and the synthesis of α-(hydroxymethyl)aspartic acid through the Strecker synthesis with $AcOCH_2COCH_2CO_2Et$[94] illustrate previously used routes. α-Vinyl analogues can be prepared through Michael addition of a 2-phenyloxazolin-5-one to $PhSO_2C{\equiv}CH$ followed by sulphone cleavage,[95] or by alkylation of a Schiff base with (*E*)- or (*Z*)-RCH=CHBr after carbanion formation with $LiNPr^i{}_2$.[96]

[83] T. Miyazawa, T. Nagai, T. Yamada, S. Kuwata, and H. Watanabe, *Mem. Konan Univ., Sci. Ser.*, 1979, **23**, 51 (*Chem. Abstr.*, 1980, **92**, 94681).
[84] R. D. Allan, *J. Chem. Res.* (*S*), 1980, 392.
[85] R. D. Allan, *Aust. J. Chem.*, 1979, **32**, 2507; R. D. Allan, G. A. R. Johnston, and B. Twitchin, *Aust. J. Chem.*, 1980, **33**, 1115.
[86] R. E. Nutt, R. G. Strachan, D. F. Veber, and F. W. Holly, *J. Org. Chem.*, 1980, **45**, 3078.
[87] D. S. Soriano, K. F. Podraza, and N. H. Cromwell, *J. Heterocycl. Chem.*, 1980, **17**, 623.
[88] J. W. Scott, A. Focella, U. O. Hengartner, D. R. Parrish, and D. Valentine, *Synth. Commun.*, 1980, **10**, 529.
[89] J. R. Dormoy, B. Castro, G. Chappuis, U. S. Fritschi, and P. Grogg, *Angew. Chem.*, 1980, **92**, 761.
[90] J. Hausler and U. Schmidt, *Liebigs Ann. Chem.*, 1979, 1881.
[91] O. Goldberg, A. Luini, and V. I. Teichberg, *Tetrahedron Lett.*, 1980, **21**, 2355.
[92] M. F. Brana, M. Garrido, M. L. Lopez, and A. M. Sanz, *J. Heterocycl. Chem.*, 1980, **17**, 829.
[93] L. Casella, A. Pasini, R. Ugo, and M. Visca, *J. Chem. Soc., Dalton Trans.*, 1980, 1655.
[94] J. J. Walsh, D. E. Metzler, D. Powell, and R. A. Jacobson, *J. Am. Chem. Soc.*, 1980, **102**, 7136.
[95] W. Steglich and H. Wegmann, *Synthesis*, 1980, 481.
[96] P. Bey and J. P. Vevert, *J. Org. Chem.*, 1980, **45**, 3249.

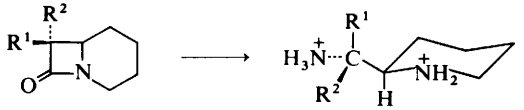
α-Heteroatom-substituted α-Amino-acids.—Good yields of α-methoxy-*N*-acetyl amino-acids are obtained through the reaction of an *N*-acetyl-*N*-benzyloxy-amino-acid ester with potassium t-butoxide and MeOH.[97] α-Bromination (NBS) and treatment with potassium thiolacetate places an acetylthio-grouping at the α-position of an *N*-acylamino-acid ester.[98]

Aliphatic Amino-acids Carrying Halogen Substituents in Side-chains.—Further examples of the use of aziridinecarboxylates for the preparation of β-fluoro-α-amino-acids, by treatment with HF–pyridine, have been reported (see Vol. 12, p. 8).[99a] The relative stereochemistry of the products has been defined[99b] by chemical correlations and *X*-ray analysis.

Aliphatic Amino-acids Carrying Hydroxy-groups in Side-chains.—Free-radical chlorination of L-valine, and hydrolysis, gives a mixture of stereoisomers from which (2*S*,3*S*)- and (2*S*,3*R*)-4-hydroxyvaline have been isolated by crystallization and hydrolysis.[100] A 34:66 *erythro*:*threo*-mixture of γ-hydroxy-DL-ornithine formed through hydrolysis of 2,5-di-amino-4-pentanolide isomers has been separated and converted into corresponding γ-hydroxyarginines.[101]

α-Amino-acids with Unsaturated Side-chains.—A new synthesis of L-vinylglycine [(*S*)-2-amino-but-3-enoic acid] from L-methionine involves conversion into the sulphoxide, followed by pyrolytic elimination of methanesulphenic acid.[102] Another example of the dehydration of *N*-benzyloxycarbonylserine or threonine into the corresponding αβ-dehydro-amino-acids employing DCCI has been reported.[103]

Synthesis of Aromatic and Heterocyclic Amino-acids.—Most examples included in this section this year, as in previous years, concern simple derivatives of the protein aromatic and heteroaromatic amino-acids, but an interesting stereospecific synthesis of a saturated heterocyclic amino-acid (14) has also been described.[104]



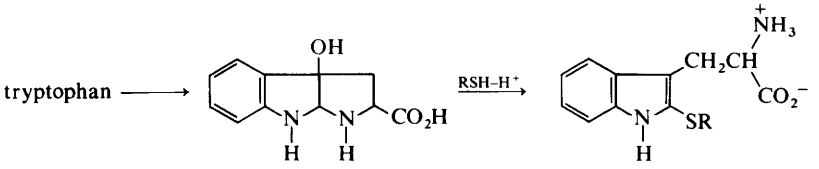
(14)
$R^1 = H$, $R^2 = CO_2^-$
$R^1 = CO_2^-$, $R^2 = H$

Tyrosine derivatives offering some interest in terms of routes for their synthesis are 3-fluoro- and 3,5-difluoro-L-tyrosine (from L-tyrosine methyl ester *via* nitration, reduction, and diazotization with $NaNO_2$–HBF_4),[105] and 3,4-dihydroxy-6-

[97] J. D. M. Herscheid, R. J. F. Nivard, M. W. Tijhuis, H. P. H. Scholten, and H. C. J. Ottenheijm, *J. Org. Chem.*, 1980, **45**, 1880.
[98] Z. Lidert and S. Gronowitz, *Synthesis*, 1980, 322.
[99] (*a*) A. Barama, R. Condom, and R. Guedj, *J. Fluorine Chem.*, 1980, **16**, 183; T. N. Wade and R. Kheribet, *J. Chem. Res.* (*S*), 1980, 210; (*b*) T. Tsuchima, T. Sato, and T. Tsuji, *Tetrahedron Lett.*, 1980, **21**, 3591; T. Tsuchima, J. Nishikawa, T. Sato, H. Tamida, K. Tori, and T. Tsuji, *ibid.*, p. 3593.
[100] J. J. Usher, *J. Chem. Res.* (*S*), 1980, 30.
[101] K. Mizusaki, H. Yamamoto, and S. Makisumi, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 2605.
[102] A. Afzali-Ardakani and H. Rapoport, *J. Org. Chem.*, 1980, **45**, 4817.
[103] M. J. Miller, *J. Org. Chem.*, 1980, **45**, 3131.
[104] B. T. Golding and A. J. Smith, *J. Chem. Soc., Chem. Commun.*, 1980, 702.
[105] K. K. Kirk, *J. Org. Chem.*, 1980, **45**, 2015.

fluorophenylalanine, formed in 25% yield from 3-methoxy-L-tyrosine ethyl ester and XeF_2.[106] Tyrosine or dopa can be converted into 5-hydroxydopa in the presence of tyrosinase.[107]

A novel route to 2-alkylthio-tryptophans [(15) → (16)][108] has been used in a synthesis of tryptathionine [16; R = $CH_2CH(NH_2)CO_2H$], a constituent of phalloidin. 5- and 7-bromotryptophans have been prepared by Fischer cyclization of the appropriate bromophenylhydrazones of 4-acetamido-4,4-bis(ethoxycarbonyl)butanals.[109]



(15) (16)

2-Trifluoromethylhistidines have been used in preparations of 2-carboxy-, 2-cyano-, and 2-ethoxycarbonyl-histidines.[110]

N-Benzoyl-α-hydroxyglycine, $PhCONHCH(OH)CO_2H$, continues to be used for the synthesis of *C*-arylglycines; a paper in the current literature describes condensations with benzimidazol-2-one and with benzo[*c*]-thiophen-2,2-dioxide.[111]

Synthesis of *N*-substituted Amino-acids.—Addition of HCN to an imine yields an α-amino-alkyl cyanide [$R^1CH{=}NR^2 + HCN \rightarrow R^1CH(CH)NHR^2$], from which *N*-mono-substituted α-amino-acids may be obtained.[112] A simple route to an *N*-mono-alkylamino-acid in which an *N*-benzyl-*N*-alkyl-L-amino-acid is debenzylated by hydrogenolysis[113] depends on the ready availability of *N*-benzyl-L-amino-acids.

Spontaneous conversion of L-lysine into N^{ε}-mono-, -di-, and -trimethyl-derivatives occurs with formaldehyde in aqueous solutions.[114]

Conversion of *N*-arylidene-amino-acid esters into oxaziridines using monoperphthalic acid provides a suitable intermediate for the synthesis of *N*-hydroxy-amino-acids through reaction with hydroxylamine.[115]

Synthesis of α-Amino-acids Containing Sulphur or Selenium.—Nucleophilic substitution of methyl α-acetamido-β-chloroacrylate with a thiol gives the corresponding β-alkylthioacrylate, whereas thiolacetic acid yields *N*-acetyl-ββ-bis(acetylthio)alanine.[116]

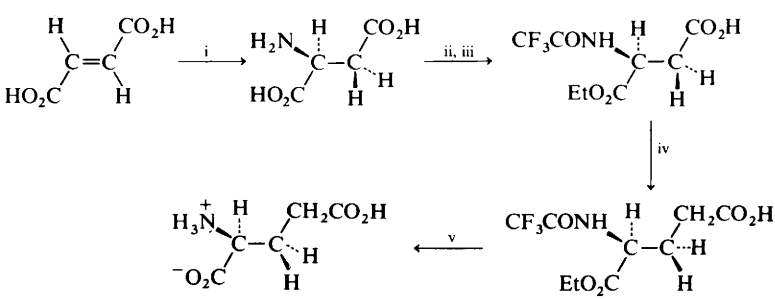
[106] G. Firnan, R. Chirakal, S. Sood, and S. Garnett, *Can. J. Chem.*, 1980, **58**, 1449.
[107] C. Hansson, H. Rorsman, and E. Rosengren, *Acta Derm.-Venereol.*, 1980, **60**, 281.
[108] W. E. Savige and A. Fontana, *Int. J. Pept. Protein Res.*, 1980, **15**, 102.
[109] M. C. Allen, D. E. Brundish, and R. Wade, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1928.
[110] H. Kimito and L. A. Cohen, *J. Org. Chem.*, 1980, **45**, 3831.
[111] M. L. Edwards, *J. Heterocycl. Chem.*, 1980, **17**, 383.
[112] S. S. Nain, N. H. Khan, and A. A. Siddiqui, *Indian J. Chem., Sect. B*, 1980, **19**, 622.
[113] J. N. Eloff, *Z. Pflanzenphysiol.*, 1980, **98**, 411.
[114] E. Tyihak, L. Trezl, and I. Rusznak, *Pharmazie*, 1980, **35**, 18.
[115] T. Polonski and A. Chimiak, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1979, **27**, 459.
[116] A. J. Kolar and R. K. Olsen, *J. Org. Chem.*, 1980, **45**, 3246.

