1

Amino-acids

BY G. C. BARRETT

1 Introduction

Chemical studies of amino-acids including structure determination, synthesis, analysis, and physical and chemical behaviour are dealt with in this Chapter. Although the objectives of most of these studies are in support of biological investigations, behaviour, metabolic studies, and papers covering the natural distribution of amino-acids are excluded, as in previous Volumes in this series.

Textbooks, Reviews, and General Articles.—Encyclopaedic[1] and other comprehensive reviews[2] have appeared. Specific topics dealt with in other reviews are: cross-linking amino-acids in proteins,[3] role and distribution of *N*-acetylaspartic acid[4] and L-canaline,[5] and biochemistry[6] and distribution in higher plants[7] of D-amino-acids. The view is developed further,[8] that the non-protein amino-acids present in unusually large amounts in many seeds may confer an evolutionary advantage by inhibiting the germination and growth of seeds of competing species. A fascinating account has been provided[9] of the activation of amino-acids by esterification to tRNA's carrying the appropriate anticodons.

Reviews dealing with topics in amino-acid chemistry are cited in the relevant sections of this Chapter.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—There is no opportunity in the space available in this Chapter to cover aspects of the distribution of the common amino-acids in the geosphere[10] and in the biosphere. The coverage concen-

[1] A. Yamamoto, 'Kirk-Othmer Encyclopaedia of Chemistry and Technology', Third Edition, ed. M. Grayson and D. Eckroth, Vol. 2, 1978, p. 376; T. Yoshida, *ibid.*, p. 410; H. Reisman, Encyclopaedia of Chemical Process Design, 1977, Vol. 3, p. 197.

[2] J.C. Johnson, 'Amino-acids Technology; Recent Developments', Noyes Data Corp., Park Ridge, New Jersey, 1978; Y. Izumi, I. Chibata, and T. Hoh, *Angew. Chem.*, 1978, **90**, 187; *Angew. Chem. Int. Ed.*, 1978, **17**, 176; J.H. Jones, in *'Comprehensive Organic Chemistry'*, ed. I.O. Sutherland, Vol. 2, 1979, p. 815; P.M. Hardy, *ibid.*, Vol. 5, ed. E. Haslam, p. 187.

[3] 'Protein Crosslinking: Nutritional and Medical Consequences', ed. M. Friedman, Plenum, New York, 1977.

[4] R. Bakhash and E. Westman, *Bios (Madison, N.J.)*, 1978, **49**, 13.

[5] G.A. Rosenthal, *Life Sci.*, 1978, **23**, 93.

[6] K. Soda, *Kagaku*, 1977, **32**, 517 (*Chem. Abs.*, 1978, **88**, 100 374).

[7] T. Ogawa, *Nippon Nogei Kagaku Kaishi*, 1978, **52**, R83 (*Chem. Abs.*, 1978, **89**, 176 255).

[8] M.F. Wilson and E.A. Bell, *Phytochemistry*, 1978, **17**, 403; E.A. Bell, *Pontif. Acad. Sci., Scr. Varia*, 1976, **41**, 571 (*Chem. Abs.*, 1979, **89**, 56 387).

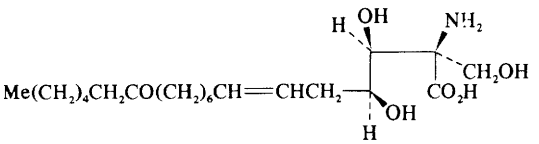
[9] E. Holler, *Angew. Chem. Int. Ed.*, 1978, **17**, 648.

[10] J.L. Bada, E.H. Man, and A.C. Walker, 'Initial Reports of The Deep Sea Drilling Project', ed. P.T. Robinson, GPO, Washington, D.C., Vol. 37, 1977, p. 633; L. Chichereau, G. Monteil, and J. Trichet, *Adv. Org. Geochem., (Proc. 7th Int. Meeting)*, ed. R. Campos and J. Goni, Empressa Nac. Adaro Invest. Minerassa, Madrid, 1977, p. 879; J.K. Whelan, *Geochim. Cosmochim. Acta*, 1977, **41**, 803.

trates on the first findings in Nature of known amino-acids, almost invariably concerning the 'uncommon' amino-acids, and on papers describing these compounds in unusual situations.

The non-protein amino-acids found in *Fagaceae* have been listed;[11] other plant sources reported are *Iris,* containing 3-(3-hydroxymethylphenyl)-L-alanine[12] (previously found in *Caesalpinia tinctoria* but of undetermined configuration) and *Iris pseudacorus,* shown to excrete 3-(3-carboxyphenyl)-alanine in large amounts into culture substrate;[13] *Afzelia bella* seeds contain (2*S*,4*R*)-dicarboxypyrrolidine and its 4-hydroxy-analogue (hydroxy-L-proline), the latter being one of the protein amino-acids but found now for the first time in the free amino-acid pool of a plant;[14] *Tephrosieae* contain 3-[2-amino-(2-imidazolin-4-yl)]alanine, enduracididine, homoarginine, γ-hydroxyhomoarginine, canavanine, and other guanidines,[15] while the report that soybean (*Glycine max.*) contains L-canavanine has been corrected[16] with the admonition that conclusions must not be drawn on the coincidence of ion-exchange retention time alone. *Lyngbya majuscula* contains (*S*)-α-butyramido-γ-butyrolactone.[17]

Microbial sources of amino-acids (excluding coverage of fermentative production[2,18a] of amino-acids, except for a representative citation of the formation of L-lysine and L-threonine by mutants of *Brevibacterium lactofermentum*[18b]) include *Streptomyces flocculus,* which synthesizes β-methyltryptophan as an intermediate *en route* to streptonigrin.[19] Configurational assignments have been made through chemical correlation and physical studies to (*S,S*)-3,5-diaminohexanoic acid (from *Clostridium sticklandii*),[20] (*S,S*)-2-amino-4-methylhex-5-enoic acid (from a New Guinea *Boletus*),[21] (*S,S*)-homoisoleucine (from *Aesculus californica*),[21] and to the antiobiotic thermozymocydin (*alias* myriocin), whose stereochemistry has been revized to the all-*cis* structure (1).[22]



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[11] T. Kasai, P.O. Larsen, and H. Sorensen, *Phytochemistry,* 1978, **17,** 1911.
[12] P.O. Larsen, F.T. Sorensen, and E. Wieczorkowska, *Phytochemistry,* 1978, **17,** 549.
[13] M.C. Cardenas and R. Kickuth, *Angew. Bot.,* 1978, **52,** 203.
[14] A. Welter, M. Marlier, and G. Dardenne, *Phytochemistry,* 1978, **17,** 131.
[15] L.E. Fellows, R.M. Polhill, and E.A. Bell, *Biochem. Syst. Ecol.,* 1978, **6,** 213.
[16] G.A. Rosenthal, *Experientia,* 1978, **34,** 1539.
[17] F.J. Marner and R.E. Moore, *Phytochemistry,* 1978, **17,** 553.
[18] (*a*) P. Pilat, *Kvasny Prum.,* 1978, **24,** 67 (*Chem. Abs.,* 1979, **90,** 74 172); (*b*) O. Tosaka, K. Takinami, and Y. Hirose, *Agric. Biol. Chem.,* 1978, **42,** 745.
[19] S.J. Gould and D.S. Darling, *Tetrahedron Letters,* 1978, 3207.
[20] F. Kunz, J. Retey, D. Arigoni, L. Tsai, and T.C. Stadtman, *Helv. Chim. Acta,* 1978, **61,** 1139.
[21] E. Gellert, B. Halpern, and R. Rudzats, *Phytochemistry,* 1978, **17,** 802.
[22] C.H. Kuo and N.L. Wendler, *Tetrahedron Letters,* 1978, 211.

β-Alanine betaine ('β-homobetaine') occurs in the white muscles of New Zealand whiptail and southern blue whiting,[23] and dopa has been found in the shell protein of *Mytilus edulis* to the extent of not less than 3.6 %.[24] Two related sponges, *Haliclona* and *Chalinopsilla,* contain L-azetidine-2-carboxylic acid;[25] this functions as an antidermatophyte towards *Trichophtyon mentagrophytes.* A dioxopiperazine incorporating *N*-methyl-$\delta\delta\delta$-trichloromethylleucine has been located in the sponge *Dysidea herbacea.*[26] Extensive studies continue[27] on the formation of *N*-(1-carboxyethyl) amino-acids in sunflower crown gall tissues incited by *Agrobacterium tumefaciens* (see also Vol. 10, p. 2), and *meso*-α-iminodipropionic acid found in squid muscle (*Todarodes pacificas*)[28a] and in the adductor muscle of the scallop *Patinopecten yessoensis*[28b] similarly incorporates a D-alanine moiety. Hypotaurine is present in the bivalve mollusc *Noetia ponderosa.*[29]

N-Terminal *N*-methylamino-acid residues in proteins described recently (Vol. 10, p. 3) have also been detected in pilin from *Pseudomonas aeruginosa*[30] and other gonoccocal and meningococcal proteins;[31] in all these examples, the residue is *N*-methylphenylalanine. *Halobacterium halobium* ferredoxin contains a single N^{ϵ}-acetyl-lysine residue.[32] γ-Carboxyglutamic acid has been found in mammalian ribosomal proteins,[33] and the occurrence of this amino-acid in vertebrates has been extended further to shark tooth,[34] fish otolith,[34] and fossil bones.[35]

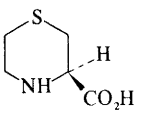
Rat-liver mitochondria are capable of the β-hydroxylation of N^{ϵ}-trimethyllysine, providing an intermediate in carnitine biosynthesis.[36]

Red feathers of the Rhode Island cock contain dopa and *S*-cysteinyldopa.[37]

New Natural Free Amino-acids.—Plant sources of new amino-acids reported in 1978 are few in number, compared with sources at other biological levels. Accumulations of 'uncommon' amino-acids have been listed for 64 species of *Caesalpineae,* showing notable species variations.[38] One of these, *Peltophorum,* contains a previously undescribed imino-acid tentatively identified as a phosphate ester of 4-hydroxypipecolic acid.[38] Lathyrine {β-(2-aminopyrimidin-4-yl)alanine} and its γ-L-glutamyl derivative have been found in *Lathyrus*

[23] S. Konosu, M. Murakami, T. Hayashi, and S. Fuke, *Nippon Suisan Gakkaishi,* 1978, **44,** 1165.
[24] J.H. Waite, and S.O. Andersen, *Biochim. Biophys. Acta,* 1978, **541,** 107.
[25] B. Bach, R.P. Gregson, G.S. Holland, R.J. Quinn, and J.L. Reichelt, *Experientia,* 1978, **34,** 608.
[26] R. Kazlauskas, P.T. Murphy, and R.J. Wells, *Tetrahedron Letters,* 1978, 4945.
[27] J.D. Kemp, *Plant Physiol.,* 1978, **62,** 26; D.W. Sutton, J.D. Kemp, and E. Hack, *ibid.,* p. 363.
[28] (*a*) M. Sato, Y. Sato, and Y. Tsuchiya, *Nippon Suisan Gakkaishi,* 1977, **43,** 1441 (*Chem. Abs.,* 1978, **88,** 84 761); (*b*) *ibid.,* 1978 **44,** 247.
[29] L.M. Amende and S.K. Pierce, *Comp. Biochem. Physiol. B,* 1978, **59B,** 257.
[30] L.S. Frost, M. Carpenter, and W. Paranchych, *Nature,* 1978, **271,** 87; W. Paranchych, L.S. Frost, and M. Carpenter, *J. Bacteriol.,* 1978, **134,** 1179.
[31] M.A. Hermodson, K.C.S. Chen, and T.M. Buchanan, *Biochemistry,* 1978, **17,** 442.
[32] T. Hase, S. Wakabayashi, H. Matsubara, L. Kerscher, D. Oesterhelt, K.K. Rao, and D.O. Hall, *J. Biochem. (Tokyo),* 1978, **83,** 1657.
[33] J.J. van Buskirk and W.M. Kirsch, *Biochem. Biophys. Res. Comm.,* 1978, **80,** 1033; 1978, **82,** 1329.
[34] K. King, *Biochim. Biophys. Acta,* 1978, **542,** 542.
[35] K. King, *Nature,* 1978, **273,** 41.
[36] J.D. Hulse, S.R. Ellis, and L.M. Henderson, *J. Biol. Chem.,* 1978, **253,** 1654.
[37] G. Agrup, C. Hansson, H. Rorsman, A.M. Rosengren, and E. Rosengren, *Acta Derm.-Venereol.,* 1978, **58,** 269.
[38] C.S. Evans and E.A. Bell, *Phytochemistry,* 1978, **17,** 1127.