The acetamidomalonate route has been used for the synthesis of 2-selenienylalanine, using 2-chloromethylselenophen as alkylating agent.[117]

Synthesis of Phosphorus-containing α-Amino-acids.—Improved preparations of N^{ε}-phospholysine and N^{ω}-phospho-arginine starting from the α-amino-acids have been described.[118]

α-Amino-acids Synthesized for the First Time.—New α-amino-acids not mentioned elsewhere in this chapter are 2-amino-(4′-hydroxy-6′-benzothiazolyl)propanoic acid,[119] 1-amino-1-carboxy-3,4-benzocyclo[2.2.2]octane,[120] and D- and L-2-(1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)glycine.[121]

Synthesis of Labelled Amino-acids.—L-[3′,5′-$^{13}C_2$]phenylalanine has been prepared from the correspondingly labelled tyrosine (synthesis displayed in Vol. 12, p. 16) by conversion into the *O*-(1-phenyltetrazol-5-yl)-derivative followed by catalytic transfer hydrogenolysis with cyclohexene.[122] Further examples of labelled amino-acids prepared for biosynthetic investigations are DL-[2-^{13}C]leucine,[123] DL-[*Me*-^{13}C]valine (starting from $^{13}CH_3OH$, thence to 2-[*Me*-^{13}C]methylthiazoline),[124] and DL-[2-^{14}C,2′,3′-$^{13}C_2$]tryptophan (prepared from [2-^{14}C]indole, [^{13}C]formaldehyde, and diethyl[2-^{13}C]malonate).[125] A route applied for the synthesis of (2*S*,3*S*)-[3-$^{2}H_1$]-, (2*S*,3*R*)-[2,3-$^{2}H_2$]-, (2*S*,3*S*,4*RS*)-[3-$^{2}H_1$, 4-$^{3}H_1$]-, and (2*S*,3*R*, 4*RS*)-[2,3-$^{2}H_2$, 4-$^{3}H_1$]-glutamic acids [126] is displayed in Scheme 2. ^{14}C-Labelled amino-acids described in recent papers include DL-[1-^{14}C]valine,[127] *N*-[*Me*-^{14}C]methyl-L-alanine,[113] N^{δ}-[*Me*-^{14}C]methylarginine,[128] β-[^{14}C]alanine from L-[^{14}C]aspartic



Scheme 2

Reagents: i, L-aspartase–NH_4Cl in $^{2}H_2O$ or $^{1}H_2O$; ii, trifluoroacetic anhydride; iii, EtOH; iv, $SOCl_2$ then CH_2N_2–Wolff rearrangement [*hν*–dioxan (aq)]; v, HCl (aq)

[117] T. Frejd, M. A. Davis, S. Gronowitz, and T. Sadeh, *J. Heterocycl. Chem.*, 1980, **17**, 759.
[118] J. M. Fujitaki, A. W. Steiner, S. E. Nichols, E. R. Helander, Y. C. Liu, and R. A. Smith, *Prep. Biochem.*, 1980, **10**, 205.
[119] I. A. Ismail, D. E. Sharp, and M. R. Chedekel, *J. Org. Chem.*, 1980, **45**, 2243.
[120] G. L. Grunewald, S. H. Kuttab, M. A. Pleiss, J. B. Mangold, and P. Soine, *J. Med. Chem.*, 1980, **23**, 754.
[121] A. Rosenthal and R. H. Dodd, *J. Carbohydr., Nucleosides Nucleotides*, 1979, **6**, 467.
[122] V. Viswanatha and V. J. Hruby, *J. Org. Chem.*, 1980, **45**, 2010.
[123] V. Viswanatha, B. Larsen, and V. J. Hruby, *Tetrahedron*, 1979, **35**, 1575.
[124] T. W. Whaley, G. H. Daub, V. N. Kerr, T. A. Lyle, and E. S. Olsen, *J. Labelled Compd., Radiopharm.*, 1979, **16**, 809.
[125] E. Leete, *J. Nat. Prod.*, 1980, **43**, 130.
[126] S. J. Field and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1979, 1163.
[127] B. Meesschaert, P. Adriaens, and H. Eyssen, *J. Labelled Compd., Radiopharm.*, 1980, **17**, 263.
[128] W. K. Paik, M. K. Paik, and S. Kim, *Anal. Biochem.*, 1980, **104**, 343.

acid mediated by aspartate 1-decarboxylase,[129] and 1-aminocyclobutanecarboxylic acid labelled in the carboxy-group.[130]

Exchange of aromatic ring protons of tyrosine using $^{2 \text{ or } 3}H_2O$, $^{2 \text{ or } 3}HCl$, and K_2PtCl_4 at 100 °C involves mainly the 3- and 5-positions,[131] and ^3H-atom bombardment is similarly specific as far as the aromatic ring is concerned but also brings about 84% exchange at the methylene protons;[132] the latter process with solid phenylalanine causes multiple exchange[133] but with alanine, predominantly α-substitution.[134] Less energetic methods have been used for the preparation of ^3H-labelled 1-(3,3-dimethylallyl)-L-tryptophan.[135]

Eighteen examples of ^{18}O-carboxy-group labelled amino-acids have been worked through, achieving 90 atom% incorporation by equilibration in $H_3{}^{18}O^+$–$H_2{}^{18}O$ at 60–70 °C during several days.[136]

Standard reactions have been used for the preparation of *m*- and *p*-[^{18}F]fluoro-DL-phenylalanines,[137] [3,5-$^{80m}Br_2$]dibromotyrosine,[138] [3,5-$^{125}I_2$]tri-iodo-L-thyronine,[139] and β-[^{131}I]iodo-D-alanine.[140] Conversion of 3-iodo- or 3,5-di-iodotyrosines into corresponding [^{211}At]astatotyrosines involves solid-state exchange reactions.[141]

[^{75}Se]Selenaproline has been prepared by the reaction of L-[^{75}Se]selenocysteine with formaldehyde.[142]

Resolution of Amino-acids.—Major areas of study have developed from long-established principles for the resolution of racemic amino-acids, employing various chiral stationary phases for liquid chromatography and exploiting the enantiospecificity of enzyme-mediated processes. At the same time, the usual methods based on separation of diastereoisomeric salts continue to be commonly used (*e.g.* resolution of DL-[1-^{14}C]lysine using L-glutamic acid[143]). A further example of the use of the principle of seeding a saturated solution with crystals of the desired enantiomer of an amino-acid has been described with a novel variation, in which *N*-acetyl-L-leucine of optical purity 92.6% is produced by asymmetric transformation of DL-leucine through seeding a reaction mixture in acetic anhydride–acetic acid with L-leucine.[144] Cram's major project on reciprocal chiral

[129] J. E. Cronan, *Anal. Biochem.*, 1980, **103**, 377.
[130] L. C. Washburn, T. T. Sun, B. L. Byrd, R. L. Hayes, and T. A. Butler, *J. Nucl. Med.*, 1979, **20**, 1055.
[131] M. Kanska and S. Drabarek, *Radiochem. Radioanal. Lett.*, 1980, **44**, 207.
[132] E. S. Filatov, M. A. Orlova, and E. F. Simonov, *Radiokhimiya*, 1980, **22**, 614.
[133] E. S. Filatov, M. A. Orlova, and E. F. Simonov, *Vestn. Mosk. Univ., Khim.*, 1980, **21**, 49 (*Chem. Abstr.*, 1980, **93**, 26749).
[134] E. S. Filatov, E. F. Simonov, A. V. Shishkov, and V. P. Mogil'nikov, *Radiokhimiya*, 1979, **21**, 909.
[135] M. F. Grundon, M. R. Hamblin, D. M. Harrison, J. N. D. Logue, M. Maguire, and J. A. McGrath, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1294.
[136] R. C. Murphy and K. L. Clay, *Biomed. Mass Spectrom.*, 1979, **6**, 309.
[137] R. W. Goulding and J. C. Clark, *J. Labelled Compd., Radiopharm.*, 1979, **16**, 145.
[138] U. A. M. Hadi, D. J. Malcome-Lawes, and G. Oldham, *Int. J. Appl. Radiat. Isot.*, 1979, **30**, 709.
[139] K. Sato and H. J. Cahnmann, *Anal. Biochem.*, 1980, **102**, 237.
[140] C.-Y. Shine and A. P. Wolf, *J. Labelled Compd. Radiopharm.*, 1980, **17**, 53.
[141] G. W. M. Visser, E. L. Diemer, and F. M. Kaspersen, *Int. J. Appl. Radiat. Isot.*, 1979, **30**, 749.
[142] S. H. Wong, R. P. Spencer, and A. Weaver, 'Radiopharm 2: Proceedings of 2nd International Symposium', ed. J. A. Sorenson, Soc. Nucl. Med. Inc., New York, 1979, p. 109.
[143] W. T. Buckley and R. R. Marquardt, *Prep. Biochem.*, 1980, **10**, 85.
[144] S. Yamada, C. Hongo, and I. Chibata, *Chem. Ind. (London)*, 1980, 539.

recognition by chiral crown ether hosts employs amino-acid perchlorates as guests, and further results have been described in the 1980 literature.[145]

Amino-acids have been resolved by ligand exchange chromatography, *e.g.* DL-[^{3}H]valine on polystyrene bonded to L-hydroxyproline, complexed with copper(II) ions;[146] DL-proline and DL-histidine, but not other amino-acids, on a similar system;[147] and related studies including uses of *N*-formyl-L-valylaminopropyl-silica.[148] More rapid hydrolysis of D-isomers of amides of DL-leucine or phenyl-alanine occurs on cross-linked polystyrene carrying L-hydroxyprolyl residues complexed with copper(II) ions.[149]

The variation of this procedure, in which the reversed-phase technique with a chiral metal chelate in the mobile phase is employed, has been applied to the resolution of DL-amino-acids [150] and dansyl-DL-amino-acids.[151]

Examples of the use of enzymes for 'resolution' of derivatives of DL-amino-acids include 5-chymotrypsin for the isolation of 5-fluoro-L-tryptophan from the DL-amino-acid methyl ester,[152] immobilized acylase for the liberation of L-methionine from the *N*-acetyl-DL-amino-acid,[153] and a related use of a D-aminoacylase from *Streptomyces olivaceus*,[154] mutant *Brevibacterium* strains for the liberation of L-amino-acids from DL-α-amino-alkyl cyanides,[155] and extensive work on the synthesis of D-amino-acids (*p*-hydroxyphenylglycine,[156] 2-thienylglycine,[157] amino-acids more generally [158]) from DL-hydantoins, *via* *N*-carbamyl derivatives, using microbial hydantoinase (*alias* dihydropyrimidinase [158]). D-α-Amino-adipic acid can be isolated after digestion of the racemate by *Pseudomonas putidea*.[159]

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—Reports of *X*-ray analysis of protein and other natural amino-acids [α-L-glutamic acid,[160] α- and β-forms of DL-methionine,[161] DL-lysine hydrochloride,[162] palythene [163] and paly-

[145] S. S. Peacock, D. M. Walba, F. C. A. Gaeta, R. C. Helgeson, and D. J. Cram, *J. Am. Chem. Soc.*, 1980, **102**, 2043.

[146] N. F. Myasoedov, O. B. Kuznetsova, O. V. Petrenik, V. A. Davankov, and Yu. A. Zolotarev, *J. Labelled Compd. Radiopharm.*, 1980, **17**, 439; V. A. Davankov, in 'Advances in Chromatography', Marcel Dekker, New York, 1980, Vol. 18, p. 139.

[147] J. Josefonvicz, D. Muller, and M. A. Petit, *J. Chem. Soc., Dalton Trans.*, 1980, 76.

[148] A. Foucault, M. Caude, and L. Oliveros, *J. Chromatogr.*, 1979, **185**, 345; A. Dobashi, K. Oka, and S. Hara, *J. Am. Chem. Soc.*, 1980, **102**, 7122; H. Okai and S. Oka, *Proceedings of 15th Peptide Symposium*, 1977, p. 11 (*Chem. Abstr.*, 1980, **93**, 120865).

[149] I. A. Yamskov, B. B. Berezin, and V. A. Davankov, *Makromol. Chem., Rapid Commun.*, 1980, **1**, 125.

[150] E. Gil-Av, A. Tishbee, and P. E. Hare, *J. Am. Chem. Soc.*, 1980, **102**, 5115.

[151] W. Lindner, J. N. LePage, G. Davies, D. E. Seitz, and B. L. Karger, *J. Chromatogr.*, 1979, **185**, 323.

[152] J. T. Gerig and J. C. Klinkenborg, *J. Am. Chem. Soc.*, 1980, **102**, 4267.

[153] W. Kuhlmann, W. Halwachs, and K. Schnegerl, *Chem.-Ing. Tech.*, 1980, **52**, 607.

[154] M. Sugie and H. Suzuki, *Agric. Biol. Chem.*, 1980, **44**, 1089.

[155] A. Arnaud, P. Galzy, and J. C. Jallageas, *Bull. Soc. Chim. Fr., Part 2*, 1980, 87.

[156] S. Shimizu and K. Yoneda, *Hakko To Kogyo*, 1980, **38**, 937.

[157] S. Shimizu, H. Shimada, S. Takahashi, T. Ohashi, Y. Tani, and H. Yamada, *Agric. Biol. Chem.*, 1980, **44**, 2233.

[158] H. Yamada, S. Shimizu, H. Shimada, Y. Tani, S. Takahashi, and T. Ohashi, *Biochimie*, 1980, **62**, 395.

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[161] T. Taniguchi, Y. Takai, and K. Sakurai, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 803.

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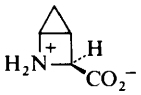
[163] D. Uemura, C. Katayama, A. Wada, and Y. Hirata, *Chem. Lett.*, 1980, 755.

thine trihydrate (see Vol. 11, p. 4),[164] and hydrochlorides of L-isoleucine, L-phenylalanine, and DL-methionine[165]] include a study of L-tyrosine found in a No. 1 Han Dynasty tomb, at Ma-Wang-Tui, China[166] (the word 'found' is tantalizingly used in the abstract source of this information). Antibiotic SF-1836 has been shown to be *trans*-2-azabicyclo[2.1.0]pentane-3*S*-carboxylic acid, (17),[167] related structurally to '2,4-methanoproline', (3), isolated recently[29] from a different source. *X*-Ray crystal analysis of D-α-amino-n-butyric acid has been reported.[168]



(17)

Derivatives of amino-acids studied by the *X*-ray technique include *N*-acetyl-L-cysteine,[169] *N*-(phosphonoethyl)glycine ('glyphosate'),[170] hydantoins of L-proline and D-allo-hydroxyproline,[171] L-arginine L-ascorbate,[172] and *N*-boc-L-phenylalanine.[173] The last-mentioned compound adopts the *E*-configuration in the solid state, although it is known to exist in the *Z*-form in solution in C^2HCl_3.[173]

After a spate of papers in the 1970's on neutron diffraction crystal analysis of amino-acids had appeared to subside, a study has been published on the γ-modification of glycine, studied at 83 K and 293 K.[174]

Nuclear Magnetic Resonance Spectrometry.—^{13}C N.m.r. studies continue to be developed to the point where routine laboratory studies can be carried out against a fully explored general background. However, scope still exists for non-routine ^1H n.m.r. studies, and pioneering work with other nuclei and new instrumentation.

Deprotonation of L-dopa as a function of p^2H is conveniently studied by ^1H n.m.r.[175] Conformational studies for amino-acids complexed to palladium(II)[176] or lanthanide cations[177] give information on rotamer equilibria concerning the C-α—C-β bond. Rotation of the amino-group has been detected in solid L-glutamic acid through ^1H n.m.r. spectrometry.[178] Wide-line n.m.r. studies have continued (see Vol. 12, p. 20), attention being paid to the solution behaviour of hippuric acid.[179]

[164] A. Furusaki, T. Matsumoto, I. Tsujino, and I. Sekikawa, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 319.
[165] B. Khawas, *Indian J. Phys. A*, 1979, **53**, 559.
[166] K.-S. Ting, *K'o Hsueh T'ung Pao*, 1980, **25**, 183 (*Chem. Abstr.*, 1980, **93**, 7014).
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[170] P. Khuuttila and H. Khuuttila, *Acta Chem. Scand., Ser. B*, 1979, **33**, 623.
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[173] J. W. Bats, H. Fuess, H. Kessler, and R. Schuck, *Chem. Ber.*, 1980, **113**, 520.
[174] A. Kvick, W. M. Canning, T. F. Koetzle, and G. J. B. Williams, *Acta Crystallogr., Sect. B*, 1980, **36**, 115.
[175] R. F. Jameson, G. Hunter, and T. Kiss, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1105.
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[177] J. Mossoyan, M. Asso, and D. Beuliau, *Org. Magn. Reson.*, 1980, **13**, 287.
[178] S. Ganapathy, C. A. McDowell, and P. Raghunathan, *J. Magn. Reson.*, 1980, **40**, 1.
[179] N. R. Jagannathan, S. Ganapathy, and R. Srinivasan, *Indian J. Pure Appl. Phys.*, 1980, **18**, 731.

Structural information with finer detail can often be obtained by approaching a problem in solution conformational behaviour with more than one physical technique, and this is well demonstrated in a ^{1}H–^{13}C n.m.r. study of *O*-acetylserine, *O*-phosphoserine and -threonine.[180] At all $p^{2}H$ values between 4 and 14, these derivatives adopt a planar W-type conformation through the H_{α}-C_{α}-C_{β}-O-P atom sequence. Multiple conformations, with similar behaviour in solution to that in the crystal state, are adopted by *N*-acetyl-D-allo-isoleucine, as shown by ^{13}C n.m.r. NT_1 values and proton–proton scalar coupling values.[181] This work establishes the potential of proton relaxation spectra for conformational analysis of amino-acids, and acyclic compounds more generally.

Characteristic ^{13}C chemical shift values as a function of solvent have been identified for *N*-acetylamino-acid methylamides[182] and corresponding esters.[183] Characteristic ^{13}C data have been carefully assembled for solutions at pH values 4.5 to 8.5, and an ambitious claim has been made that these data allow both qualitative and quantitative analysis of mixtures of the twenty common protein amino-acids.[184] A similar study[185] assesses the microscopic protonation behaviour of lysine and hydroxylysine. ^{13}C N.m.r. studies of amino-acids in the solid state have advanced significantly,[186,187] high-resolution data revealing splitting of the C-α resonance associated with nearby structural features, which may therefore possibly be identified.[187]

Natural abundance ^{15}N n.m.r. of α- and ω-amino-acids in protic solvents reveals an upfield shift for the N^{α}-resonance as a result of protonation.[188] The scope for natural abundance ^{17}O n.m.r. analysis of amino-acids has been explored.[189]

Optical Rotatory Dispersion and Circular Dichroism.—C.d. spectra of L-phenylalanine, and its *N*-acetyl and alkyl ester derivatives,[190] and interpretation of c.d. spectra of amino-acid alkyl esters in terms of conformational equilibria[191] extend studies described in earlier volumes of this *Specialist Periodical Report*, and need no further description here. In one of these studies, scope for magneto-c.d. study was offered and pursued.[190] The chromophores in these simple derivatives correspond to those in peptides and proteins, and their c.d. spectra are useful models for the contributions of individual amino-acid residues to the overall chirospectroscopic behaviour of disordered conformations of polypeptides. Long-chain *N*-acyl derivatives of L-glutamic acid and L-valine yield c.d. spectra in

[180] L. Pogliani, D. Zeissow, and C. Krueger, *Tetrahedron*, 1979, **35**, 2867.
[181] N. Niccolai, M. P. Miles, S. P. Hehir, and W. A. Gibbons, *J. Am. Chem. Soc.*, 1980, **102**, 1412.
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[184] V. I. Svergun, S. V. Tarabakin, and V. P. Panov, *Khim.-Farm. Zh.*, 1980, **14**, 104 (*Chem. Abstr.*, 1980, **92**, 193790).
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[186] M. H. Frey and S. J. Opella, *J. Chem. Soc., Chem. Commun.*, 1980, 474.
[187] C. J. Groombridge, R. K. Harris, K. J. Packer, B. J. Say, and S. F. Tanner, *J. Chem. Soc., Chem. Commun.*, 1980, 174.
[188] H. R. Kricheldorf, *Org. Magn. Reson.*, 1979, **12**, 414.
[189] B. Valentine, T. St. Amour, R. Walter, and D. Fiat, *Org. Magn. Reson.*, 1980, **13**, 232.
[190] T. Komiyama and M. Miwa, *Int. J. Quantum Chem.*, 1980, **18**, 527; *Koen Yoshishu Bunshi Kozo Sogo Toronkai*, 1979, 532 (*Chem. Abstr.*, 1980, **93**, 167034).
[191] O. Korver and T. J. Liefkens, *Tetrahedron*, 1980, **36**, 2019.

solution that are not typical of simple acyl derivatives, however, and have been interpreted to reveal the formation of chiral aggregates.[192]

The conversion of amino-acids into 'chromophoric derivatives' for the purpose of configurational or conformational assignments is also a long-established field of study, and the application of previously studied *N*-dithiocarbethoxy-β-amino-acids[193] and fluorescamine derivatives[194] to new configurational assignments has been described. Thus, (+)-(2-furyl- and -thienyl)-β-alanines have the L-configuration;[193] 1-pyrrolinones from L-amino-acids show a positive Cotton effect in the wavelength range 300—324 nm, and a negative Cotton effect in the range 263—290 nm.[194] A substantial study with the same objectives has been published for the chiral iso-indoles formed between D- or L-amino-acids and *o*-phthaldialdehyde with 2-mercaptoethanol.[195] A positive Cotton effect centred near 340 nm characterizes the L-configuration for all common amino-acids except alanine, tryptophan, aspartic acid, and histidine;[195] the same long path must now be trodden as in earlier studies of other chromophoric derivatives of amino-acids, to try to understand the reasons for exceptions to an empirical rule linking sign of Cotton effect with absolute configuration, but the relatively high sensitivity offered by the *o*-phthaldialdehyde derivatives (2×10^{-5}M) may be a sufficient encouragement to pursue these studies.