japonicus.[39] L-1,4-Thiazane-3-carboxylic acid (2) is a constituent of the alga *Heterochordaria abietina*,[40] while tetra-acetylclionamide (an N^{α}-acetyl-6-bromotryptophanamide from the sponge *Cliona celata*)[41] and four families of *N*-acyl β-methylene-β-alanine esters from an unidentified black sponge[42] are closer relatives of well-known natural free amino-acids. Particularly interesting structures have been established for palythine (3; R = H)[43] and derivatives palythene (3; R = *trans*-MeCH=CH–) and palythinol (3; R = $HOCH_2CHMe$) from the zoanthid *Palythoa tuberculosa*.[44] Palythene is optically-inactive.[43] While undeniably an amino-acid, antibiotic I-851 (4) is a structural type normally outside the scope of this Chapter; this chloramphenicol antagonist



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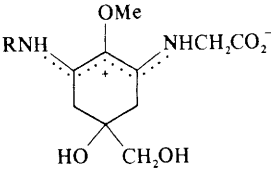

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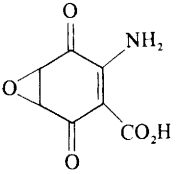


(4)

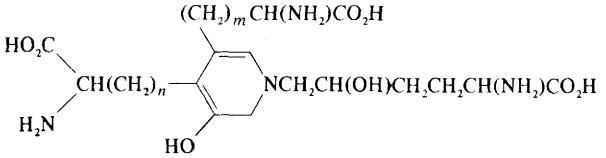
was isolated from *Streptococcus fulvoviolaceus*.[45] Related culture studies have led to the isolation of 4-[(*E*)-4′-hydroxy-3′-methylbut-2′-enyl]-L-tryptophan, in addition to the previously reported 4-dimethylallyltryptophan, from cell-free extracts of *Claviceps paspali* with isopentenyl pyrophosphate and L-tryptophan.[46] *Flavobacterium rigense* accumulates *O*-2-hydroxypropyl homoserine in a medium containing propane-1,2-diol as sole carbon source.[47]

New amino-acids from normal human urine are α-amino-γ,δ-dihydroxyadipic acid[48] and α-hydroxy-β-keto-γ-aminobutyric acid.[49]

New Amino-acids from Hydrolysates.—The main interest in this section continues to be found in novel protein cross-linking residues and in peptide antibiotics.

Bovine bone collagen contains pyridinoline (5; $n + m = 3$); further studies are in hand to resolve the structural ambiguities.[50] U.v. irradiation (253 nm) induces cross-links, tentatively identified as α-*S*-cysteinylthymine (6), between

[39] S. Hatanaka and S. Kaneko, *Phytochemistry*, 1978, **17**, 2027.
[40] K. Kawauchi, T. Kosuru, S. Ota, and T. Suzuki, *Nippon Suisan Gakkaishi*, 1977, **43**, 1293.
[41] R.J. Andersen, *Tetrahedron Letters*, 1978, 2541.
[42] M.B. Yunker and P.J. Scheuer, *Tetrahedron Letters*, 1978, 4651.
[43] S. Takano, D. Uemura, and Y. Hirata, *Tetrahedron Letters*, 1978, 2299.
[44] S. Takano, D. Uemura, and Y. Hirata, *Tetrahedron Letters*, 1978, 4909.
[45] Y. Imagawa, S. Shima, A. Hirota, and H. Sakai, *Agric. Biol. Chem.*, 1978, **42**, 681.
[46] R.J. Petroski and W.J. Kelleher, *Lloydia*, 1978, **41**, 332.
[47] S. Yamada, K. Nabe, T. Ujimaru, N. Izuo, and M. Chibata, *Appl. Environ. Microbiol.*, 1978, **35**, 1046.
[48] S. Yuasa, *Biochim. Biophys. Acta*, 1978, **540**, 93.
[49] M. Kinuta, *Biochim. Biophys. Acta*, 1978, **542**, 56.
[50] D. Fujimoto, T. Moriguchi, T. Ishida, and H. Hayshi, *Biochem. Biophys. Res. Comm.*, 1978, **84**, 52.



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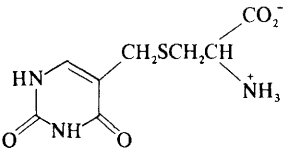

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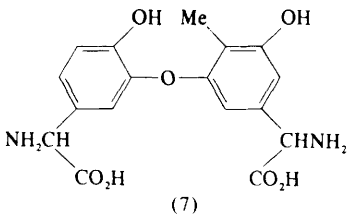
protein and polynucleotide in phage fd gene 5 protein complexes with fd DNA or poly(dT).[51]

Further progress has been made in structural assignments to amino-acids released from ristomycins[52,53] and related antibiotics (vancomycin, *alias* vancosamine[54]) on hydrolysis. The oxygen-bridged phenylglycine derivative (7; ristomycinic acid[52]) and the biphenyl (8; actinoidinoic acid [52]) have been identified as components of ristomycins, and the evidence for the presence of two β-hydroxychlorotyrosine, three oxygenated phenylglycines, on *N*-methylleucine, and an aspartic acid residue in vancomycin, has been reviewed.[54] N^{β}-Methyl-L-β-arginine $H_2NC(=NH)NHCH_2CH_2CH(NHMe)CH_2CO_2H$ is a constituent of the newly discovered antibiotic LL-BM547β.[55]

3 Chemical Synthesis and Resolution of Amino-acids

General Methods of Synthesis of α-Amino-acids.—Alkylation of glycine derivatives is formally a general synthetic route to α-amino-acids but is fre-



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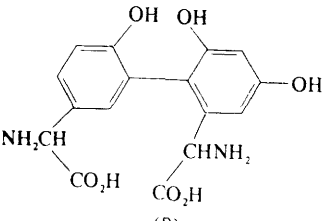


(8)

[51] P. Paradiso, Y. Nakashima, and W. Konigsberg, 'Biomolecules: Structure and Function', ed. P.F. Agris, Academic Press, New York, 1977, p. 581.
[52] G.S. Katrukha, P.B. Terentev, B. Diarra, and E.S. Gershtein, *Khim. prirod. Soedinenii*, 1978, 141.
[53] C.M. Harris, J.J. Kibby, and T.M. Harris, *Tetrahedron Letters*, 1978, 705.
[54] G.M. Sheldrick, P.G. Jones, O. Kennard, D.H. Williams, and G.A. Smith, *Nature*, 1978, **271**, 223.
[55] S. Nomoto and T. Shiba, *Chem. Letters*, 1978, 589.

quently inefficient, owing to low reactivity. When activated halides are used as alkylating agents to overcome this source of low yields, appreciable amounts of unwanted $\alpha\alpha$-dialkylated products may be formed. Phase transfer alkylation of the stable Schiff base $Ph_2C{=}NCH_2CO_2Et$ is something of an improvement over earlier methods based on this process[56] (though some α-benzylphenylalanine is formed using benzyl halides), and the corresponding Schiff base $Ph_2C{=}NCH_2CN$ can be employed analogously with non-activated halides.[57] Examples of the related azlactone synthesis[58,59] and other examples of the use of an aldehyde to create an amino-acid side-chain $Me_2CHCHO \rightarrow Me_2CHCH(OH)CN \rightarrow Me_2CHCH(OSOCl)CN \rightarrow$ DL-valine;[60] methyl isocyanoacetate with R^1COR^2 giving cycloalk-1-enylglycines after hydrolysis,[61] or Schiff bases of $\alpha\beta$-dehydro-amino-acids $R_2NCH{=}NC(=CR^1R^2)CO_2Me$ *via* Michael addition of the amine to the isocyanoacrylate, followed by synchronous elimination – insertion of the amine into the isocyano-group[62]). Related applications of methyl isocyanoacetate [$\rightarrow RCOCH(NH_2)CO_2Me$ with RCOCl or $(RCO)_2O$, thence to the substituted oxazole-4-carboxylate and to the erythro-β-hydroxyamino-acid after hydrolysis and hydrogenation over Pt, or $NaBH_4$ reduction;[63] $CNCH_2CONR^1R^2 + MeCHO \rightarrow$ *trans*-5-methyloxazolin-2-yl-4-carboxamide $\rightarrow$ N^{α}-formyl-DL-threoninamides with formic acid[64]] illustrate novel routes to β-hydroxy-α-amino-acids. Condensation of a methyl 2-isocyanoalkanoate with 1-phenylethylamine, followed by cyclization (BuLi) gives the corresponding 4-substituted *N*-(l-phenylethylimidazolidin-5-one), which can be alkylated further, giving $\alpha\alpha$-dialkyl-α-amino-acids.[65] Further examples of Ugi four-component condensation reactions, leading to N^{α}-benzyl-amino-acid amides,[66,67] have been described; one of these studies[67] involves CO_2 as a reactant.

Carbon–carbon bond formation methods described in the preceding paragraph include several processes in which bis-alkylation is avoided, as is also the case with the acetamidomalonate route.[21,68–73] 2-Amino-4-methylhexanoic acid,[21] 2-amino-4-(2-aminoethoxy)-*trans*-but-3-enoic acid[68] and its 4-methoxy analogue,[73] and acetyltyrosine[69] have been prepared by this method, and 3,5-

[56] M.J. O'Donnell, J.M. Boniece, and S.E. Earp, *Tetrahedron Letters*, 1978, 2641.
[57] M.J. O'Donnell and T.M. Eckrich, *Tetrahedron Letters*, 1978, 4625.
[58] E.I. Karpeiskaya, E.S. Neupokoeva, L.F. Godunova, I.P. Murina, A.P. Kharchevnikov, and E.I. Klabunovskii, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1978, 1368; L.F. Godunova, E.S. Neupokoeva, E.I. Karpeiskaya, and E.I. Klabunovskii, *ibid.*, p. 1363.
[59] M.A. Rakshinda and N. H. Khan, *Indian J. Chem.*, 1978, **16B**, 634.
[60] J.W. Davis, *J. Org. Chem.*, 1978, **43**, 3980.
[61] M. Suzuki, K. Nunami, and N. Yoneda, *J.C.S. Chem. Comm.*, 1978, 270.
[62] M. Suzuki, K. Nunami, T. Moriya, K. Matsumoto, and N. Yoneda, *J. Org. Chem.*, 1978, **43**, 4933.
[63] M. Kirihata, H. Tokumori, I. Ichimoto, and H. Ueda, *Nippon Nogei Kagaku Kaishi*, 1978, **52**, 271.
[64] Y. Ozaki, K, Matsumoto, and M, Miyoshi, *Agric. Biol. Chem.*, 1978, **42**, 1565.
[65] U. Schöllkopf, H.H. Hansberg, I. Hoppe, M. Segal, and U. Reiter, *Angew. Chem.*, 1978, **90**, 136.
[66] H.R. Divanfard, Z. Lysenko, P.-C. Wang, and M.M. Joullie, *Synthesis Comm.*, 1978, **8**, 269.
[67] E. Haslinger, *Monatsh. Chem.*, 1978, **109**, 749.
[68] D.D. Keith, R. Yang, J.A. Tortora, and M. Weigele, *J. Org. Chem.*, 1978, **43**, 3713.
[69] L. Florval, S.B. Ross, S.O. Ogren, and N.E. Stjernstrom, *Acta Pharm. Suec.*, 1978, **15**, 13.
[70] H.J. Teuber, H. Krause, and V. Berariu, *Annalen*, 1978, 757.
[71] W. Oppolzer and H. Andres, *Tetrahedron Letters*, 1978, 3397.
[72] G.A. Dilbeck, L. Field, A.A. Gallo, and R.J. Gargiulo, *J. Org. Chem.*, 1978, **43**, 4593.
[73] D.D. Keith, J. A. Tortora, and R. Yang, *J. Org. Chem.*, 1978, **43**, 3711.

di-t-butyltyrosine and (3,5-di-t-butyl-4-hydroxyphenyl)glycine have been prepared *via* the rarely-used formamidomalonate.[70]

An interesting synthesis of arylglycines from the corresponding aldehyde, bromoform, and NH_3–KOH–$LiNH_2$ involves a bromo-oxirane intermediate.[74] Further examples of arylglycine synthesis using β-hydroxyglycine derivatives have been described.[75]

Electroreductive amination of α-keto-acids in aqueous NH_4OH–NH_4Cl at mercury electrodes proceeds with low yields in some cases owing to competing condensation reactions.[76] Radical amination of corresponding hydroxy acids in aqueous NH_4OH under γ-irradiation has been shown to produce alanine, β-alanine, glycine, aspartic acid, and α- and γ-aminobutyric acids.[77]

Synthesis of β-Amino-acids and Higher Homologues.—Mention is made in the preceding section, and elsewhere in this Chapter,[78,79] of the synthesis of homologues of α-amino-acids. Little scope exists for generalizations covering synthetic methods for these compounds, but the use of the Arndt–Eistert reaction for conversion of N^{α}-toluene-*p*-sulphonyl-N^{δ}-Boc-L-ornithine into the β-lysine derivative, followed by treatment with *O*-methyl-*N*-nitroisourea, in the synthesis of N^{α}-methyl-L-β-arginine,[55] and electroreduction of cyanoacetic acid to β-alanine,[80] illustrate two standard methods.