The c.d. of representative *N*-5- or -6-benzofuroxanyl-L-amino-acids has been reported.[196]

The use of c.d. or polarimetry for quantitative analysis is rarely considered, but a technique for the estimation of an amino-acid in the presence of its methyl ester, and simultaneous determination of the optical purity of the constituents of the mixture, has been worked out. Reaction with the cobalt complex of *NN'*-ethylenebis(acetylacetonimine) at pH 7 gives coloured species for the two constituents whose absorption spectra are sufficiently different to allow the separate contributions of the two species to the c.d. spectra to be measured.[197]

Mass Spectrometry.—The main content of this section in previous volumes has been a good indication of the advance of the frontiers of mass spectrometric analysis, year by year. This has been so because of the difficulty in obtaining spectra for amino-acids themselves, and the eagerness with which new instrumental techniques have been applied in this area. However, most of the analytical laboratories relying on commercially available spectrometers have continued to convert amino-acid mixtures into volatile derivatives, and new examples are 2,2-bis(difluorochloromethyl)oxazolidinones, formed from an amino-acid with bis-(difluorochloromethyl)ketone,[198] and fluorescamine derivatives, for which field

[192] K. Sakamoto and M. Hatano, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 339.
[193] S. Kuwata, T. Yamada, T. Shinogi, N. Yamagami, F. Kitabashi, T. Miyazawa, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3326.
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[195] N. A. Voskova, V. V. Romanov, N. V. Sumbatyan, G. A. Korschunova, and Yu. P. Shvachkin, *Bioorg. Khim.*, 1980, **6**, 731; V. V. Romanov, N. A. Voskova, and Yu. P. Shvachkin, *Khim. Prir. Soedin.*, 1980, 132.
[196] M. M. El-Abadelah, A. A. Anani, Z. H. Khan, and A. M. Hassan, *J. Heterocycl. Chem.*, 1980, **17**, 213.
[197] N. Spassky, M. Reix, M. O. Sepulchre, and J. P. Guette, *Analusis*, 1980, **8**, 130.
[198] R. Liardon, U. Ott-Kuhn, and P. Husek, *Biomed. Mass Spectrom.*, 1979, **6**, 381.

desorption techniques are well suited.[199] Mass spectrometric methods combined with g.l.c. separation would be resorted to for identification as well as quantitation of trace amounts, and brain tissue samples have been analysed in this way after conversion of their constituent amino-acids, with [1,2-$^{13}C_2$, ^{15}N]glycine as internal standard, into *N*-hexafluorobutyryl hexafluoroisopropyl esters.[200] Chemical ionization m.s. techniques can provide the same information for biological fluids containing ng or pg levels of amino-acids.[201]

Problems of interpretation of mass spectra of amino-acid derivatives have also featured in this section in previous volumes, and further study of the rearrangement of trimethylsilyl esters of *N*-acylglycines has been published.[202]

Other Physical and Theoretical Studies.—A number of i.r./Raman spectroscopic papers describe continuing studies of specifically deuteriated α-amino-acids (L-alanine,[203] L- and DL-cysteine[204]) aimed at assignments of vibrational modes. Polarized Raman spectra of α-glycine, L- and DL-alanine[205] continue recently described applications (see Vol. 12, p. 21) of this single-crystal variation of the standard technique. Conformational assignments to *N*-acetylamino-acid esters in different solvents based on i.r. data are usefully supported by n.m.r. studies.[206]

Physical adsorption of α-amino-acids on to clay (sodium montmorillonite) has formed the basis of a persistent theory of enantiomer discrimination leading to the present predominance of the L-isomers in proteins, and i.r. data for these adsorbates have been published.[207] No selective adsorption of protein amino-acids by clay from a solution containing also some non-protein amino-acids could be demonstrated, tending to dispose of a theory accounting for the relatively limited range of protein building blocks.[208] Bentonite has been shown to bind L-leucine, L-aspartic acid, and D-glucose through different contact geometry from that adopted for their respective enantiomers.[209]

Simple physical properties of amino-acid solutions continue to be determined, often by sophisticated methods, including solubilities in water–ethanol,[210] viscosities in water–MeCN,[211] dissociation constants in formic acid–butanone or acetic acid–butanone,[212] and Kerr effect studies of a series of eighteen amino-acids with an attempt to interpret the data in terms of conformational preferences of side-chains.[213] Thermodynamic properties that have been studied include heat

[199] K. E. Murray and D. I. Ingles, *Chem. Ind.* (*London*), 1979, 476.
[200] A. Lapin and M. Karobath, *J. Chromatogr.*, 1980, **193**, 95.
[201] J. M. L. Mee, *Am. Lab.* (*Fairfield, Conn.*), 1980, **12**, 55 (*Chem. Abstr.*, 1980, **93**, 91 335).
[202] P. V. Fennessey and S. S. Tjoa, *Org. Mass Spectrom.*, 1980, **15**, 202.
[203] D. M. Byler and H. Susi, *Spectrochim. Acta, Part A*, 1979, **35**, 1365; H. Susi and D. M. Byler, *J. Mol. Struct.*, 1980, **63**, 1.
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[207] Yu. I. Tarasevich, V. S. Rak, and E. G. Sivalov, *Teor. Eksp. Khim.*, 1980, **16**, 351.
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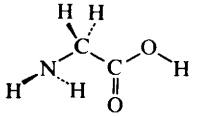
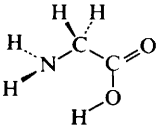
[210] J. C. McGowan and A. Mellors, *J. Appl. Biochem.*, 1979, **1**, 423.
[211] N. C. Dey, B. K. Saikia, and I. Haque, *Can. J. Chem.*, 1980, **58**, 1512.
[212] A. P. Kreshkov, B. B. Tanganov, A. N. Yarovenko, and T. Kh. Batorova, *Zh. Fiz. Khim.*, 1980, **54**, 105; L. Pardeshi and R. A. Bhobe, *J. Indian Chem. Soc.*, 1980, 57, 442 (*Chem. Abstr.*, 1980, **93**, 81 570).
[213] G. Khanarian and W. J. Moore, *Aust. J. Chem.*, 1980, **33**, 1727.

capacities of transfer of amino-acids and peptides from water to aqueous urea,[214] heats of dilution of aqueous solutions of *N*-acetylamino-acid amides,[215] heats of mixing of aqueous solutions of glycine with corresponding solutions of alkali metal chlorides (Li^+ interacts with the amino-acid in a different manner compared with the other cations),[216] partial molar enthalpies of amino-acids in aqueous solutions,[217] and a useful discourse on the thermodynamic parameters (ΔG −7.67, ΔH −9.9, and ΔS −7.5 kcal mol^{-1}) for zwitterion formation $H_2NCHRCO_2H \rightleftharpoons H_3\overset{+}{N}CHRCO_2^-$.[218]

Molecular orbital calculations have received a substantial boost in persistently supporting a preferred conformation, (18), for glycine,[219] in spite of evidence from microwave spectroscopy favouring (19). The assignments, referring to behaviour in the gas phase, are important in terms of spectroscopic analysis of interstellar vapours, and more sensitive microwave techniques have now[220] detected (18) and possibly (20) for gaseous glycine. More routine m.o. calculations relating to



(18)



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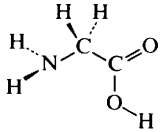

(20)

conformational energies have been reported for glycine, covering both zwitterionic and neutral forms,[221a] and for *N*-acetyldehydroalanine methylamide, revealing very different energy profiles for various conformations when compared with the alanine analogue.[221b] Comparisons have been made[221c] between the conformational behaviour of glycine and alanine with that of β-heteroatom-substituted homologues serine, cysteine, and threonine. The solvation structure around L-serine in aqueous solution provides an interesting challenge for energy calculations,[222] and mutual interactions of a different kind are explored for lattice energy calculations for α-, β-, and γ-glycine.[223]

5 Chemical Studies of Amino-acids

Racemization.—Although the two main strands of study, the use of racemization data for amino-acids in determinations of age for relatively recent fossils, and racemization mechanisms, are both well represented in the 1980 literature, there is increasing interest in mechanistic studies. Perhaps this exposes some shortcomings in knowledge of factors which influence the racemization kinetics of free or

[214] K. P. Prasad and J. C. Ahluwalia, *Biopolymers*, 1980, **19**, 273.
[215] G. M. Blackburn, T. H. Lilley, and E. Walmsley, *J. Chem. Soc., Faraday Trans. 1*, 1980, **76**, 915.
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[221] (*a*) P. Palla, C. Petrongolo, and J. Tomasi, *J. Phys. Chem.*, 1980, **84**, 435; (*b*) D. Ajo, G. Granozzi, E. Tondello, and A. Del Pra, *Biopolymers*, 1980, **19**, 469; (*c*) L. R. Wright and R. R. Borkman, *J. Am. Chem. Soc.*, 1980, **102**, 6207.
[222] S. Roman and E. Clementi, *Int. J. Quantum Chem.*, 1980, **17**, 1007.
[223] J. L. Derissen and J. Voogd, *J. Phys. Chem.*, 1980, **84**, 2035.

protein-bound amino-acids, which diminish the reliability of the age determinations.

D : L-Ratios for amino-acids, particularly aspartic acid, from human and animal protein sources, taken with racemization rate constants, could be most reliable for samples which are very young (on the fossil time scale), since for dental tissues [224] and other proteins with low turnover rates [225] from living sources, at least the racemization has taken place in constant temperatures. This area has been reviewed.[224-227] The problems with much older samples are starkly revealed in a study of the D : L-isomer ratio for aspartic acid from collagen of Dead Sea scroll parchment;[228] up to 60% of this amino-acid had racemized but D : L-ratios varied widely even for samples taken from the same scroll.

A substantial volume has appeared of conference proceedings concerned with studies of amino-acids from geological and biological sources.[6] Most of the papers cover deductions from racemization data, presented by all leading workers in the field.[229]

The racemization rates of amino-acids differ widely, but this variation does not reflect electronic effects relayed to the chiral centre.[230] Such a conclusion does not exclude a role for polar or polarizable groups in facilitating proton transfer from the chiral centre to an incoming base, and also leaves the reader in an unsatisfied state since the presumption that steric effects account for the variations in racemization rates does not seem to be related obviously to the facts.

Withdrawal of the proton from the chiral centre in L-histidine by the imidazole π-nitrogen atom has been concluded to be the cause of the pronounced racemization that accompanies DCCI-mediated coupling reactions of protected derivatives of this amino-acid, and which occurs after conversion of the derivatives into corresponding *O*-acyl iso-ureas.[231,232] Racemization kinetics for series of simple α-amino-acids in aqueous solutions over the pH range -1 to 12 at 142 °C are subject to three distinct influences: an acid catalysed process at pH values less than 1, a pH-independent region at pH values between 3 and 6.5, and a second pH-independent region between 9 and 12.[233]

New examples of racemizations of amino-acids in aqueous alkali through equilibration of Λ-β_2-[Co(tetra-ammine)(amino-acid)]$^{2+}$ complexes,[56,234] and a distantly related technique, the use of a polymeric salicylaldehyde capable of reversible Schiff base formation with copper(II) complexes of amino-acids,[235] have been published.

[224] B. Szabuniewicz, *Czas. Stomatol.*, 1980, **33**, 23 (*Chem. Abstr.*, 1980, **93**, 25278).
[225] F. Pautet, *Pathol. Biol.*, 1980, **28**, 325.
[226] J. L. Bada and S. E. Brown, *Trends Biochem. Sci.* (*Pers. Ed.*), 1980, **5**, p. iii.
[227] N. Hamda, *Kagaku To Seibutsu*, 1980, **18**, 678 (*Chem. Abstr.*, 1981, **94**, 60205).
[228] S. Weiner, Z. Kustanovich, E. Gil-Av, and W. Traub, *Nature* (*London*), 1980, **287**, 820.
[229] *Inter alia*: T. C. Hoering, in ref. 6, p. 193; B. J. Katz and E. H. Man, *ibid.*, p. 215; K. A. Kvenvolden, *ibid.*, p. 223; J. L. Bada and M. Y. Shou, *ibid.*, p. 235; E. M. Jope, *ibid.*, p. 23; D. W. von Endt, *ibid.*, p. 297; K. M. Towe, *ibid.*, p. 65.
[230] G. G. Smith and B. Silva del Sol, *Science* (*Wash. D.C.*), 1980, **207**, 765.
[231] J. H. Jones, M. I. Ramage, and M. J. Witty, *Int. J. Pept. Protein Res.*, 1980, **15**, 301.
[232] J. H. Jones, Lecture at Meeting of the Peptide and Protein Group of The Chemical and Biochemical Societies, University of Sussex, 3 April 1981.
[233] P. M. Shou and J. L. Bada, *Naturwissenschaften*, 1980, **67**, 37.
[234] M. Yamaguchi, S. Yamamatsu, T. Furusawa, S. Yano, M. Saburi, and S. Yoshikawa, *Inorg. Chem.*, 1980, 2010.
[235] I. A. Yamskov, V. E. Tikhonov, and V. A. Davankov, *Bio-org. Khim.*, 1980, **6**, 885.