Asymmetric Synthesis.—Increased interest in this topic, though using well-established principles, is noticeable. Asymmetric hydrogenation of α-acylaminoacrylic acids catalysed by chiral Rh^{I}–phosphine complexes[81] now interests several research groups. Optical purity 73—93 %[81] and 'reaching 100 %'[82] have been recorded in the synthesis of enantiomers of alanine[81,83] and phenylalanine.[82,85–87] Optically-active β-amino-acids are available through the same process applied to β-acylamino-acrylates.[88] L-Dopa and L-phenylalanine can be obtained from the corresponding azlactones by hydrogenation over Pd in the presence of (*S*)-phenylethylamine.[58]

Hydrogenolysis is a key step in asymmetric transamination procedures employing chiral benzylamines. Asymmetric synthesis of alanine from ethyl pyruvate *via* the Schiff base formed with (*R*)-$H_2NCHPhCO_2R$, on treatment with H_2–Pd to give the secondary amine (highest optical purity with a bulky ester grouping R), acid hydrolysis, and hydrogenolysis, leads to the L-isomer

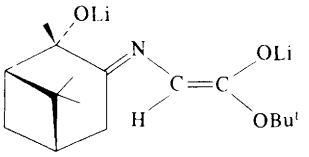
[74] E.L. Compere and D.A. Weinstein, *Synthesis*, 1977, 852.
[75] D. Ben-Ishai, J. Altman, Z. Bernstein, and N. Peled, *Tetrahedron*, 1978, **34**, 467; A. Schouteeten, Y. Christidis, and G. Mattioda, *Bull. Soc. chim. France*, 1978, 248.
[76] E.A. Jeffrey and A. Meisters, *Austral. J. Chem.*, 1978, **31**, 73; E.A. Jeffrey, O. Johansen, and A. Meisters, *ibid.*, p. 79.
[77] K. Ema, T. Kato, and M. Shinagawa, *Radioisotopes*, 1978, **27**, 445.
[78] D.H. Rich, E.T. Sun, and A.S. Boparai, *J. Org. Chem.*, 1978, **43**, 3624.
[79] J.A. Yankeelov, K.F. Fok, and D.J. Carothers, *J. Org. Chem.*, 1978, **43**, 1623.
[80] V. Krishnan, K. Ragapathy, and H.V.K. Udupa, *J. Appl. Electrochem.*, 1978, **8**, 169.
[81] K. Hanaki, K. Kashiwabara, and J. Fujita, *Chem. Letters*, 1978, 489.
[82] J. Vilim and J. Hetflejs, *Chem. prumysl*, 1978, **28**, 135 (*Chem. Abs.*, 1978, **89**, 180 317).
[83] K. Herrmann, *Nachr. Chem., Tech. Lab.*, 1978, **26**, 651 (*Chem. Abs.*, 1978, **89**, 215 729).
[84] N. Takaishi, H. Imai, C.A. Bertelo, and J.K. Stille, *J. Amer. Chem. Soc.*, 1978, **100**, 264.
[85] G. Descotes, D. Lafont, and D. Sinou, *J. Organometallic Chem.*, 1978, **150**, C14.
[86] R. Jackson and D.J. Thompson, *J. Organometallic Chem.*, 1978, **159**, C29.
[87] T. Masuda and J.K. Stille, *J. Amer. Chem. Soc.*, 1978, **100**, 268.
[88] K. Achiwa and T. Soga, *Tetrahedron Letters*, 1978, 1119.

predominantly when hydrogenation is carried out at −10 ° to +20 °C, but to the D-enantiomer at higher temperatures.[89] Chiral serines are obtained in optical purity 28—39 % by successive hydrolysis and hydrogenolysis of the chiral aziridinecarboxylates formed between αβ-dibromopropionates and (*R*)-phenylethylamine;[90] aspartic acids have been obtained analogously from dibromosuccinates in rather better optical yields.[91] Only low optical yields (2—18 %) have so far been achieved by alkylation of *N*-[(*S*)-phenylethyl]-formamidoacetonitrile followed by hydrolysis and hydrogenolysis, indicating minimal stereoselectivity in the deprotonation step (NaH) preceding alkylation.[92] Alkylation of the di-anion (9) from (+)-pinanone and glycine t-butyl ester followed by hydrolysis gives the corresponding D-amino-acid t-butyl ester with regeneration of the chiral reagent,[93] which is an improvement relative to corresponding steps in the transamination of α-keto acids with the hydrazide analogue of (−)-ephedrine[94] and with a chiral pyridoxamine analogue.[95] Asymmetric alkylation of (+)-menthyl α-isocyanopropionate has been described.[96]

Asymmetric synthesis of β-amino-acids *via* Reformatsky reactions using (−)-menthyl bromoacetate and chiral Schiff bases $R^1CH{=}NR^2$[R^2 = (*R*)-phenylethyl] favours the (*S*)-enantiomers to the extent of 2—28 %.[97]

Prebiotic Synthesis; Model Reactions.—As in the preceding section, there are more papers eligible for citation here, compared with previous Volumes, but most of the work described involves development of familiar approaches.

The current work of virtually all the active research groups in this field has been collected in a '*Proceedings*' volume.[98] The temptation to select material from this volume for discussion has been resisted, since any selection would have been unrepresentative, but the material has been published already in the primary sources.



(9)

[89] K. Harada and Y. Kataoka, *Chem. Letters*, 1978, 791; *Tetrahedron Letters*, 1978, 2103.
[90] K. Harada and I. Nakamura, *J.C.S. Chem. Comm.*, 1978, 522.
[91] K. Harada and I. Nakamura, *Chem. Letters*, 1978, 9.
[92] K. Harada, M. Tamura, and S. Suzuki, *Bull. Chem. Soc. Japan*, 1978, **51**, 2171.
[93] T. Oguri, N. Kawai, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.*, 1978, **26**, 803.
[94] H. Takahashi, H. Neguchi, K. Tomita, and H. Otomasu, *Yakugaku Zasshi*, 1978, **98**, 618.
[95] H. Kuzuhara, T. Komatsu, and S. Emoto, *Tetrahedron Letters*, 1978, 3563.
[96] M. Kirihata, S. Mihara, I. Ichimoto, and H. Ueda, *Agric. Biol. Chem.*, 1978, **42**, 185.
[97] M. Furukawa, T. Okawara, Y. Noguchi, and Y. Terewaki, *Chem. Pharm. Bull.*, 1978, **26**, 260.
[98] 'Origins of Life,' Proceedings of the 2nd ISSOL Meeting 1977, ed. H. Noda, Bus. Cent. Acad. Soc., Tokyo, 1978; *inter alia* see I. Draganic, Z. Draganic, A. Shimoyama, and C. Ponnamperuma, p. 53 (*Chem. Abs.*, 1979, **90**, 49 864); A. Shimoyama, N. Blair, and C. Ponnamperuma, p. 95; C.S. Matthews, p. 123.

Perhaps the most interesting work, from a chemist's viewpoint, is the oligomerization of HCN in 0.1M aqueous solutions at pH 9.2, subsequent hydrolysis giving purines, pyrimidines, and amino-acids.[99] This topic has been discussed in earlier volumes in this series, as have studies of contact glow-discharge electrolysis, extended to the amination of aliphatic cyanides in aqueous NH_4OH[100] and the cyanation of aliphatic amines,[100] hydroxyamino-acid formation from alkenoic acids in aqueous NH_4OH,[101] β-hydroxyaspartic acid formation from maleic and fumaric acids,[101] and conversion of β-amino-acids formed in this way into α-amino-acids (through excision of the α-methylene or -methine grouping[102]). Electric discharge through CH_4–NH_3–H_2O mixtures gives amino-nitriles in very low yields[103] and analogous mixtures subjected to high-frequency plasmolysis[104] give products in proportions influenced by catalysts, pH, and the characteristics of the energy source. Aqueous solutions of metal cyanides exposed to multi-krad doses from a ^{60}Co source give uncharacterized material which releases five protein amino-acids and five non-protein amino-acids on hydrolysis.[105a] Similar treatment of aqueous MeCN yields glycine, lysine, aspartic acid, serine, and glutamic acid,[106,105b] while EtCN gives nine amino-acids.[105b] γ-Irradiation of cyanamide, H_2NCN, in aqueous solutions gives arginine and other compounds.[107]

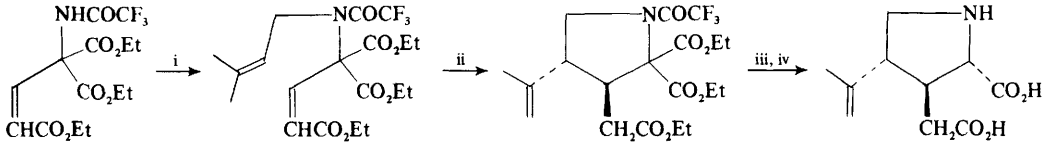
Glycine is formed in 2.5 % aqueous formaldehyde, saturated with N_2 and subjected to ultrasonic sound.[108]

Protein Amino-acids and Other Naturally-occurring Amino-acids.—The preceding sections have described synthetic methods which have been used, more often than not, for the synthesis of naturally-occurring amino-acids. This section deals with specific rather than general synthetic methods.

The synthesis of lysine from ε-caprolactam,[109] and the routes available to canaline [$H_2NOCH_2CH_2CH(NH_2)CO_2H$][110] have been reviewed. Full details of the synthesis of DL-capreomycidine (Vol. 10, p. 9) from *threo*-β-hydroxy-ornithine have been published.[111]

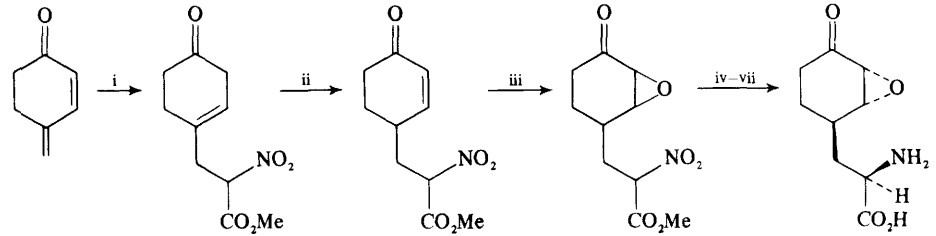
Trichloroethene and amino-acetonitrile are intermediates in the synthesis of glycine from tetrachloroethanes in aqueous NH_4OH at 160—170 °C.[112] Mechanistically interesting syntheses have also been reported for α-allokainic

[99] J.P. Ferris, P.C. Joshi, E.H. Edelson, and J.G. Lawless, *J. Mol. Evol.*, 1978, **11**, 293.
[100] K. Harada, S. Suzuki, and H. Ishida, *BioSystems*, 1978, **10**, 247.
[101] K. Harada, S. Suzuki, and H. Ishida, *Experientia*, 1978, **34**, 17.
[102] S. Suzuki, M. Tamura, J. Teresawa, and K. Harada, *Bio-org. Chem.*, 1978, **7**, 111.
[103] H. Ebisawa, S. Mankino, E. Miyoshi, T. Shirai, and S. Yanagisawa, *Nippon Kagaku Kaishi*, 1978, 351 (*Chem. Abs.*, 1978, **88**, 169 292).
[104] C.I. Simionescu, S. Dumitriu, V.I. Popa, V. Bulacovschi, and B. Simionescu, *Rev. Roumaine Chim.*, 1978, **23**, 89.
[105] (*a*) Z. Draganic, I. Draganic, A. Shimoyama, and C. Ponnamperuma, *Origins of Life*, 1977, **8**, 371; (*b*) *ibid.*, p. 377.
[106] M.J. Shushtarian, *Q. Bull. Fac. Sci., Tehran Univ.*, 1976, **8**, 19 (*Chem. Abs.*, 1978, **88**, 90 006).
[107] Z.D. Draganic, I.G. Draganic, and S.V. Jovanovic, *Radiation Res.*, 1978, **75**, 508.
[108] A.V. Sokolskaya, *Zhur. obshchei Khim.*, 1978, **48**, 1407.
[109] M. Ciha, E. Stefanikova, and A. Zvakova, *Chem. listy*, 1978, **72**, 1066.
[110] T. Korpela, J. Lundell, and P. Pasanen, *Org. Prep. Proced. Int.*, 1977, **9**, 57.
[111] T. Wakamiya, K. Mizuno, T. Ukita, T. Teshima, and T. Shiba, *Bull. Chem. Soc. Japan*, 1978, **51**, 850.
[112] M. Inoue and S. Enomoto, *Chem. Letters*, 1978, 1231.