General Reactions of Amino-acids.—Following discussion of relatively drastic treatment, such as pyrolysis, reactions in strong mineral acid solutions, and oxidation, this section is divided, much as in previous volumes, between reactions at amino- and carboxy-groups, and uses in heterocyclic synthesis.

Formation of relatively large amounts of HCN during the pyrolysis of proline and glutamic acid at 850 °C, compared with other amino-acids, has been noted.[236] Hydrocarbons, CO, CO_2, and NH_3 are the major products. A kinetic study has been made [237] of the pyrolysis of a mixture of eight amino-acids at 178 °C through periods of up to 170 h, noting the accumulation of polymeric products which cannot be hydrolysed under peptide bond cleavage conditions (6M-hydrochloric acid at 105 °C during 12 h).[237] An important study [238] has shown that the presence of 0.01% NaN_3 during acid hydrolysis of proteins is responsible for destruction of tyrosine, phenylalanine, and histidine, and the generation of side-products overlapping arginine on the amino-acid analyser trace; in test mixtures, methionine sulphone was also destroyed. At the same time, the aspartic acid content was augmented by up to 15%.[238]

The sulphur-containing amino-acids, and tyrosine, tryptophan, and histidine, were the only protein amino-acids to undergo oxidation at a graphite anode.[239] Contact glow discharge electrolysis of β- and γ-amino-acids brings about their stepwise oxidative degradation, ascribed to the generation of hydroxy-radicals.[240] An interesting consequence is the formation of α-amino-acids; for example, the formation of glycine from β-alanine *via* isoserine and aminopyruvic acid. This observation is relevant to model reactions for the prebiotic synthesis of α-amino-acids, already known to be formed from simple precursors under contact glow discharge electrolysis,[64] since it is now conceivable that higher homologues of the protein amino-acids may have been formed first during the events leading to the genesis of life.

Labile *N*-hydroxymethyl derivatives that form at pH values above 9.2 in solutions of formaldehyde and amino-acids even at low concentrations are increasingly favoured at higher pH values,[241] and conditions for the condensation of 2 mol formaldehyde per mol amino-acid are eventually reached.

Condensation of pyridoxal with representative amino-acids (alanine, arginine, and methionine) is accelerated by reversed micelles.[242]

α-*N*-Nitroso-*N*-alkylamino-acids suffer decarboxylation under irradiation by u.v. light, yielding corresponding amidoximes, but β-amino-acid analogues are not photolabile.[243]

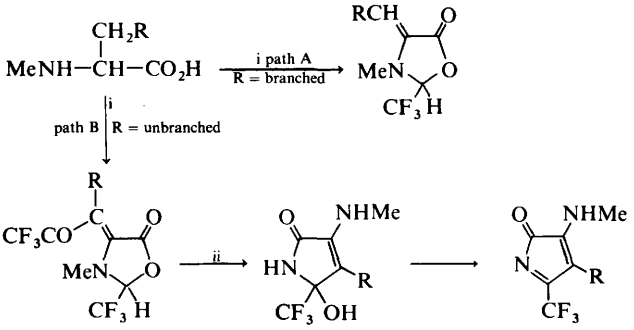
Nitrosation of alanine or α-aminobutyric acid with $NaNO_2$ and HF in pyridine yields the corresponding 2-fluoroalkanoic acids with retention of configuration, whereas phenylalanine, tyrosine, and threonine give the 3-fluoroalkanoic acids

[236] N. F. Haidar, J. M. Patterson, M. Moors, and W. T. Smith, *J. Agric. Food Chem.*, 1981, **29**, 163.
[237] G. A. Lavrent'ev, A. S. Timoshchenko, T. F. Strigunkova, and I. A. Egorov, *Dokl. Akad. Nauk SSSR*, 1980, **251**, 486.
[238] J. M. Walker, J. R. B. Hastings, and E. W. Johns, *J. Chromatogr.*, 1980, **189**, 106.
[239] V. Brabec and V. Mornstein, *Biophys. Chem.*, 1980, **12**, 159.
[240] K. Harada and J. Terasawa, *Chem. Lett.*, 1980, 441.
[241] Y. Kitamoto and H. Maeda, *J. Biochem. (Tokyo)*, 1980, **87**, 1519.
[242] H. Kondo, H. Yoshinaga, and J. Sunamoto, *Chem. Lett.*, 1980, 973.
[243] Y. L. Chow, D. P. Horning, and J. Polo, *Can. J. Chem.*, 1980, **58**, 2477.

resulting from stereospecific 1,2-shift of the β-aryl or -hydroxy-group.[244] β-Branched aliphatic amino-acids (valine, isoleucine) give product mixtures indicating reluctant 1,2-methyl shift reactivity.

Isocyanides formed from *N*-formylamino-acid benzyl esters using $POCl_3$ and Et_3N undergo radical-induced reductive de-amination with tri-n-butyltin hydride in the presence of azo-bis-isobutyronitrile.[245] High-yield procedures for the *N*-acetylation of amino-acid esters have been described.[246]

Further examples of cycloaddition [247] and Michael addition [248] reactions of α-amino-acid ester imines, leading to pyrrolines and pyrrolidines, respectively, have been described. Continuing studies are also represented in cyclization of *N*-methylamino-acids by trifluoroacetic anhydride[249] (Scheme 3) in which an interesting pair of reaction pathways is revealed, which depend respectively on whether the amino-acid side-chain is branched (path A) at the β-carbon atom, or not (path B).

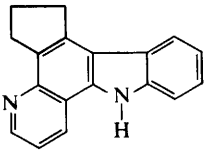


Scheme 3

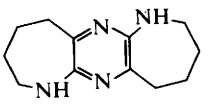
Reagents: i, trifluoroacetic anhydride; ii, NH_3

Specific Reactions of Natural Amino-acids.—Further indication of the importance of studying pyrolytic breakdown of protein amino-acids is provided in *X*-ray identification of (21) as the potent mutagen formed from L-lysine.[250a] The non-mutagenic pyrazine (22) has also been isolated from L-lysine hydrochloride pyrolysates.[250b] Pyrolysis of histidine and of 3-methylhistidine at 770 °C yields imidazole and 1-methylimidazole respectively.[251] Maillard reaction of L-lysine with D-glucose (reaction at 105 °C in aqueous solution during 6 h) gives the pyrrole (23).[252a] A further example of the conversion of one L-amino-acid into another is RuO_4 oxidation of an *N*-acyl proline to the pyroglutamic acid, thence to glutamic acid.[252b]

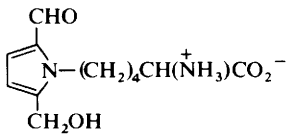
[244] R. Keck and J. Retey, *Helv. Chim. Acta*, 1980, **63**, 769.
[245] D. H. R. Barton, G. Bringmann, and W. B. Motherwell, *Synthesis*, 1980, 68.
[246] M. Dymicky, *Org. Prep. Proced. Int.*, 1980, **12**, 207.
[247] R. Grigg and J. Kemp, *Tetrahedron Lett.*, 1980, **21**, 2461.
[248] R. Grigg, J. Kemp, J. Malone, and A. Tangthougkum, *J. Chem. Soc., Chem. Commun.*, 1980, 648.
[249] U. Hess and W. A. Koenig, *Liebigs Ann. Chem.*, 1980, 611.
[250] (*a*) K. Yamaguchi, Y. Iitaka, K. Shudo, and T. Okamoto, *Acta Crystallogr., Sect. B*, 1980, **36**, 176; (*b*) V. S. Gann, A. L. Y. Lau, and H. H. Wassermann, *Tetrahedron Lett.*, 1980, **21**, 2679.
[251] R. M. Smith, G. A. Solabi, W. P. Hayes, and R. J. Stretton, *J. Anal. Appl. Pyrolysis*, 1980, **1**, 197.
[252] (*a*) T. Nakayama, F. Hayase, and H. Kato, *Agric. Biol. Chem.*, 1980, **44**, 1201; (*b*) S. Yoshifuji, H. Matsumoto, K. Tanaka, and Y. Nitta, *Tetrahedron Lett.*, 1980, **21**, 2963.



(21)



(22)



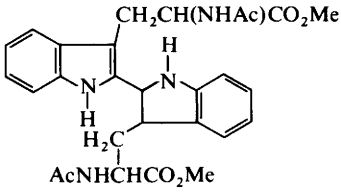
(23)

Liberation of ethylene from amino-acids has important botanical consequences, and its formation from 1-aminocyclopropanecarboxylic acid in tobacco leaves is inhibited by light.[253] Radiolytically produced oxygen radicals HO• and $O_2^{\bullet-}$ cause the formation of ethylene from methionine or *S*-adenosylmethionine.[254] Other detailed studies involving aliphatic amino-acids are decarboxylation kinetics of γ-carboxyglutamic acid in comparison with those of aminomalonic acid and β-carboxyaspartic acid ($t_{\frac{1}{2}}$ 8.6, 1.2, and 1.7 min, respectively),[255] and several studies of cysteine and its derivatives. Normal protein hydrolysis conditions convert cystine into 'thiocystine' [bis(2-amino-2-carboxyethyl) trisulphide], which is the source of sulphenyl cations RS^+ capable of initiating the breakdown of tryptophan in the protein hydrolysate.[256] Thiocystine has been detected in biological systems, and mechanisms for its breakdown into cystine have been described.[257] Enzyme systems capable of mediating the breakdown of sulphur-containing amino-acids have been discussed.[258] 'Cystine disulphoxide' is actually the thiolsulphonate $HO_2CCH(NH_2)CH_2SSO_2CH_2CH(NH_2)CO_2H$,[259] and the current position[260] in which 'α-disulphoxides' RS(O)S(O)R remain detectable only as transient intermediates in a limited number of organosulphur reactions remains intact. The common reagents used for the reductive cleavage of cystine are applicable for the corresponding reaction with selenocystine.[261]

Excepting a reference to the tyrosinase-catalysed oxidation of dopa and 5-*S*-cysteinyldopa to initiate the formation of pigments in higher species,[262] the other papers covering aromatic amino-acids which have been selected for citation deal with heterocyclic side-chain chemistry. Oxidation processes with tryptophan

[253] S. Gepstein and K. V. Thimann, *Planta*, 1980, **149**, 196.
[254] M. Saran, W. Bors, C. Michel, and E. F. Elstner, *Int. J. Radiat. Biol., Relat. Stud. Phys., Chem., Med.*, 1980, **37**, 521 (*Chem. Abstr.*, 1980, **93**, 128 827).
[255] P. V. Hauschka, E. B. Henson, and P. M. Gallop, *Anal. Biochem.*, 1980, **108**, 57.
[256] T. Ohta and T. Nakai, *Agric. Biol. Chem.*, 1979, **43**, 2419.
[257] R. Abdolrasulina and J. L. Wood, *Bio-org. Chem.*, 1980, **9**, 253; in 'Natural Sulfur Compounds', Proceedings of the 3rd International Meeting 1979, ed. D. Cavallini, G. E. Gaull, and V. Zappia, Plenum Press, New York, 1980, p. 483 (*Chem. Abstr.*, 1980, **93**, 162 967).
[258] D. Cavallini, G. Federici, S. Dupre, C. Cannella, and R. Scandurra, *Pure Appl. Chem.*, 1980, **52**, 147.
[259] T. Obuka, S. Yuasa, M. Kinuta, and R. Akagi, *Physiol. Chem. Phys.*, 1980, **12**, 45.
[260] G. C. Barrett, in 'Organic Compounds of Sulphur, Selenium, and Tellurium', ed. D. R. Hogg (Specialist Periodical Reports), The Chemical Society, London, 1980, Vol. 5, p. 67.
[261] J. N. Burnell, J. A. Karle, and A. Shrift, *J. Inorg. Biochem.*, 1980, **12**, 343.
[262] S. Ito, E. Novellino, F. Chioccara, G. Misuraca, and G. Prota, *Experientia*, 1980, **36**, 822.

(conversion into oxindolylalanine in dimethylsulphoxide–acetic acid media [263]) and its derivatives [dimer (24) and its stereoisomer are formed from *N*-acetyltryptophan methyl ester in TFA;[264] electro-oxidation of *N*-acetyltryptophanamide also brings about dimerization,[265] as does photo-oxidation of the amino-acid itself[266]]



(24)

are accompanied by descriptions of indole-substitution reactions (attack by sulphenyl cations,[256] and by t-butyl cations liberated during de-protection of N^{α}-boc-tryptophan derivatives in TFA–ethanedithiol [267]) as topics of recent papers. Kinetics of de-tritiation of C-2[^{3}H]histidine derivatives [268] and ^{1}H–^{2}H exchange at the same site [269] have been studied and interpreted as a reflection of the influence of nearby groupings.

Specific Reactions and Properties of Amino-acids Related to Biochemical Processes.—This section is intended to be read with the preceding and following sections if a general view is sought of recent literature on some biochemical aspects of the chemistry of the amino-acids. Interactions of L-tryptophan with nucleic acids induced by light [270] have been studied, an extension of one of several lines of inquiry on this general topic. Acetone-sensitized photo-coupling between *N*-acetyltryptophan methyl ester and 5-bromo-1,3-dimethyluracil leads to the corresponding 2-substituted indoles.[271] Apparent dissociation constants of AMP–amino-acid ester complexes in aqueous solutions correlate well with features of the genetic code and with the frequencies of occurrence of amino-acids as constituents of proteins;[272] another aspect of the same topic underlies a study of the relative rates of non-enzymic activation of hydrophobic amino-acids by ATP.[273]

Effects of Electromagnetic Radiation on Amino-acids.—Three major topics stand out from a broad view on the literature of this topic: a study of radicals formed through high-energy irradiation of amino-acids; the finer details of the absorption and re-emission of u.v. light by tryptophan, that archetypal 3-substituted indole;

[263] W. E. Savige and A. Fontana, *Int. J. Pept. Protein Res.*, 1980, **15**, 285.
[264] K. Hashizume and Y. Shimonishi, *Proceedings of 17th Peptide Symposium*, 1979, p. 77 (*Chem. Abstr.*, 1980, **93**, 168 577).
[265] C. Jakubowicz, R. Vallot, L. T. Yu, and J. Reynaud, *C. R. Seances Acad. Sci., Ser. C.*, 1980, **290**, 377.
[266] C. Sconfienza, A. Van de Vorst, and G. Jori, *Photochem. Photobiol.*, 1980, **31**, 351.
[267] Y. Masui, N. Chino, and S. Sakakibara, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 464.
[268] J. A. Elvidge, J. R. Jones, R. Salih, M. Shandala, and S. E. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 1980, 447.
[269] J. H. Bradbury, B. E. Chapman, M. W. Crompton, R. S. Norton, and J. S. Teh, *J. Chem. Soc., Perkin Trans. 2*, 1980, 693.
[270] A. E. Reeve and T. R. Hopkins, *Photochem. Photobiol.*, 1980, **31**, 223, 413.
[271] S. Ito, I. Saito, and T. Matsuuro, *J. Am. Chem. Soc.*, 1980, **102**, 7535.
[272] J. Reuben and F. E. Polk, *J. Mol. Evol.*, 1980, **15**, 103.
[273] D. W. Mullins and J. C. Lacey, *Biochem. Biophys. Res. Commun.*, 1980, **96**, 491.

and attempts to demonstrate differential degradation of enantiomers of amino-acids under irradiation.

γ-Irradiation of aqueous solutions of L-valine [274] and other simple aliphatic α-amino-acids glycine, alanine, leucine, and isoleucine [275] creates short-lived radicals whose breakdown products have been studied by h.p.l.c.[274] Spin-trapping using 2-methyl-2-nitropropane [275] has proved to be a useful method for locating the site of the unpaired electron in the initial products of irradiation, though e.s.r. and ENDOR techniques have been generally applied in related studies: γ-irradiation of L-alanine generates the radical cation structure $—\dot{C}(OH)O^-$ from the carboxy-group;[276] while the amino-group and the α-carbon atom are also implicated, for example in the pulse radiolysis of deuteriated glycine, alanine, and α-aminoisobutyric acid;[277] hydrated electrons liberated in aqueous solutions of phenylalanine react with the amino-acid at sites partly determined by the pH of the reaction mixture.[278] Solid samples can also suffer degradation under γ- or *X*-irradiation, and current studies have involved alanine single crystals doped with copper(II) salts,[279] L-aspartic [280] and L-glutamic acids,[281] *N*-acetyl-DL-alanine,[282] and L-proline hydrate and its thiazolidine analogue.[283] In the last-mentioned study, e.s.r. and ENDOR monitoring indicate the possibility of de-amination under *X*-irradiation, a process established previously with acyclic aliphatic amino-acids.

Although radiation at the levels to which meteorites must be exposed is sufficient to cause appreciable racemization of amino-acids,[284] as well as partial destruction or interconversions of some amino-acids (see earlier volumes of this *Specialist Periodical Report*), previous claims that enantiomeric amino-acids are degraded at different rates under irradiation now seem to be discounted by counter-claims generated by attempts to extend the topic. This leaves an open question; the fact that a wide variety of amino-acids is present in some meteorites but all in racemic form does not necessarily invalidate a hypothesis that these objects have travelled from some extra-terrestrial source on which one enantiomeric form of the amino-acids predominates. But the so-called Vester–Ulbricht theory, that the predominance of one enantiomeric form is associated with unequal rates of degradation of D- and L-isomers by electromagnetic radiation with dissymmetric characteristics, is not standing up well to experimental study. ^{32}P-β-Radiolysis of DL-tryptophan shows no evidence of asymmetric degradation,[285] in contrast to results reported in 1976, whose authors have offered comments in support of their original claims.[286] Radiolysis over a period in which complete destruction of

[274] K. Makino, *J. Phys. Chem.*, 1980, **84**, 1016.
[275] K. Makino, *J. Phys. Chem.*, 1980, **84**, 1968; F. Moriya, K. Makino, N. Suzuki, S. Rokushika, and H. Hatano, *J. Phys. Chem.*, 1980, **84**, 3085.
[276] L. Kevan, S. Schlick, K. Toriyama, and M. Iwasaki, *J. Phys. Chem.*, 1980, **84**, 1950.
[277] P. O. Samskog, G. Nilsson, A. Lund, and T. Gillbro, *J. Phys. Chem.*, 1980, **84**, 2819.
[278] R. F. Lakhary and P. Krebs, *Chem. Phys. Lett.*, 1980, **70**, 469.
[279] R. Calvo, S. B. Oseroff, and H. C. Abache, *J. Chem. Phys.*, 1980, **72**, 760.
[280] M. Ogawa, K. Ishigure, and K. Oshima, *Radiat. Phys. Chem.*, 1980, **16**, 289.
[281] M. Ogawa, K. Ishigure, and K. Oshima, *Radiat. Phys. Chem.*, 1980, **16**, 281.
[282] J. C. Haynes, S. Kuroda, K. Matsuki, and I. Miyagawa, *Radiat. Res.*, 1980, **84**, 426.
[283] W. H. Nelson and D. R. Taylor, *J. Chem. Phys.*, 1980, **72**, 524.
[284] R. M. Lemmon and W. A. Bonner, *Stud. Phys. Theor. Chem.*, 1979, **7**, 47.
[285] W. A. Bonner, N. E. Blair, and J. J. Flores, *Nature (London)*, 1979, **281**, 150.
[286] W. Darge, I. Laczko, and W. Thiemann, *Nature (London)*, 1979, **281**, 151.

tryptophan is brought about causes destruction to the extent of only 20—30% when applied to DL-leucine, and careful analysis has established that there is no asymmetric bias in the case of leucine.[287] However, less energetic (u.v.) irradiation of DL-tryptophan derivatives and determination of the equilibrium constants for excimer formation reveal a chiral discrimination energy of 0.7 kcal mol^{-1},[288] thus providing a new basis for speculation on mechanisms accounting for the predominance of L-amino-acids.

The remarkable result stated in the preceding sentence is one culmination of a vigorous field of photochemical study of aromatic and heteroaromatic amino-acids. Recent papers cited here all concern tryptophan solutions, whose u.v. chemiluminescence, phosphorescence, and fluorescence have been studied,[289] and whose light instability has been described in precautionary terms for the attention of those engaged in clinical research.[290] Kinetics of fluorescence decay of photo-excited tryptophan in aqueous solutions have provided useful detailed information,[291-293] notably [293] an interpretation of data consistent with double exponential kinetics, originating in two distinct conformations of the amino-acid.

6 Analytical Methods

Gas–Liquid Chromatography.—While the g.l.c. technique in itself is rapid and accurate after calibration for a particular purpose, the derivatization procedure which is an essential precursor to the analysis of amino-acid mixtures may introduce errors of precision. This is because yields in the reactions used to convert amino-acids into volatile derivatives may vary from one amino-acid to the next, or even from one procedure to a repetition on the same sample. This is overstating the case somewhat, but clearly there is scope for uncertainty in the accuracy of quantitative analysis of amino-acids by g.l.c., and attempts have been made to introduce modifications which compensate for artifactual errors. In the 'enantiomer labelling' technique,[294] an aliquot of a solution of known amounts of *the enantiomers* of the amino-acids present in the sample is added, in the form of their isopropyl esters, to act as internal standards. Esterification, clean-up, and *N*-acylation are then followed by g.l.c. on a chiral stationary phase, noting variations in proportions of the internal standards from their actual concentrations. The accuracy and precision of the novel variation is claimed to be equal to, or better than, the performance of the ion-exchange analyser,[294] although much preliminary work is required to establish conditions leading to fully resolved g.l.c. peaks.