Reagents: i, NaH–1-bromo-3-methylbut-1-ene; ii, 170 °C, 10 min; iii, 0.5M NaOH, reflux; iv, H_3O^+

Scheme 1



(10)

Reagents: i, $NO_2CH_2CO_2Me$; $PhCH_2\overset{+}{N}Me_3\bar{O}H$; ii, 1% aq.HCl–DMSO (50% conversion; separate on silica gel); iii, alkaline H_2O_2–MeOH– –20 °C; iv, H_2–Raney Ni–Ac_2O; v, silica gel chromatography; vi, alkaline hydrolysis (1.25M NaOH, 5 °C, 15 min); vii, hog kidney acylase

Scheme 2

acid (Scheme 1; in which the ingredients for an ene reaction are set up, starting from diethyl *N*-trifluoroacetylaminomalonate),[71] and for the antibiotic anticapsin (10; for which the obvious starting point, a reduced tyrosine, was precluded by the ease of intramolecular addition of the amino-function to the conjugated ketone).[113]

Stereospecific synthesis of the chiral lactone ester analogue establishes the all-*cis* stereochemistry (1) for the antibiotic thermozymocydin (myriocin).[22] The synthesis of (3*S*,5*S*)-5-hydroxyhexahydropyrazine-3-carboxylic acid, a constituent of the monamycin antibiotics, has been established.[114] The starting point, pyridazino[1,2-*b*]phthalazine, proved to be a suitable substrate for stereospecific introduction of the hydroxy- and carboxy-groups, the phthaloyl residue being cleaved at the end of the synthesis with hydrazine.

The pepstatin constituent, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid ('statine'), has been synthesized from Boc-L-leucine methyl ester *via* Bu^t_2AlH reduction to the leucinal, followed by addition of $LiCH_2CO_2Et$ and conventional subsequent steps.[78]

Aliphatic Amino-acids.—Hydrogenation of (*R*)-phenylglycine over $Pd(OH)_2$ around neutral pH gives (*R*)-cyclohexylglycine.[115]

α-Alkyl Analogues of Protein Amino-acids.—A synthesis of α-methylarginine, produced by Streptomyces X-11837,[116] involves the cleavage of 2-methyl-2-acetamidovalerolactone with 30% HBr, and elaborating the resulting $R(CH_2)_3CMe(NHAc)CO_2H$ (R = Br) successively into intermediates [R = PhCH=N(O)] and (R = HONH).[117] An asymmetric synthesis of α-methylornithine depends on Michael addition of (+)-menthyl α-isocyanopropionate carbanion to acrylonitrile.[96]

α-Hetero-atom Substituted α-Amino-acids.—Full details are reported for the conversion of *N*-acetylamino-acid methyl esters into α-methoxy-analogues using Bu^tOCl and MeOH.[118] α-Chloro-α-acylamido-acids formed[119] with AcCl from α-hydroxy-α-acylamidoacids (*i.e.* amide–glyoxylic acid adducts)[75] can be converted into alkoxy-, alkythio-, or alkanesulphonyl-analogues through nucleophilic substitution.[119]

α-Halogenoalkyl-α-amino-acids.—Alkylation of the carbanion derived from benzylidenealanine methyl ester with halogenomethanes gives α-halogenomethyl α-amino-acids.[120]

Aliphatic Amino-acids Carrying Hydroxy Groups in Side-chains.—Hydroxyalkylation of *N*-salicylideneglycine ethyl ester by acetaldehyde in the presence of base gives the threonine derivative, exemplifying further this well-studied

[113] R.W. Rickards, J.C. Rodwell, and K.J. Schmalzl, *J.C.S. Chem. Comm.*, 1977, 849.
[114] C.H. Hassall and K.L. Ramachandran, *Heterocycles*, 1977, **7**, 119.
[115] M. Tamura and K. Harada, *Synthesis Comm.*, 1978, **8**, 345.
[116] T.C. Demny and H. Maehr, U.S.P. 4 061 542 (*Chem. Abs.*, 1978, **88**, 72 986).
[117] H. Maehr and M. Leach, *J. Antibiotics*, 1978, **31**, 165.
[118] H. Poisel, *Chem. Ber.*, 1978, **111**, 3136.
[119] D. Matthies, *Synthesis*, 1978, 53.
[120] P. Bey and J.P. Vevert, *Tetrahedron Letters*, 1978, 1215.

addition reaction (see also Vol. 10, p. 10).[121] A different approach to converting one amino-acid into another is the chiral synthesis of Boc-D-homoserine lactone through treatment of *N*-Boc-*S*-methyl-D-methionone trimethylsilyl ester with $KOBu^t$ in refluxing THF.[122]

A new synthesis of γ-hydroxyglutamic acid involves hydrogenolysis of 3-ethoxycarbonyl-5-methoxycarbonylisoxazoline (the 1,3-dipolar adduct of methyl acrylate and ethyl cyanoformate-*N*-oxide).[123]

To examples elsewhere in this Chapter of higher homologous hydroxyalkyl-amino-acids can be added the synthesis of 4-amino-3-hydroxybutanoic acid.[124]

α-Amino-acids with Unsaturated Side-chains.—The two main structural types covered by this section, 'αβ-dehydro-amino-acids' $R^1R^2C{=}C(NH_2)CO_2H$, and alkenyl or alkynyl amino-acids, are represented increasingly regularly in the literature. Dehydration of *N*-protected serine and threonine esters can be performed with Ph_3P and diethyl azodicarboxylate, giving the protected dehydro-amino-acids ($R^1 = R^2 = H$) and ($R^1 = H$, $R^2 = Me$), respectively.[125] Amino-substituted analogues ($R^1 = H$, $R^2 = NH_2$) can be prepared from ethyl pyruvate, after conversion into the protected dehydro-alanine $CH_2{=}CH(NHZ)CO_2Et$, by successive *N*-bromination (NBS), *N*-azidation (NaN_3) and spontaneous N→C shift, and Raney nickel reduction.[126]

A novel approach to the synthesis of α-vinyl-α-amino-acids depends on the exclusive α-alkylation of the carbanion formed with $LiN(SiMe_3)_2$ from the Schiff base of a dehydro-amino-acid ($R^1 = H$, $R^2 = Me$): $\overbrace{CH_2{\cdots}CH{\cdots}C}^{-}(N{=}CHPh)CO_2Me \rightarrow CH_2{=}CHCR(N{=}CHPh)CO_2Me$.[127] In a related approach,[128] the di-anion formed from *N*-ethoxycarbonyl trimethyl-silyglycine methyl ester by treatment with $LiNPr^i_2$ in HMPA undergoes mono-alkylation to give α-ethynyl-α-amino-acids. This synthon rearranges into the allenic isocyanate $Me_3SiCH{=}C{=}C(N{=}C{=}O)CO_2Me$ when used in an attempt to prepare the first member of the series, 2-aminobutynoic acid.[128]

Aromatic and Heterocyclic Amino-acids.—Aromatic and heteroaromatic side-chains may be introduced into glycine derivatives through general synthetic routes described in an earlier section, and this section deals with subsequent modifications to side-chains, and the construction of heteroaromatic rings from acyclic precursors.

Benzylideneazlactone on treatment with hydroxylamine gives the ring-opened adduct PhCH(NHOH)CH(NHCOPh)CONHOH, from which β-

[121] Y.N. Belokon, V.M. Belikov, V.A. Maksakov, N.G. Faleev, and E.A. Paskonova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1978, 1026.
[122] R.D.G. Cooper, F. Jose, L. McShane, and G.A. Koppel, *Tetrahedron Letters*, 1978, 2243.
[123] T. Kusumi, H. Kakisawa, S. Suzuki, K. Harada, and C. Kashima, *Bull. Chem. Soc. Japan*, 1978, **51**, 1261.
[124] M. Pinzi and G. Pifferi, *J. Pharm. Sci.*, 1978, **67**, 120.
[125] H. Wojciechowska, R. Pawlowicz, R. Andruszkiewicz, and J. Grzybowska, *Tetrahedron Letters*, 1978, 4063.
[126] C. Shin, K. Watanabe, H. Ohmatsu, and J. Yoshimura, *Tetrahedron Letters*, 1978, 4535.
[127] W.J. Greenlee, D. Taub, and A.A. Patchett, *Tetrahedron Letters*, 1978, 3999.
[128] P. Casara and B.W. Metcalf, *Tetrahedron Letters*, 1978, 1581.

aminophenylalanine can be obtained by hydrogenation (over Pd–C) followed by acid hydrolysis.[129]

L-Histidine carrying a trifluoromethyl group at C-2 of the imidazole ring can be obtained by Bamberger cleavage of the amino-acid followed by recyclization in refluxing TFAA.[130] Fischer indolization of *N*-acetyl-L-glutamic *γ*-semialdehyde with *p*-methoxy- or -phenoxy-phenylhydrazine gives the corresponding 5-methoxy- or -phenoxy-L-tryptophans.[131] A synthesis of racemic *α*-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid, an analogue of ibotenic acid, has been accomplished, the key step being the cyclization of the corresponding oxime.[132]

***N*-Substituted Amino-acids.**—Continuing efforts aimed at the synthesis of *N*-methylamino-acids are represented by a paper from one of the main innovators in this field, demonstrating reductive *NN*-dimethylation of Boc-amino-acids in a MeOH–O_2–Pd/C brew, equivalent to methanolic formaldehyde.[133] *N*-Hydroxytyrosine has been synthesized from the keto-acid oxime by $Na(CN)BH_3$ reduction.[134] *N*-(Alkylthiomethyl)amino-acids are readily obtained using formaldehyde and a thiol.[135]

Excluding citations of *N*-protected amino-acids prepared for use in peptide synthesis, two topics remain, *viz.* the preparation of a wide variety of *N*-alkylated DL-phenylalanines by reduction of the corresponding Schiff bases,[136] and continuing Australian work (preparation of *N*-substituted analogues)[137] on L-mimosine (3-hydroxy-4-oxo-1,4-dihydropyrid-1-yl)-L-alanine, an amino-acid known to cause defleecing of sheep.[138]

***α*-Amino-acids Containing Sulphur.**—Carefully worked out procedures, some amounting to improvements of known methods, have been described for the synthesis of heterocyclic analogues of cysteine, norcysteine, and homocysteine (*e.g.* 1,3-thiazane-6-carboxylic acid).[139] 5-Mercapto-4,4-dimethyl-2-aminopentanoic acid, a bis homologue of penicillamine has been synthesized from acetamidomalonate and $Me_2C{=}CHCH_2CL$, followed by BF_3-catalysed addition of $PhCH_2SH$ (the first example of BF_3-catalysed Markownikoff addition of a thiol to an alkene).[72] 3-Alkyl analogues of the same homologue have been prepared through the azlactone route, using $R^1SCR^2R^3CH_2COR^4$ and hippuric acid.[59]

[129] R.M. Ali and N.H. Khan, *Synthesis Comm.*, 1978, **8**, 497.
[130] H. Kimoto, K.L. Kirk, and L.A. Cohen, *J. Org. Chem.*, 1978, **43**, 3403.
[131] S. Iriuchijima and G. Tsuchihashi, *Agric. Biol. Chem.*, 1978, **42**, 843.
[132] S.B. Christensen and P. Krogsgaard-Larsen, *Acta Chem. Scand.*, 1978, **B32**, 27.
[133] F.M.F. Chen and N.L. Benoiton, *Canad. J. Chem.*, 1978, **56**, 150.
[134] B.K. Malik and I.J. McFarlane, *Indian J. Chem. B*, 1978, **16B**, 76.
[135] K. Ito, R. Komaki, and M. Sekiya, *Chem. Pharm. Bull.*, 1977, **25**, 3385.
[136] S. Takemura, H. Terauchi, K. Kowata, K. Nakano, Y. Okumura, K. Li, and Y. Inamori, *Yakagaku Zasshi*, 1978, **98**, 869.
[137] F.H.C. Stewart, *Austral. J. Chem.*, 1978, **31**, 1861.
[138] P.J. Reis, *Austral. J. Agric. Res.*, 1978, 29, 1043; P.J. Reis and D.A. Tunks, *ibid.*, p. 1057; P.J. Reis, D.A. Tunks, and A.M. Downes, *ibid.*, p. 1065.
[139] N.J. Lewis, R.L. Inloes, J. Hes, R.H. Matthews, and G. Milo, *J. Med. Chem.*, 1978, **21**, 1070.

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

Compound	*Ref.*
$N^{\beta}N^{\beta}$-Dialkylasparagines	140
$N^{\gamma}N^{\gamma}$-Dialkylglutamines	140
L-Cysteic acid sulphonamides, H-Cys(SO$_2$NHR)-OH (R = *O*-alkyl; CH_2CH_2Cl; $CH_2C_6H_4$-*p*-SO_2F; $NHCOC_6H_4$-*p*-SO_2F; *trans*-NHCOCH=CHCO$_2$Et)	141
2′-Deoxyadenosyl-L-homocysteine	142
(2,3-Epoxycyclohexyl)glycine	143
(3,5-Di-t-butyl-4-hydroxyphenyl)glycine	144
N-Bromoacetyl-*p*-azido-L-phenylalanine methyl ester	145
3-(OR)-4(OR′)-Dopas (R = Me, Et, Pr, or Bu^s; R′ = H, Me, or Bu)	146
5-Fluorodopa{(3,4-dihydroxy-5-fluorophenyl)alanine}	147
2-Arylazo-L-histidines	148
4-Arylazo-L-histidines	148
2,4-Bis-arylazo-L-histidines	148

Labelled Amino-acids.—The synthesis and metabolism of chirally labelled α-amino-acids have been reviewed.[149]

Further studies of ^{3}H-labelling of amino-acids by ^{3}H atoms formed by microwave discharge in $^3H_2$ (see also Vol. 10, p. 16) have been described;[150,151] ^{2}H atoms at 77 K degrade *ca.* 40 % of a sample of D-alanine, giving also α- and β-exchanged products in the ratio 88 : 12.[151] [2-^{2}H]-3-Fluoro-DL-alanine is formed by reductive amination in aqueous NH_4OH containing NaB_2H_4 with highly specific incorporation of the label.[152] (1*R*)-[1-^{3}H,^{2}H]-3-phenylpropanol was formed from (1*S*)-[1-^{2}H]-3-phenylpropanol, [1-^{3}H]ethanol, and yeast alcohol dehydrogenase, and transformed into (4*R*)-[4-^{3}H,^{2}H]-DL-homoserine

[140] T. Caplaneris, P. Cordopatis, J. Matsoukas, and D. Theodoropoulos, *Tetrahedron*, 1978, **34**, 969.
[141] S. Brynes, G.J. Burckhart, and M. Mokotoff, *J. Med. Chem.*, 1978, **21**, 45.
[142] Y. Wang and H.P.C. Hogenkamp, *J. Org. Chem.*, 1978, **43**, 998.
[143] M. Dzieduszycka, M. Smelkowski, T. Czarnowska, and E. Borowski, *Pol. J. Chem.*, 1978, **52**, 933.
[144] F.R. Hewgill and R.J. Webb, *Austral. J. Chem.*, 1977, **30**, 2565.
[145] Y.S. Klausner, A.M. Feigenbaum, N. De Groot, and A.A. Hochberg, *Arch. Biochem. Biophys.*, 1978, **185**, 151.
[146] J. Barth and C.G. Wermuth, *Bull. Soc. chim. France*, 1977, 956.
[147] E.S. Garnett, G. Firnau, P.K.H. Chan, S. Sood, and L.W. Belbeck, *Proc. Nat. Acad. Sci. U.S.A.*, 1978, **75**, 464.
[148] G. Montagnoli, O. Pieroni, L. Nannicini, and A. Muttini, *Gazzetta*, 1977, **107**, 409.
[149] R.J. Parry, *Bio-org. Chem.*, 1978, **2**, 247.
[150] B.W. Wessels, D.J. McKean, N.C. Lien, C. Shinnick, P.M. De Luca, and O. Smithies, *Radiat. Res.*, 1978, **74**, 35.
[151] E.F. Siminov, M.S. Unukovich, E.S. Filatov, and A.V. Shishkov, *Khim. vysok. Energii*, 1978, **12**, 8.
[152] U.H. Dolling, A.W. Douglas, E.J.J. Grabowski, E.F. Schoenewaldt, P. Sohar, and M. Sletzinger, *J. Org. Chem.*, 1978, **43**, 1634.

[the (4*S*)-diastereoisomer was formed analogously], but 2-phenylethanol underwent negligible H-exchange and a corresponding synthesis of asymmetrically [^{3}H,^{2}H]-labelled serine was not feasible.[153]

Nine carboxyl-^{11}C-labelled amino-acids have been prepared by an appropriately rapid synthesis (high temperature, high pressure) from K^{11}CN, NH_4Cl, and $(NH_4)_2CO_3$ (a modified Bucherer–Strecker synthesis),[154] also used[155] under more normal laboratory conditions for the synthesis of DL-[1-^{13}C]methionine. *S*-Adenosyl-L-methionines, labelled in the *S*-methyl group by either ^{2}H or ^{13}C have been prepared from the methionines and *Saccharomyces cerevisiae*.[156] ^{13}C-or ^{14}C-Enriched CO_2 and HCHO have been used, with the reduced form of cobaloxime, to synthesize *N*-bromomethylphthalimide, from which labelled glycines have been prepared.[157] While ^{14}C-β-bromo-α-aminobutyric acid, can be prepared from ^{14}C-L-threonine by conventional substitution reactions,[158] the ^{125}I-γ-iodo-analogues and the corresponding γ-iodo-α-aminopentanoic acid are unstable and are rapidly de-iodinated.[158] Unusual synthetic methodology is involved in the route to [1-^{14}C]-*N*-benzyloxycarbonylphenylalanine methyl ortho-ester: $Ph(CH_2)_2Cl \rightarrow Ph(CH_2)_2{}^{14}C(OMe)=NCl \rightarrow PhCH_2CH(NHZ)$-$^{14}C(OMe)_3$[159] while more conventional steps (and eight of them) are involved in the synthesis of the ^{14}C-labelled nitrogen mustard p-$[Cl(^{14}CH_2)_2]_2N$-$C_6H_4CH_2CH(NH_2)CO_2H$ from L-phenylalanine.[160]

Small quantities of labelled N^6-methyl-lysine can be prepared from [*U*-^{14}C]lysine with a *Neurospora crassa* or *Salmonella typhimurium* culture, or in an alternative labelling pattern from reductive methylation of a protein with $^{14}CH_2O$ at pH 9.0, followed by hydrolysis and ion-exchange separation.[161] A new synthesis of ^{14}C-labelled azaserine from *N*-trifluoroacetyl-L-serine benzyl ester and ^{14}C-labelled glycine proceeds *via* esterification of the *Z*-glycine, generation by HNO_2 of the diazoacetate after de-protection of the glycyl moiety, and use of acylase I for enantiospecific deacylation.[162]

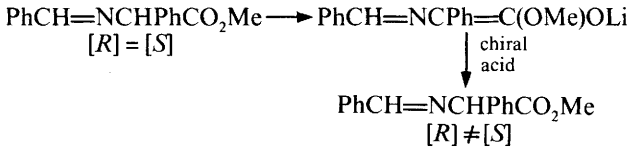
To citations in the preceding paragraphs for halogen isotope-labelled amino-acids can be added the synthesis of ^{18}F-5-fluorodopa, employing known methodology.[147]

Resolution of Amino-acids.—Judging by the scope of the 1978 literature, many hours were spent in many laboratories in the resolution of amino-acids. As usual, most of the procedures are based on well-known physical separations of diastereoisomeric salts. Space is found here for mention of only one paper in this area, because of its special interest; preferential separation from a solution of an *N*-benzyloxycarbonyl-DL-amino-acid and (±)-ephedrine seeded with the

[153] C. Fuganti, O. Ghiringhelli, and P. Grasselli, *Experientia*, 1978, **34**, 297.
[154] R.L. Hayes, L.C. Washburn, B.W. Wieland, T.T. Sun, J.B. Anon, T.A. Butler, and A.P. Callahan, *Internat. J. Appl. Radiat. Isotopes*, 1978, **29**, 186.
[155] B.D. Andersen, *J. Labelled Comp. Radiopharm.*, 1978, **14**, 589.
[156] M.F. Hegazi, R.T. Borchardt, S. Osaki, and R.L. Schowen, *Nucleic Acid Chem.*, 1978, **2**, 889.
[157] G.L. Blackmer and C.-W. Tsai, *J. Organometallic Chem.*, 1978, **155**, C17.
[158] H.F. Kung, S. Gilani, and M. Blau, *J. Nuclear Med.*, 1978, **19**, 393.
[159] C.R. Partington and M.P. Mertes, *J. Labelled Comp. Radiopharm.*, 1978, **14**, 223.
[160] C. Nicolas and D. Godeneche, *J. Labelled Comp. Radiopharm.*, 1978, **14**, 205.
[161] W.K. Paik, P. Dimaria, E. Pearson, and S. Kim, *Analyt. Biochem.*, 1978, **90**, 262.
[162] T.J. Curphey and D.S. Daniel, *J. Org. Chem.*, 1978, **43**, 4666.

desired chiral salt is a process which amounts to the simultaneous resolution of two pairs of racemates.[163]

Enantioselective protonation of the lithium enolate of the Schiff base of a DL-amino-acid methyl ester can be brought about to the extent of 62 % enantiomeric enhancement, when using a di-acyltartaric acid with bulky acyl groups:[164]



Column techniques involving a chiral stationary phase continue to receive attention, particularly the use of polystyrene beads to which an amino-acid residue is covalently attached, capable of complexing copper(II) ions.[165] This technique (co-ordination exchange chromatography), applied to amino-acid resolution, offers much scope for experimental appraisal of factors involved, a process already largely completed by Cram and co-workers in their distantly related work on the resolution of amino-acid ester salts with macrocyclic chiral polyethers.[166]

Enantiomeric discrimination, the principle underlying enzymic 'resolution' of DL-amino-acid derivatives, is also the basis of attempts to account for the ascendance of L-amino-acids after 'chance' synthesis in prebiotic times. Two different approaches using hydantoins as substrates produce interesting contrasts, with the hydrolysis of racemic hydroxybutylhydantoin in aqueous $Ba(OH)_2$ at 121 °C followed by incubation with D-amino-acid oxidase giving a mixture of L-amino-acid and the corresponding α-keto-acid,[167] while incubation of DL-5-(2-methylthioethyl)hydantoin with *Pseudomonas striata* gives *N*-carbamyl-D-methionine.[168] Examples of conventional methods are the use of hog renal acylase in the last stage of the total synthesis of L-2-amino-4-methoxy-*trans*-but-3-enoic acid (from *Pseudomonas aeruginosa*),[73] discriminatory metabolism by *Escherischia coli* of *N*-phenylacetyl-DL-methionine and N^{α}-phenylacetyl-N^{ϵ}-benzoyl-DL-lysine giving the L-amino-acids through cleavage of the N^{α}-acyl group[169] (this hydrolytic process is catalysed by benzylpenicillinacylase,[170] and may be useful in the assignment of absolute configur-

[163] C.-H. Wong and K.-T. Wang, *Tetrahedron Letters*, 1978, 3813.
[164] L. Duhamel and J.C. Plaquevent, *J. Amer. Chem. Soc.*, 1978, **100**, 7415.
[165] D. Muller, M.A. Petit, A. Szubarga, and J. Jozefonovicz, *Compt. rend. Acad. Sci., Ser. C*, 1977, **285**, 531; J. Jozefonovicz, M.A. Petit, and A. Szubarga, *J. Chromatog.*, 1978, **147**, 177; V.A. Davankov, Y.A. Zolotarev, and A.B. Tevlin, *Biorg. Khim.*, 1978, **4**, 1164; I.A. Yamskov, B.B. Berezin, V.E. Tikhonov, L.A. Belchich, and V.A. Davankov, *ibid.*, p. 1170; V.A. Davankov and Y.A. Zolotarev, *J. Chromatog.*, 1978, **155**, 285, 295, 303; I.A. Yamskov, B. Berezin, and V.A. Davankov, *Makromol. Chem.*, 1978, **179**, 2121.
[166] J.M. Timko, R.C. Helgeson, and D.J. Cram, *J. Amer. Chem. Soc.*, 1978, **100**, 2828.
[167] B.A. Kern and R.H. Reitz, *Agric. Biol. Chem.*, 1978, **42**, 1275.
[168] H. Yamada, S. Takahashi, Y. Kii, and H. Kumagai, *J. Ferment. Technol.*, 1978, **56**, 484.
[169] Y. Kameda and Y. Kameda, *Chem. Pharm. Bull.*, 1978, **26**, 2236.
[170] D. Rossi, A. Romeo, and G. Lucente, *J. Org. Chem.*, 1978, **43**, 2576.

ation to α-amino-acids), and the use of immobilized trypsin for the preparation of L-lysine from DL-lysine methyl ester.[171] This enzyme, in the free form, was shown earlier[172] to release D-lysine from DL-lysine ethyl ester; it seems unlikely that the change of ester grouping, or a modified specificity brought about by immobilizing the enzyme, can reverse the enantiospecificity of trypsin, as these results imply, and the lack of evidence for stereochemical purity of the product from the recent study[171] suggests that a re-appraisal is required.