Routines for derivatization of amino-acids have been described,[295] and many examples of the favoured combinations of *N*-acyl and esterifying groups have

[287] N. E. Blair and W. A. Bonner, *J. Mol. Evol.*, 1980, **15**, 21.
[288] C. D. Tran and J. H. Fendler, *J. Am. Chem. Soc.*, 1980, **102**, 2923.
[289] J. Slawinski, M. Elbanowski, and D. Slawinska, *Photochem. Photobiol.*, 1980, **32**, 253.
[290] M. Kenney, R. F. Lambe, D. A. O'Kelly, and A. Darragh, *Clin. Chem. (Winston-Salem, N.C.)*, 1980, **26**, 1511.
[291] G. S. Beddard, G. R. Fleming, G. Porter, and R. J. Robbins, *Philos. Trans. R. Soc. London, Ser. A*, 1980, **298**, 321; R. J. Robbins, G. R. Fleming, G. S. Beddard, G. W. Robinson, P. J. Thistlethwaite, and G. J. Woolfe, *J. Am. Chem. Soc.*, 1980, **102**, 6271.
[292] K. P. Ghiggino, G. R. Mant, D. Phillips, and A. J. Roberts, *J. Photochem.*, 1979, **11**, 297.
[293] A. G. Szabo and D. M. Rayner, *J. Am. Chem. Soc.*, 1980, **102**, 554.
[294] H. Frank, A. Rettenmeier, H. Weicker, G. J. Nicholson, and E. Beyer, *Clin. Chim. Acta*, 1980, **105**, 201.
[295] I. M. Moodie, *Lab. Pract.*, 1980, **29**, 1074.

again been reported. *N*-Trifluoroacetyl amino-acid n-butyl esters[296-298] and corresponding n-propyl,[299] isopropyl,[300] or hexafluoroisopropyl esters[301] have been used, as have *N*-heptafluorobutyryl n-propyl esters,[302] their isopropyl esters,[303] and particularly their isobutyl esters.[304-306] Users of the g.l.c. method are tending to quote the precedent source from which they obtained practical details for the preparation of derivatives (*e.g.* ref. 307 quoted in 306 for the conversion of amino-acid mixtures into *N*-heptafluorobutyryl isobutyl esters), and this tends to encourage moves towards uniformity of operations. Trimethylsilyl derivatives are less favoured now, although trimethylsilylation has been advocated for the quantitative analysis of 3-methylhistidine,[308] and trimethylsilyl esters of *NO*-bis(trifluoroacetylated)hydroxyamino-acids have been used.[309] Amino-acids yield 2-trifluoromethyloxazolin-5-ones on treatment with trifluoroacetic anhydride, and these are useful in g.l.c.–m.s. studies.[310]

Points of interest from these derivatization procedures are the problems arising with sulphur-containing amino-acids,[296, 298, 305] and three independent approaches to the g.l.c. analysis of 3-methylhistidine.[299, 304, 308] δ-Amino-n-valeric acid has been advocated as an internal standard for g.l.c. studies.[301]

Resolution of amino-acid enantiomers by g.l.c. methods can be accomplished either by the conversion of the D : L-mixture into a pair of diastereoisomers, for example using an optically active *N*-acyl or ester grouping, or through the use of a chiral stationary phase. An example of the former approach, esterification of *N*-isobutoxycarbonyl-DL-histidine using (+)-pantoyl-lactone, yields diastereoisomers which can be separated completely.[311] Grafting L-valine t-butylamide on to a cyanosilicone for use as a stationary phase, a further example of a well investigated methodology, has been found to be satisfactory for resolution of derivatized D : L-amino-acid pairs,[312] and other examples can be found in papers cited earlier in this chapter,[300] including several papers published in the form of Conference Proceedings.[6, 229]

The success of the derivatization approach for the g.l.c. of amino-acids has meant that the pyrolysis–g.l.c. technique has been largely ignored. In any case, the complex mixture of simple pyrolysis products which can arise (*cf.* ref. 236) renders this approach of little value in diagnostic work, although it is the only g.l.c.

[296] J. Kvalraag and T. Tjoernhom, *Nord. Jordbrugsforsk.*, 1980, **62**, 281 (*Chem. Abstr.*, 1980, **93**, 200 284).
[297] C. Perier, M. C. Ronziere, A. Rattner, and J. Frey, *J. Chromatogr.*, 1980, **182**, 155.
[298] E. Bailey, F. B. Farmer, and J. H. Lamb, *J. Chromatogr.*, 1980, **200**, 145.
[299] L. Cotellessa, F. Marcucci, D. Corni, P. Sfondrini, L. Colombo, E. Mussini, and F. Poy, *J. Chromatogr.*, 1980, **221**, 149.
[300] N. Oi, O. Hiroaki, H. Shimida, M. Horiba, and H. Kitahara, *Bunseki Kagaku*, 1980, **29**, 270.
[301] T. Asakura and M. Matsuda, *Jikeikai Med. J.*, 1980, **27**, 63.
[302] T. Yoneda, *Anal. Biochem.*, 1980, **104**, 247.
[303] M. A. Kirkman, M. M. Burrell, P. J. Lea, and W. R. Mills, *Anal. Biochem.*, 1980, **101**, 364.
[304] T. W. Larsen and R. F. Thornton, *Anal. Biochem.*, 1980, **109**, 137.
[305] S. L. MacKenzie and A. J. Finlayson, *J. Chromatogr.*, 1980, **187**, 239.
[306] H. Sedova and M. Kahler, *Kvasny Prum.*, 1980, **26**, 193.
[307] S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 1979, **171**, 195; 1979, **173**, 53.
[308] H. Vielma and J. Mendez, *J. Chromatogr.*, 1980, **196**, 166.
[309] G. Michael, *J. Chromatogr.*, 1980, **188**, 251.
[310] V. Ferrito, R. Borg, J. Eagles, and G. R. Fenwick, *Biomed. Mass Spectrom.*, 1979, **6**, 499.
[311] M. Makita, Y. Ohkaru, and S. Yamamoto, *J. Chromatogr.*, 1980, **188**, 408.
[312] T. Saeed, P. Sandra, and M. Verzele, *J. Chromatogr.*, 1979, **186**, 611.

approach available for amino-acid betaines,[313] many of which (*e.g.* glycine betaine $Me_3\overset{+}{N}CH_2CO_2^-$) occur in plants.

Ion-exchange Chromatography.—As in previous volumes, no comprehensive coverage is attempted for this topic. While it is considered to be appropriate to exclude routine acquisition of results of a type familiar to readers, novel work concerning instrumentation (including microprocessor control and computer-assisted operations) is also felt to be beyond the scope of this report.

The opportunity has been taken to comment on textbook errors on the methodology of amino-acid analysis by ion-exchange, and to describe improvements in buffer composition.[314] Less common amino-acids for which ion-exchange separation techniques have been established are aminomalonic, β-carboxyaspartic, and γ-carboxyglutamic acids,[255] diaminopimelic acid,[315] and the group of sulphonic acids taurine, *S*-sulphocysteine, cysteic acid, and *S*-sulphothiocysteine.[316] Identification of hydroxylysine, and its glycoside, and 3-methylhistidine in urine has been studied.[317]

Thin-layer and Paper Chromatography.—The current literature on this topic amounts to consolidation of established techniques with minor modifications. Two multi-author books include chapters on t.l.c. of amino-acids.[318, 319]

Rapid t.l.c. methods have been established for the identification of hydroxylysine and hydroxyproline in mixtures,[320] the separation on cellulose of tyrosine from its mono-, di-, tri-, and tetra-iodo derivatives,[321] and the estimation of tryptophan in human plasma.[322] Solvent systems and the effects of pH have been investigated for the t.l.c. on silica gel[323] and on DEAE-cellulose[324] of representative amino-acids. Dansylamino-acids,[325] phenylthiohydantoins[326, 327] and their *p*-(*NN*-dimethylaminophenylazo)-analogues,[328] and methylthiohydantoins[327] are described in a small selection from a larger number of papers dealing with t.l.c. identification of commonly used amino-acid derivatives. It is becoming more common to perform parallel t.l.c. and h.p.l.c. analyses with these derivatives, since resolutions of groups of close-running amino-acid phenylthiohydantoins, for example, which are not possible by one technique are often achieved by the other.[327, 328]

[313] W. D. Hitz and A. D. Hanson, *Phytochemistry*, 1980, **19**, 2371.
[314] J. Svasti, *Trends Biochem. Sci. (Pers. Ed.)*, 1980, **5**, p. viii.
[315] S. Pongor and K. Baintner, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1980, **15**, 1.
[316] T. Ubuka, M. Kinuta, K. Akagi, and S. Kiguchi, *J. Chromatogr.*, 1980, **188**, 442.
[317] D. T. Di Ferrante, N. Y. Wilson, and C. S. Leach, *J. Chromatogr.*, 1980, **187**, 271.
[318] J. G. Heathcote, in 'Densitometry in Thin-Layer Chromatography', ed. J. C. Touchstone and J. Sherma, Wiley, New York, 1979.
[319] T. Omori, in 'Instrumentation for H.P.T.L.C., Proceedings of 1st International Symposium', ed. W. Bertsch, S. Hara, and R. E. Kaiser, Huethig, Heidelberg, 1980, p. 275.
[320] Z. Buzas, B. Polyak, and L. Boross, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1980, **15**, 173.
[321] M. Lederer, *J. Chromatogr.*, 1980, **194**, 270.
[322] H. K. L. Hundt, E. C. Clark, and H. C. Van der Linde, *J. Chromatogr.*, 1980, **182**, 110.
[323] I. Kalnina, L. Krauja, and T. M. Sheveleva, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1980, 76 (*Chem. Abstr.*, 1980, **92**, 226127).
[324] I. Kalnina and L. Krauja, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1980, 71 (*Chem. Abstr.*, 1980, **92**, 226126).
[325] J. C. Wesenberg and J. E. Walpole, *Mikrochim. Acta*, 1980, **2**, 1.
[326] C.-Y. Yang, *Hoppe-Seyler's Z. Physiol. Chem.*, 1980, **361**, 1599.
[327] M. J. Horn, P. A. Hargrave, and J. K. Wang, *J. Chromatogr.*, 1979, **180**, 111.
[328] K. J. Wilson, K. Rodger, and G. J. Hughes, *FEBS Lett.*, 1979, **108**, 87.

Microcrystalline cellulose can effect the resolution into enantiomers of the protein aromatic and heteroaromatic amino-acids.[329] Although this is a well known attribute of cellulose, it is as well to have this reminder that a pair of spots on a t.l.c. plate may originate in this way.

Two-dimensional paper chromatography of amino-acids has been described [330] and, though not in itself a new method of course, full details are presented of development in one direction, a second development in the same direction with a different solvent, and finally the use of a third solvent system in the second direction. This method, together with the use of strontium nitrate–ninhydrin spray reagent, has been claimed to give good separations of mixtures of common amino-acids.

High-performance Liquid Chromatography.—Many more papers year by year are devoted to analysis of amino-acids and their derivatives by h.p.l.c., and some novel variations of standard methodology accompany a substantial body of routine work. Scope continues to exist for improved procedures for detection of constituents emerging from h.p.l.c. columns, and this aspect is represented in the 1980 literature.

Detailed description is given of techniques for h.p.l.c. analysis of free amino-acids,[331] including tryptophan [332–335] (detection based on its fluoresence) and γ-amino-butyric acid [336] (detection based on derivatization with *o*-phthaldialdehyde–mercaptoethanol leading to a one pmole sensitivity limit). Prior conversion of a sample into fluorescent derivatives has been used for the h.p.l.c. analysis of amino-acids in synaptosomal extracts [337] and for 3-methylhistidine estimation [338, 339] (using the iso-indoles formed from amino-acids with *o*-phthaldialdehyde and a thiol). The same approach has been used for the estimation of dopa at 45 pmole sensitivity, but using fluorescamine as the reagent.[340] Alternative derivatization procedures involve *N*-substitution reactions of an equally familiar kind, illustrated in h.p.l.c. analysis of lysine as its 2,4-dinitrophenyl derivative,[341] dansylamino-acids,[342–345] and a less commonly used relative, dabsyl derivatives [346] (useful for the estimation of amino-acids in urine based on their maximal absorbance at 425 nm).