Some theoretical considerations, that enantioselection favouring the survival of L-amino-acids in prebiotic times was due to intrinsic energy differences between enantiomeric configurations, or that elliptically polarized radiation was responsible for preferential destruction of the D-enantiomer,[173] can be linked with recent experimental results (see Volume 10, p. 17 and earlier Volumes). β-Irradiation in itself has been deduced to be insufficiently energetic to discriminate between amino-acid enantiomers[174] and there is experimental support for this[175] in the finding that crystalline ^{14}C-labelled DL-amino-acids do not undergo asymmetric degradation on β-radiolysis, in contrast with accelerated electrons reported earlier. ^{60}Co-γ-Irradiation of enantiomers of leucine, and aqueous solutions of their Na salts, causes degradation, and *ca.* 5 % of the residual amino-acid was racemized when this process was continued to the extent of 68 % degradation.[176] Little or no racemization accompanied γ-radiolysis of other amino-acids, as their hydrochlorides, in aqueous solution.[177] Perhaps the experiment with more promise, along these lines, will be the irradiation of a DL-amino-acid obtained in a crystalline modification involving an asymmetric distribution of paired enantiomers.

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—*X*-Ray crystal analysis of both naturally occurring [L-methionine hydrochloride,[178] L-cystathionine,[179] (2*RS*, 4RS)-2-hydroxy-4-aminopentanoic acid,[180] and lycoperdic acid[181]] and closely related amino-acids [LL- and *meso*-di-aminopimelic acid hydrochlorides,[182] *p*-nitrophenyl-DL-alanine hydrochloride,[183] and 4′-methoxy-3,5,3′-trimethyl-L-thyronine (a halogen-free thyroxine analogue[184])] has been reported. The conformation of the latter was

[171] P. Monsan and G. Durand, *Biochim. Biophys. Acta,* 1978, **523,** 477.
[172] J.E. Purdie, R.E. Demayo, J.H. Seely, and N.L. Benoiton, *Biochim. Biophys. Acta.,* 1972, **268,** 523.
[173] B. Norden, *J. Mol. Evol.,* 1978, **11,** 313.
[174] C. Fajszi and J. Csege, *Origins of Life,* 1977, **8,** 277.
[175] W.A. Bonner, R.M. Lemmon, and H.P. Noyes, *J. Org. Chem.,* 1978, **43,** 522.
[176] W.A. Bonner and R.M. Lemmon, *J. Mol. Evol.,* 1978, **11,** 95.
[177] W.A. Bonner and R.M. Lemmon, *Bio-org. Chem.,* 1978, **7,** 175.
[178] B. De Blasio, V. Pavone, and C. Pedone, *Cryst. Struct. Comm.,* 1977, **6,** 845.
[179] C.S. Chen, T. Srikrishnan, and R. Parthasarathy, *Biochim. Biophys. Acta,* 1978, **538,** 534.
[180] L. Brehm and T. Honore, *Acta Cryst.,* 1978, **B34,** 2359.
[181] J. Lamotte, B. Oleksyn, L. Dupont, O. Dideberg, H. Campsteyn, M. Vermeire, and N. Rmugenda-Banga, *Acta Cryst.,* 1978, **B34,** 3635.
[182] S.E. Hull, O. Kennard, H.J. Rogers, and M.V. Kelemen, *Acta Cryst.,* 1977, **B33,** 3832.
[183] K.K. Chacko, S. Swaminathan, S. Parthasarathy, and S. Natarajan, *Acta Cryst. B,* 1978, **B34,** 1208.
[184] V. Cody, *Science,* 1978, **201,** 1131.

very similar to that of the 3,5,3′-tri-iodo analogue, suggesting that no special role is played by the iodine atoms in sustaining a physiologically significant conformation.

Amino-acid derivatives *N*-acetyl-α-amino-isobutyric acid methylamide,[185] *N*-acetyl-*S*-nitroso-DL-penicillamine,[186] and alaproclate $H_2NCHMeCO_2CMe_2$-CH_2-*p*-Cl-C_6H_4, a potent inhibitor of 5-hydroxytryptamine accumulation in brain,[187] have also been subjected to *X*-ray analysis. Crystal data for 27 *N*-acylamino-acid derivatives reveal the existence of intermolecular hydrogen bonds between the carboxyl proton and the acyl oxygen atom in the free acids, but a variety of hydrogen bonding patterns for corresponding amides, in which intermolecular bonding between the N–H proton of the acylamido-group and the oxygen atom of the amide group is preferred.[188]

N.M.R. Spectrometry.—Much of the work in this area continues along well-established lines of enquiry, with solution conformational studies predominating.

The influence of pH on the solution conformations of leucine, isoleucine, and valine[189] and of arginine and lysine[190] has been assessed through variable temperature ^{1}H-n.m.r. studies. Lysine adopts an intramolecularly hydrogen bonded structure in aqueous solutions;[190] four distinct zwitterionic forms of arginine can be discerned, each at maximum concentration at pH values $\leqslant$ 1, 8, 11—12, and $\geqslant$ 13.[190] Related studies of the protonation of the imidazole moiety of N^{α}-acetylhistidine methylamide have been described[191] (see also Vol. 10, p. 19). Increasing amounts of adenosine-5′-monophosphate cause upfield shifts of imidazole proton resonances of L-histidine, interpreted[192] to indicate interaction of the solutes through stacking involving the heteroaromatic groupings.

Full details (see Vol. 10, p. 19) of ^{1}H-n.m.r. studies of phenylalanine derivatives show their remarkable conformational dependence on solvent polarity.[193]

Structural information with either more detail or greater reliability can usually be obtained by subjecting a sample to scrutiny by more than one physical technique. This may involve the co-operative use of ^{1}H- and ^{13}C-n.m.r., as employed in a study of the *cis–trans* isomerism of acylsarcosine esters,[194] ^{1}H-, ^{13}C-, and ^{15}N-n.m.r. in continuing studies (see Vol. 10, p. 19) of the assessment of the populations of the three staggered C-1–C-2 rotamers in solutions of [4,5-$^2H_2$]-, [1-^{13}C,2,4,5-$^2H_3$]- and [^{15}N, 2,3,4-$^2H_3$*]-labelled leucine,[195] and ^{1}H-n.m.r. and Raman spectroscopic studies, showing that

[185] A. Aubry, J. Protas, G. Boussard, M. Marraud, and J. Neel, *Biopolymers*, 1978, **17**, 1693.
[186] L. Field, R.V. Dilts, R. Ravichandran, P.G. Lenhert, and G.E. Carnahan, *J.C.S. Chem. Comm.*, 1978, 249; G.E. Carnahan, P.G. Lenhert, and R. Ravichandran, *Acta Cryst.*, 1978, **B34**, 2645.
[187] U.H. Lindberg, S.B. Ross, S.-O. Thorberg, and S.-O. Ögren, *Tetrahedron Letters*, 1978, 1779.
[188] C.-S. Chen and R. Parthasarathy, *Internat. J. Peptide Protein Res.*, 1978, **11**, 9.
[189] V.P. Tikhonov and N.A. Kostromina, *Teor. i eksp. Khim.*, 1977, **13**, 496.
[190] V.P. Tikhonov and N.A. Kostromina, *Ukrain khim. Zhur.*, 1978, **44**, 451.
[191] M. Tanokura, M. Tasumi, and T. Miyazawa, *Chem. Letters*, 1978, 739.
[192] H.H. Mautsch and K. Neurohr, *F.E.B.S. Letters*, 1978, **86**, 57.
[193] J. Kobayashi and U. Nagai, *Biopolymers*, 1978, **17**, 2265.
[194] H.R. Kricheldorf and G. Schilling, *Makromol. Chem.*, 1977, **178**, 3115.
[195] A.J. Fischman, H.R. Wyssbrod, W.C. Agosta, and D. Cowburn, *J. Amer. Chem. Soc.*, 1978, **100**, 54.

4-aminobutanoic acid is flexible in aqueous solution, with the three main staggered rotamers equally populated, over a wide range of pH, while 4-amino-3-hydroxybutanoic acid exists in the *trans–trans* and *gauche–trans* rotameric forms predominantly at neutral pH, and in the *trans–trans* conformation at other pH values.[196]

The effects of pH on ^{13}C-n.m.r. chemical shifts and spin–spin coupling constants of [*U*-^{13}C]aspartic and glutamic acids have been assessed.[197]

Cyclic α-imino-acids have provided interesting results to reward the efforts expended in their study by n.m.r. methods. Papers from some leading investigators describe calculations of *exo–endo* ratios and their pH dependence for proline,[198] and similar studies of L-thiazolidine-4-carboxylic acid[199] which, with its enlarged ring size compared with proline, adopts an average planar conformation at pH 6.1 but with *exo* and *endo* puckered conformations predominating at lower pH values. Concentrated aqueous solutions of proline show some of the characteristics of hydrophilic colloids,[200] and ^{1}H-n.m.r. studies show that solute ordering, through a stacking structure sustained by strong hydrogen bonds with solvent, offers a plausible explanation.[200]

^{15}N-N.m.r. studies can provide information concerning interactions involving the α-amino-group, and of nitrogen-containing functional groups in side-chains, through the sensitivity of chemical shift to electronic and conformational changes, as well as through interpretations of ^{15}N–^{13}C and ^{15}N–^{1}H coupling constants.[201] Effects of pH on the proton distribution in arginine[202,203] and complex formation involving this amino-acid with Cl^-, PO_4^{3-}, and ATP,[203] are described in recent papers; Roberts and co-workers used samples containing ^{15}N at natural abundance.[202,203]

Continuing studies of solid amino-acids (proton-selective spin-lattice relaxation rates[204]) and metal-binding properties, of methylmercury compounds to tryptophan,[205] of lanthanide ions to γ-carboxyglutamic acid (binding involves both γ-carboxy groups),[206] and of manganese(II) ions to amino-acids (resulting in selective broadening of proton resonances in order $\alpha > \beta > \gamma$).[207]

Assignment of absolute configuration and enantiomeric purity can be accomplished through n.m.r. study of Eu(fod)$_3$ complexes of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl derivatives of amino-acids.[208]

[196] K. Tanaka, H. Akutsu, Y. Ozaki, Y. Kyogoku, and K. Tomita, *Bull. Chem. Soc. Japan*, 1978, **51**, 2654.
[197] R.E. London, T.E. Walker, V.H. Kollmann, and N.A. Matwiyoff, *J. Amer. Chem. Soc.*, 1978, **100**, 3723.
[198] K. Jankowski, F. Soler, and M. Ellenberger, *J. Mol. Struct.*, 1978, **48**, 63.
[199] F. Piriou, K. Lintner, T.H. Lam, F. Toma, and S. Fermandjian, *Tetrahedron*, 1978, **34**, 553.
[200] B. Schobert and H. Tschesche, *Biochim. Biophys. Acta*, 1978, **541**, 270.
[201] F. Blomberg and H. Rueterjans, *Jerusalem Symp. Quantum Chem. Biochem.*, 1978, **11**, 231.
[202] R.E. London, T.E. Walker, T.W. Whaley, and N.A. Matwiyoff, *Org. Magn. Resonance*, 1977, **9**, 598; I. Yavari and J.D. Roberts, *Biochem. Biophys. Res. Comm.*, 1978, **83**, 635.
[203] K. Kanamori, A.H. Cain, and J.D. Roberts, *J. Amer. Chem. Soc.*, 1978, **100**, 4979.
[204] E.R. Andrew, W.S. Hinshaw, M.G. Hutchins, and R.O.I. Sjöblom, *Mol. Phys.*, 1977, **34**, 1695; N. Niccolai, M.P. Leon de Miles, S.P. Hehir, and W.A. Gibbons, *J. Amer. Chem. Soc.*, 1978, **100**, 6528; U. Pande and R.C. Gupta, *Indian J. Pure Appl. Phys.*, 1977, 15, 777.
[205] P. Svejda, A.H. Maki, and R.R. Anderson, *J. Amer. Chem. Soc.*, 1978, **100**, 7138.
[206] R. Sperling, B.C. Furie, M. Blumenstein, B. Keyt, and B. Furie, *J. Biol. Chem.*, 1978, **253**, 3898.
[207] E. Tiezzi and G. Valensin, *Biofizika*, 1976, **21**, 401.
[208] F. Yasuhara, K. Kabuto, and S. Yamaguchi, *Tetrahedron Letters*, 1978, 4289.

Optical Rotatory Dispersion and Circular Dichroism.—Conventional approaches are reflected in interpretations of sign of longest-wavelength Cotton effect of a chromophoric derivative of an amino-acid with absolute configuration. *N*-Dithioethoxycarbonyl-L-*β*-amino-acids show negative $n \rightarrow \pi^*$ Cotton effects in $CHCl_3$ or benzene solutions,[209] and Schiff bases $Me_2CHCR^1{=}NCHR^2CO_2R^3$ show a c.d. maximum at 240 nm.[210]

Increasing interest in vibrational c.d. studies, involving absorption and Raman scattering, can be discerned through the general literature, and although complexities of peak assignments are fully displayed in studies of L-alanine,[211] some assignments to C–H stretching modes have been possible in this case.

Mass Spectrometry.—An increasing variety of ionization techniques is being assessed through fundamental studies of many compound types, including amino-acids. Chemical ionization (c.i.m.s.) studies of free amino-acids involve additional problems in interpretation since solvated protonated species are formed when the samples are volatilized by rapid heating in NH_3, leading to (4M + 1) ions in some cases.[212] The most recent paper culled from the literature[213] covers c.i.m.s. of arginine hydrochloride using isobutane. Field desorption (f.d.m.s.) studies of free amino-acids (methionine[214] and a representative group of amino-acids[215]) cover continuing scrutiny of origins of fragment ions[214] and effects of emitter temperature on fragment ion intensities.[215] Negative-ion mass spectra using 2—4 eV electrons of 20 free amino-acids have been compared with positive-ion spectra obtained using 6—16 eV electrons.[216] Absolute secondary-ion emission yields have been reported[217] for parent-like secondary ions [$(M + H)^+$ or $(M-H)^-$] and large characteristic fragments produced through this variation of the ion–molecule ionization procedure.[218]

Amino-acid derivatives chosen for their higher volatility are featured in the majority of recent papers. Per-trimethylsilylated amino-acids,[219] triazines,[220] and phenylthiohydantoins derived from amino-acids[221] have not displaced *N*-acyl amino-acid esters as the most commonly used derivatives (*N*-acetylamino-acidmethyl[222] and n-propyl esters,[223] *N*-trifluoroacetyl-amino-acid n-butyl esters,[223] *N*-heptafluorobutyryl-amino-acid n-butyl esters,[223] *N*-acetyl-amino-

[209] T. Yamada, S. Kuwata, and H. Watanabe, *Tetrahedron Letters*, 1978, 1813.
[210] H. Takahashi and H. Otomasu, *Chem. Pharm. Bull.*, 1978, **26**, 466.
[211] M. Diem, P.J. Gotkin, J.M. Kupfer, and L.A. Nafie, *J. Amer. Chem. Soc.*, 1978, **100**, 5644.
[212] J.S. Gaffney, R.C. Pierce, and L. Friedman, *Internat. J. Mass Spectrom. Ion Phys.*, 1977, **25**, 439.
[213] R.J. Cotter, *Analyt. Chem.*, 1979, **51**, 317.
[214] J. van der Greef, N.M.M. Nibbering, H.R. Schulten, and W.D. Lehmann, *Z. Naturforsch. Anorg. Chem. Org. Chem.*, 1978, **33b**, 770.
[215] H.U. Winkler, H.B. Linden, and H.D. Beckley, *Adv. Mass Spectrom.*, 1978, **7A**, 104.
[216] D. Voigt and J. Schmidt, *Biomed. Mass Spectrom.*, 1978, **5**, 44.
[217] A. Benninghoven, D. Jaspers, and W. Sichtermann, *Adv. Mass Spectrom.*, 1978, **7B**, 1433.
[218] A. Benninghoven and W.K. Sichtermann, *Analyt. Chem.*, 1978, **50**, 1180.
[219] K.R. Leimer, R.H. Rice, and C.W. Gehrke, *J.Chromatog.*, 1977, **141**, 355.
[220] K. Maekawa, E. Taniguchi, and E. Kuwano, *Org. Mass Spectrom.*, 1978, **13**, 4.
[221] J.B. Laudenslager and L.P. Theard, *Adv. Mass Spectrom.*, 1978, **7B**, 1388.
[222] J.M.L. Mee and B. Halpern, '4th Int. Symposium on Recent Developments in Mass Spectrometry in Biochemistry and Medicine', ed. A. Frigerio, Plenum, New York, Vol. 1, 1978, p. 291.
[223] P. Padieu, J. Desgres, B.F. Maume, G. Vander Velde, and R.S. Skinner, *Adv. Mass Spectrom.*, 1978, **7B**, 1604.

acid *p*-nitrobenzyl esters,[224] and *N*-benzoyl-amino-acid methyl esters,[225] have been featured), and further examples of amino-acid derivatives employed in analysis are described in the later section dealing with gas – liquid chromatography. Some of these studies involve c.i.m.s.[222,223,225] techniques, and dissociation resonance capture[224] (avoiding extensive fragmentation) and Penning ionization variations.[221] Application of the derivatization approach to the analysis of γ-aminobutyric acid has been demonstrated.[226]

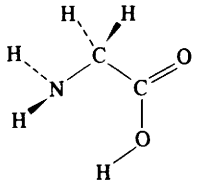
Other Physical and Theoretical Studies.—The major collective headings for papers described in this section, as in previous Volumes, are: spectroscopic studies not covered in preceding sections; classical studies of fundamental physical properties, including those with possible relevance to abiotic synthesis of peptides; and molecular orbital calculations.

Raman spectroscopic studies of tryptophan in neutral aqueous solutions,[227] of solid β-alanine, taurine, and ciliatine,[228] and of L-histidine in aqueous solutions[229] have been reported. The tautomer ratio $N^{\tau}:N^{\pi}$ = *ca.* 4 : 1 for imidazole-protonated L-histidine, from which a difference in stability of *ca.* 1 kcal mol^{-1} has been calculated for these tautomers.[229] Among microwave spectroscopic studies[230—233] are results for glycine interpreted to show that a compact conformation (11) is adopted in the vapour phase.[231—233] This is in disagreement with the conformation (12) deduced from *ab initio* calculations[231,233] (see Volume 10, p. 22) but it has been reasoned[231] that both conformations should exist in the vapour phase, but that (12) should not contribute significantly to the observed spectra. Ordered layers formed through adsorption of amino-acids on copper (100) and copper (101) have been revealed by electron-diffraction.[234] Photoelectron spectroscopy of amino-acids and their methyl esters yields the generalization that the gas-phase electronic structure of the amino-acid can be deduced from results obtained for its ester.[235] Ultrasonic absorption spectra have been reported for aqueous solutions of L-proline and of L-serine.[236]

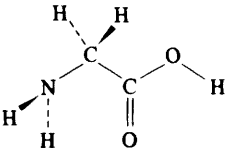
A large number of papers[237] have been published covering solutions (solubility of common amino-acids in water;[237] influence of electrolytes on solubility;[238] free energy of interaction of glycine with LiCl, NaCl, and CsCl in

[224] C.L. Brown and C.L. Chan, *Adv. Mass Spectrom.*, 1978, **7A**, 371.
[225] G. Höfle, G. Höhne, J. Respondek, and H. Schwarz, *Org. Mass Spectrom.*, 1977, **12**, 477.
[226] J.D. Huizinga, A.W. Teelken, F.A.J. Muskiet, H.J. Jeuring, and B.G. Wolthers, *J. Neurochem.*, 1978, **30**, 911.
[227] A.Y. Hirakawa, Y. Nishimura, T. Matsumoto, M. Nakanishi, and M. Tsuboi, *J. Raman Spectroscopy*, 1978, **7**, 282.
[228] C. Garrigou-Lagrange, *Canad. J. Chem.*, 1978, **56**, 663.
[229] I. Ashikawa and K. Itoh, *Chem. Letters*, 1978, 681.
[230] E.J. Croom, R. Shack, J.C.W. Sheppard, and R.J. Sheppard, *J. Microwave Power*, 1977, **12**, 111 (*Chem. Abs.*, 1978, **88**, 70 693).
[231] H.L. Sellers and L. Schafer, *J. Amer. Chem. Soc.*, 1978, **100**, 7728.
[232] R.D. Suenram and F.J. Lovas, *J. Mol. Spectroscopy*, 1978, **72**, 372.
[233] R.D. Brown, P.D. Godfrey, J.W.V. Storey, and M.P. Bassez, *J.C.S. Chem. Comm.*, 1978, 547.
[234] L.L.A. Fanasoska, J.C. Buchholz, and G.A. Somorjai, *Surface Sci.*, 1978, **72**, 189.
[235] L. Klasinc, *Internat. J. Quantum Chem., Quantum Biol.*, 1978, **5**, 373.
[236] N. Inoue, *Japanese J. Appl. Phys.*, 1978, **17**, 1699; *Acustica*, 1978, **40**, 91 (*Chem. Abs.*, 1978, **89**, 163 929).
[237] H.B. Bull, K. Breese, and C.A. Swenson, *Biopolymers*, 1978, **17**, 1091.
[238] A.J.H. Hernandez, *Acta Cient. Venez.*, 1978, **29**, 158.

aqueous solution;[239] dissociation constants for $H_3\overset{+}{N}(CH_2)_nCO_2H$, $n = 1—10$, interpreted in terms of a conformation intermediate between the random-coil and fully-extended states;[240] dissociation constants of α-amino-acids in various media[241]) and thermodynamic properties (heats of sublimation of sarcosine and L-proline;[242] heats of dissociation of proline in different electrolytes;[243] heat capacities of amino-acids in aqueous solutions;[244] heats of solution of optically-active amino-acids in water at 298. 15 K, several kJ mol^{-1} lower than those for corresponding DL-forms, except for threonine;[245] apparent molal heat capacities of amino-acids in aqueous mannitol or glycerol;[246] and apparent molal volumes and adiabatic compressibilities of amino-acids in water at 25 °C[247]). Reverse osmosis separation of amino-acids has been demonstrated, using a cellulose acetate membrane.[248]



(11)



(12)

Adsorption studies range from fundamental properties relevant to laboratory techniques (adsorption of aliphatic amino-acids at a mercury–water interface;[249] adsorption of amino-acids at the electrode inhibits the anodic oxidation of glucose[250]) to uptake on solids [251—253] with possible relevance to abiotic condensation. Glycine is adsorbed weakly by apatite,[251] and aspartic acid (but not tryptophan) is adsorbed on monodispersed chromium hydroxide sol.[252] Aqueous micelles (hexadecyltrimethylammonium hydroxide) are more effective concentrating media at pH 8 for amino-acids than clays.[253] Preferential