Derivative formation of a different kind has been used for the determination of

[329] S. Yuasa, A. Shimada, K. Kameyama, M. Yasui, and K. Adzuma, *J. Chromatogr. Sci.*, 1980, **18**, 311.
[330] A. Abbasi, R. Ali, and Z. H. Zaidi, *J. Biochem. Biophys. Methods*, 1980, **3**, 311.
[331] R. Schuster, *Anal. Chem.*, 1980, **52**, 617.
[332] O. Beck and T. Hesselgren, *J. Chromatogr.*, 1980, **181**, 100.
[333] T. Flatmark, S. Wahlstroem Jacobsen, and J. Haavik, *Anal. Biochem.*, 1980, **107**, 71.
[334] H. R. McKim and W. G. Dewhurst, *Proc. West. Pharmacol. Soc., 23rd Meeting*, 1980, p. 291 (*Chem. Abstr.*, 1980, **93**, 14 565).
[335] H. F. Baker, M. H. Joseph, and R. M. Ridley, *J. Pharmacol.*, 1980, **70**, 133P.
[336] T. A. Hare and N. V. B. Manyam, *Anal. Biochem.*, 1980, **101**, 349.
[337] K. Lenda and G. Svenneby, *J. Chromatogr.*, 1980, **198**, 516.
[338] Z. Friedman, H. W. Smith, and W. S. Hancock, *J. Chromatogr.*, 1980, **182**, 414.
[339] S. J. Wassner, J. L. Schlitzer, and J. B. Li, *Anal. Biochem.*, 1980, **104**, 284.
[340] B. Tabakoff and R. F. Black, *J. Neurochem.*, 1980, **34**, 1707.
[341] N. Muhammad and J. A. Bodnar, *J. Liq. Chromatogr.*, 1980, **3**, 529.
[342] S. Kobayashi and K. Imai, *Anal. Chem.*, 1980, **52**, 424.
[343] S. K. Lam and F. K. Chow, *J. Liq. Chromatogr.*, 1980, **3**, 1579.
[344] H. Engelhardt and S. Kromidas, *Naturwissenschaften*, 1980, **67**, 353.
[345] W. Lindner, *Naturwissenschaften*, 1980, **67**, 354.
[346] J.-K. Liu and C.-H. Wang, *Clin. Chem.* (*Winston-Salem, N.C.*), 1980, **26**, 579.

the enantiomeric purity of tri- and tetra-iodothyronines, in which these amino-acids are converted into diastereoisomer mixtures through coupling with L-leucine,[347] then separated by h.p.l.c. An alternative resolution procedure, in which a chiral stationary phase is used (L-prolinamide [345] or L-valinamide [346] bonded to silica gel) for the separation of D : L-dansylamino-acid pairs, is not the only other variation of the chromatographic resolution technique, since dansylamino-acids can be resolved using an eluent containing a copper(II)–L-proline complex.[343] An example of the determination of enantiomeric purity concerning one of the less common amino-acids has been reported for penicillamine.[348]

Substantial studies continue to be reported on h.p.l.c. analysis of phenylthiohydantoins [327,349–352] and *p*-(*NN*-dimethylaminophenylazo)-analogues.[328,353] Although the mention here of a few points of interest does not do justice to the useful details to be found in each of the papers, attention to the separation of glutamic and aspartic acid derivatives,[351] and a maximum time of 30 min for the separation of all 20 protein amino-acid derivatives,[350] can be highlighted. Use of the dimethylaminoazobenzenethiohydantoins allows sensitivity levels of 5—10 pmole to be reached.[353]

Fluorimetry.—Ammonia released from glutamine by heating in dilute sulphuric acid at 100 °C can be assayed by absorption into an *o*-phthaldialdehyde–mercaptoethanol reaction mixture, yielding fluorescence in proportion to the glutamine content of the sample.[354] The reagent system is extremely sensitive, and careful cleaning of glassware is essential for accurate results.[355]

Mention has been made in preceding sections of analytical exploitation of fluorescence-forming reactions. Further examples are reported for the fluorimetric estimation of phenylthiohydantoins using the pyridoxamine–lead(II) acetate reagent,[356] for the formation of fluorescent spots on thin-layer chromatograms by thiamine and sodium hypochlorite for the detection of these compounds,[352] and for the estimation of γ-aminobutyric acid or glutamic acid based on the formation of a fluorescent chelate with ninhydrin in the presence of a copper(II) salt.[357]

Other Separation Methods.—Low-voltage electrophoresis in acidic media, in combination with concurrent chromatographic separation on cellulose layers, has been advocated [358] for identification of mixtures containing 1—10 nmole levels of

[347] E. P. Lankmayr, K. W. Budna, and F. Nachtmann, *J. Chromatogr.*, 1980, **198**, 471.
[348] F. Nachtmann, *Int. J. Pharm.*, 1980, **4**, 337.
[349] L. E. Henderson, T. D. Copeland, and S. Oroszlan, *Anal. Biochem.*, 1980, **102**, 1; N. D. Johnson, M. W. Hunkapiller, and L. E. Hood, *Anal. Biochem.*, 1979, **100**, 335; C. Zalut and H. W. Harris, *Biochem. Biophys. Res. Commun.*, 1980, **2**, 155; J. Simmons and D. H. Schlesinger, *Anal. Biochem.*, 1980, **104**, 254; R. Somack, *Anal. Biochem.*, 1980, **104**, 464; S. E. Gotfredsen and R. W. A. Oliver, *Carlsberg Res. Commun.*, 1980, **45**, 35; I. V. Nazimove and N. B. Levina, *Bio-org. Khim.*, 1980, **6**, 343; T. Greibrokk, E, Jensen, and G. Ostvold, *J. Liq. Chromatogr.*, 1980, **3**, 1277; S. M. Rose and B. D. Schwartz, *Anal. Biochem.*, 1980, **107**, 206; L. Sottrup-Jensen, T. E. Petersen, and S. Magnusson, *ibid.*, p. 456.
[350] J. Fohlman, L. Rask, and P. A. Peterson, *Anal. Biochem.*, 1980, **106**, 22.
[351] J. U. Harris, D. Robinson, and A. J. Johnson, *Anal. Biochem.*, 1980, **105**, 239.
[352] T. Kinoshita, K. Murayama, and A. Tsuji, *Chem. Pharm. Bull.*, 1980, **28**, 1925.
[353] J. Y. Chang, A. Lehmann, and B. Wittman-Liebold, *Anal. Biochem.*, 1980, **102**, 380.
[354] T. Z. Liu and H. Khayam-Bashi, *Clin. Chem. (Winston-Salem, N.C.)*, 1980, **26**, 700.
[355] D. J. Shute, *Med. Lab. Sci.*, 1980, **37**, 173.
[356] T. Kinoshita and K. Murayama, *Jpn. Kokai Tokkyo Koho* 80 36 740 (*Chem. Abstr.*, 1980, **93**, 91 531).
[357] C. Pfister and H. J. Wolney, *Acta Histochem.*, 1980, **67**, 195.
[358] R. L. Munier and S. Mennier, *Anal. Biochem.*, 1979, **100**, 254.

amino-acids. High-voltage electrophoresis following conventional procedures has been used for the determination of proline and its 3- and 4-hydroxy-derivatives in biological samples.[359] Isotachophoresis techniques are suitable for the estimation of *S*-(carboxymethyl)cysteine in urine.[360] Reverse osmosis across a DDS-cellulose acetate membrane from binary aqueous solutions containing L-alanine, and the effects of other amino-acids on the permeation of this compound have been described.[361]

Determinations of Specific Amino-acids.—Nearly all the citations in this section refer to specific enzyme-based procedures, but this is not a realistic picture of this topic, since colorimetric assays are still fashionable, and have been largely located in earlier sections of this chapter.

Modified ninhydrin colour-forming reactions have been established for the assay of mixtures containing proline, hydroxyproline, and hydroxylysine.[362] Further development of spectrophotometric assay of hydroxyproline in tissue (see also Vol. 11, p. 2) has been reported,[363] and an improvement of established nitroprusside colorimetry of cystine in urine has been developed.[364]

Enzyme-catalysed degradations of amino-acids which have been applied for specific estimation procedures include: a bacterial ω-amino-acid–pyruvate amino-transferase together with lactate dehydrogenase for estimation of L-alanine;[365] leucine dehydrogenase used for the quantitative determination of branched-chain amino-acids;[366] lysine decarboxylase in immobilized form for an automated assay of L-lysine;[367] nmole level assay of L-ornithine employing ornithine amino-transferase with Δ^1-pyrroline-5-carboxylate reductase;[368] and microassay of cysteinesulphinic acid through enzymic conversion into lactate with glutamate oxalacetate transaminase with α-ketoglutarate and NADP(H).[369]

Microbioassay of L-leucine[370] or L-phenylalanine[371] through metabolism by *Leuconostoc mesenteroides* followed by use of an immobilized lactate oxidase electrode illustrates the continuing development of potentiometric sensor methods. The lactate sensor used in these studies is based on an oxygen electrode which is coated with the immobilized enzyme. A related example applied to the assay of L-histidine uses an ammonia-sensing electrode coated with immobilized *Pseudomonas*, and is based on the stoicheiometry 2 mol $NH_3 \equiv$ 1 mol histidine.[372] This technique has been refined through the isolation of the enzyme (histidine ammonia-lyase) and its immobilization on the electrode.[373]

[359] S. C. G. Tseng, R. Stern, and D. E. Nitecki, *Anal. Biochem.*, 1980, **102**, 291.
[360] H. Kodama, M. Yamamoto, and K. Sasaki, *J. Chromatogr.*, 1980, **183**, 226.
[361] O. Tozawa and D. Nomura, *Nippon Kagaku Kaishi*, 1980, 127 (*Chem. Abstr.*, 1980, **93**, 26742).
[362] N. Blumenkrantz, *Clin. Biochem. (Ottawa)*, 1980, **13**, 177.
[363] C. A. Edwards and W. D. O'Brien, *Clin. Chim. Acta*, 1980, **104**, 161.
[364] A. Uhlemann and J. E. Peters, *Z. Med. Laboratoriumsdiagn.*, 1980, **21**, 302.
[365] K. Yonaha and S. Toyama, *Anal. Biochem.*, 1980, **101**, 504.
[366] G. Livesey and P. Lund, *Biochem. J.*, 1980, **188**, 705.
[367] A. Tanaka, N. Hagi, N. Itoh, and S. Fukui, *J. Ferment. Technol.*, 1980, **58**, 391.
[368] T. Matsuzawa, M. Ito, and I. Ishiguro, *Anal. Biochem.*, 1980, **106**, 1.
[369] A. Baba, S. Yamagami, H. Mizuo, and H. Iwata, *Anal. Biochem.*, 1980, **101**, 288.
[370] T. Matsunaga, I. Karube, N. Teraoka, and S. Suzuki, *Nippon Kagaku Kaishi*, 1980, 1537 (*Chem. Abstr.*, 1980, **93**, 234467).
[371] I. Karube, T. Matsunaga, N. Teraoka, and S. Suzuki, *Anal. Chim. Acta*, 1980, **119**, 271.
[372] R. R. Walters, B. E. Moriarty, and R. P. Buck, *Anal. Chem.*, 1980, **52**, 1680.
[373] R. R. Walters, P. A. Johnson, and R. P. Buck, *Anal. Chem.*, 1980, **52**, 1684.

All these enzyme-mediated assays depend on a quantitation stage, and a spectrometric determination of NADP(H) released through the degradation of meso-αε-diaminopimelate by the specific D-amino-acid dehydrogenase has been adopted in this case; alternatively, the conversion of the NADP(H) into a formazan preceding spectrophotometry may be considered.[374]

[374] H. Misono and K. Soda, *Agric. Biol. Chem.*, 1980, **44**, 2125.