[239] B.P. Kelley and T.H. Lilley, *J.C.S. Faraday I*, 1978, **74**, 2771, 2779.
[240] J.T. Edward, P.G. Farrell, J.L. Job, and B.-L. Poh, *Canad. J. Chem.*, 1978, **56**, 1122.
[241] H. Matsui, *Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1977*, **45**, 734 (*Chem. Abs.*, 1978, **88**, 116 547); A.P. Kreshkov, N.S. Aldarova, A.N. Yarovenko, B.B. Tanganov, K.N. Shulunova, T.K. Batorova, and T.E. Batlaeva, *Zhur. fiz. Khim.*, 1978, **52**, 456; O.I. Zinovev and L.F. Pulatova, *ibid.*, p. 631.
[242] R. Sabbah and M. Lafitte, *Bull. Soc. Chim.*, 1978, 50.
[243] V.P. Vasilev, L.A. Kochergina, and T.B. Sokolova, *Zhur. obshchei Khim.*, 1978, **48**, 650.
[244] J.C. Ahluwalia, C. Ostiguy, G. Perron, and J.E. Desnoyers, *Canad. J. Chem.*, 1977, **55**, 3364.
[245] M. Matsumoto and K. Amaya, *Chem. Letters*, 1978, 87.
[246] G. Di Paola and B. Belleau, *Canad. J. Chem.*, 1978, **56**, 1827.
[247] F.J. Millero, A. Lo Surdo, and C. Shin, *J. Phys. Chem.*, 1978, **82**, 704.
[248] O. Tozawa and D. Nomura, *Nippon Kagaku Kaishi*, 1978, 1291.
[249] T. Kakinchi and M. Senda, *J. Electroanal. Chem. Interfacial Electrochem.*, 1978, **88**, 219.
[250] J.R. Rao, G.J. Richter, G. Luft, and F. von Sturm, *Biomaterials, Medical Devices, and Artificial Organs*, 1978, **6**, 127.
[251] C. Rey, J.C. Trombe, and G. Montel, *J. Chem. Research (S)*, 1978, 188.
[252] H. Kumanomido, R.C. Patel, and E. Matijevic, *J. Colloid Interface Sci.*, 1978, **66**, 183.
[253] D.W. Armstrong, R. Seguin, and J.H. Fendler, *J. Mol. Evol.*, 1977, **10**, 241.

adsorption of the L-enantiomer has been established for the DL-α-amino-propionitrile-*d*-quartz system.[254] Direct correlations exist between the hydrophobicity or hydrophilicity of the homocodonic amino-acids and their anticodon nucleotides.[255]

Conformational energy calculations for cystine,[256] glycine,[257] and for other amino-acids[258] continue using established methods[256,257] together with attempts to break new ground.[258] The equilibrium conformation of the glycine zwitterion is planar, stabilized by an intramolecular hydrogen bond, and is 29 kcal mol^{-1} above the energy of the corresponding conformation of the non-zwitterion form. A hydration energy of *ca.* 50 kcal mol^{-1} can be deduced for the gas phase zwitterion.[257]

5 Chemical Studies of Amino-acids

Racemization.—The main areas of interest developed in recent years provide the basis of all papers published in 1978 on this topic. Analytical procedures for assessing accurate enantiomer ratios have been developed to meet the quantitative demands of these studies, and the reader is referred to Section 6 and to the coverage earlier in this Chapter (on preferential degradation of one enantiomer in a racemic mixture) for a complete review.

Interpretation of D–L-ratios for amino-acids present in fossil materials[259] in terms of the age of the samples has been criticized because the ratios may have been influenced by differential leaching of enantiomers over the intervening period. A study[35] of the relationship between γ-carboxyglutamic acid content of fossil bones and their leaching histories suggests a basis for checking this aspect. High D–L-ratios are found for aspartic acid present in water-insoluble protein from cataracts, suggesting a reason for the precipitation.[260] A review has appeared[261] on the more general topic of racemization of amino-acid residues in proteins in relationship to signs of ageing. Variations in D–L-aspartic acid ratios for samples of plants uniformly preserved for *ca.* 11 000 years in an ancient packrat midden have been observed,[262] implying either that complex relationships exist between bound aspartic acid and the lignin–cellulose matrix, or that the neighbouring sequence to the protein-bound aspartic acid residue in these samples controls the racemization rate of that residue.[262] A similar species-dependent racemization rate for isoleucine has been demonstrated for fossil planktonic foraminifera.[263]

Mechanistic studies relevant to metabolic processes have been described for copper(II) catalysed racemization of Schiff bases derived from L-amino-acids

[254] S. Morimoto, K. Kawashiro, and H. Yoshida, *Origins of Life*, 1977, **8**, 355.
[255] A.L. Weber and J.C. Lacey, *J. Mol. Evol.*, 1978, **11**, 199; J.R. Jungck, *ibid.*, p. 211.
[256] L.A. Eslava, J.B. Putnam, and L. Pedersen, *Internat. J. Peptide Protein Res.*, 1978, **11**, 149.
[257] Y.C. Tse, M.D. Newton, S. Vishveshwara, and J.A. Pople, *J. Amer. Chem. Soc.*, 1978, **100**, 4329.
[258] J. Snir, R.A. Nemenoff, and H.A. Scheraga, *J. Phys. Chem.*, 1978, **82**, 2527.
[259] P.M. Masters and J.L. Bada, *Advances in Chemistry Series*, No. 171 (1978), p. 117.
[260] P.M. Masters, J.L. Bada, and J.S. Zigler, *Proc. Nat. Acad. Sci. U.S.A.*, 1978, **75**, 1204.
[261] L. Poplin and R. De Long, *Gerontology*, 1978, **24**, 365.
[262] M.H. Engel, J.E. Zumberge, B. Nagy, and T.R. Van Devender, *Phytochemistry*, 1978, **17**, 1559.
[263] K. King and C. Neville, *Science*, 1977, **195**, 1333.

and salicylaldehyde,[264] pyruvic acid,[265] or pyridoxal.[266] Oxazolone intermediates are plausible sources of the racemization observed for L-proline in trifluoroacetic anhydride[267] or aroyl-L-leucine *p*-chlorophenyl esters in basic media.[268]

The racemization of amino-acid derivatives has been reviewed.[269]

General Reactions.—De-aminative bromination of α-amino-acids using $NaNO_2$–NaBr with retention of configuration in the cases of 3,5-dichloro-L-tyrosine[270a] and L-aspartic acid[270b] is accounted for by neighbouring group participation of the phenoxide and α-carboxy groups, respectively. Inversion of configuration is observed in the corresponding reaction with α-methyl- and αβ-dimethylaspartates.[270b] This stereospecific process is exploited in the conversion of D-leucine into (*R*)-2-bromo-4-methylpentanoic acid for the synthesis of (*S*)-2-(2-aminoethylthio)-4-methylpentanoic acid.[79] There are relatively few unexplored reactions, apparently, of free amino-acids, and routine work covering the decomposition kinetics of crystalline amino-acids,[271] the Maillard reaction (fusion of an amino-acid with glucose[272,273] or a triglyceride[273] at 100—120 °C), and conversion into aldehydes through heating with $KMnO_4$,$K_2Cr_2O_7$ in acid media, or $KBrO_3$ or $KClO_4$ in alkaline media[274] has been described.

Results with amino-acid derivatives include oxidative decarboxylation of *N*-acylamino-acid *p*-nitrophenyl esters with *m*-chloroperbenzoic acid,[275] preparation of *N*-acylamino-acid t-butylthioesters *via* $LiSBu^t$,[276] esterification of *N*-acylamino-acids with MeOH or EtOH with BF_3–Et_2O (amino-acids give methyl esters in 14–82 % yield by this procedure),[277] formation of difluoroboron heterocycles from *N*-alkylamino-acids and BF_3–Et_2O,[278] conversion of *N*-(*o*-nitrophenylsulphenyl)amino-acid amides into corresponding nitriles without racemization using $POCl_3$ and pyridine,[279] and conversion of amino-acid esters into hydroxamic acids.[280] *N*-Acyłamino-acids, after conversion into

[264] M. Ando and S. Emoto, *Bull. Chem. Soc. Japan*, 1978, **51**, 2366; Y.N. Belokon, M.M. Dolgaya, M.B. Saporovskaya, V.I. Tararov, and V.M. Belikov, *J. Mol. Catal.*, 1978, **4**, 289; V.M. Velikov, Y.N. Belokon, V.A. Karginov, N.S. Martinkova, and M.B. Saporovskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 1276.

[265] R.D. Gillard and P. O'Brien, *J.C.S. Dalton*, 1978, 1444.

[266] M.-D. Tsai, H.J.R. Weintraub, S.R. Byrn, C.-J. Chang, and H.G. Floss, *Biochemistry*, 1978, **17**, 3183.

[267] I. Tomida and T. Kuwahara, *Agric. Biol. Chem.*, 1978, **42**, 1059.

[268] J.P. Morawiec and I.Z. Siemion, *Roczniki Chem.*, 1977, **51**, 1867.

[269] M. Lebl and K. Jost, *Chem. listy*, 1978, **72**, 252.

[270] (*a*) K. Koga, M.J. Tzuoh, and S. Yamada, *Chem. Pharm. Bull.*, 1978, **26**, 178; (*b*) Y. Murakami, K. Koga, and S. Yamada, *ibid.*, p. 307.

[271] G.W.C. Hung, *Thermochim. Acta*, 1978, **23**, 233.

[272] F. Orsi and E. Dworschak, *Acta Ailment. Acad. Sci. Hung.*, 1978, **7**, 41.

[273] S. Saito and K. Shirai, *Nippon Daigaku Nojuigakubu Gakujutsu Kenkyu Hokoku*, 1978, **35**, 186 (*Chem. Abs.*, 1978, **89**, 24 752).

[274] L. Lipparini and M.R. Cavana, *Rass. Chim.*, 1978, **30**, 19 (*Chem. Abs.*, 1978, **89**, 161 856).

[275] G. Lucente, F. Pinnen, and G. Zanotti, *Tetrahedron Letters*, 1978, 3155.

[276] U. Schmidt and F. Steindl, *Synthesis*, 1978, 544.

[277] T. Yamada, N. Isono, A. Inui, T. Miyazawa, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Japan*, 1978, **51**, 1897.

[278] J. Halstrom, E. Nebelin, and E.J. Pedersen, *J. Chem. Research (S)*, 1978, 80.

[279] K. Kawashiro, H. Yoshida, and S. Morimoto, *Bull. Chem. Soc. Japan*, 1977, **50**, 2956.

[280] H. Hjeds and T. Honore, *Acta Chem. Scand.*, 1978, **B32** 187.

oxazol-5-ones, can be alkylated to give α-alkyl analogues $R^1CONHCR^2R^3$-CO_2H, after hydrolysis: $Pb(OAc)_4$ oxidation causes loss of the acylamido and carboxy groups, yielding ketones R^2COR^3.[281] This new ketone synthesis portrays amino-acids as carbonyl synthons. *N*-Thiobenzoylamino-acids give 5-(2′-benzamidoacyloxy)-2-phenylthiazoles, and corresponding amides give 5-trifluoroacetamido analogues, with trifluoroacetic anhydride;[282] these reactions involve *S*-trifluoroacetyl intermediates, since corresponding esters are converted into benzamido-analogues.

1,3-Dipolar reactivity of amino-acid Schiff bases (see Vol. 10, p. 27) has been illustrated further, in reactions with acrylonitriles to give pyrrolidines,[283] and in reactions of pyridoxal derivatives of possible metabolic significance.[284]

The chemical basis of colorimetric analysis procedures for amino-acids has been studied. The formation of 1-alkyl-and aryl-thio-2-alkylisoindoles from *o*-phthalaldehyde, a thiol, and an amino-acid[285,286] proceeds at maximum rate at pH 9.7—10,[287] but for amino-acid esters the maximum rate is at pH 5—6. This permits the quantitative analysis of mixtures containing amino-acids and their esters.[288] The *o*-phthalaldehyde procedure is ten times more sensitive than the ninhydrin reaction,[287] but the latter can be rendered 2—3 times more sensitive by the addition of Fe^{II}, Mn^{II}, or Mo^{II} salts, bringing the lower detection limit down to 3 nmol.[289] The basis of the colour reaction between amino-acids and *o*-acetylbenzophenone,[290] and that of hydroxyproline, chloramine T, and 4-dimethylaminbenzaldehyde,[291] has been assessed.

Specific Reactions of Natural Amino-acids.—A group of papers which have the common feature of reporting reactions of side-chain functional groups in natural amino-acids is collected in this section. Conversion of *N*-trityl-*O*-toluene-*p*-sulphonyl-L-serine benzyl ester into the L-aziridine-2-carboxylate,[292] decarboxylation of kainic acid (the C-4 epimer of the product shown in Scheme 1) with sodium periodate,[293] the relative stability of 4-hydroxyproline towards HNO_2 or chloramine-T, compared with the 3-hydroxy-isomer,[294] formation of homolanthionine, homocysteic acid, and homolanthionine sulphone from DL-homocystine and H_2O_2 in aqueous HCl,[295] EDTA-stimulation of Cu^{2+}-catalysed autoxidation of cysteine,[296] oxidation of methionine derivatives to sulphoxides by H_2O_2, $NaIO_4$, $NaBrO_3$, *N*-chlorosuccinimide, or chloramine T,

[281] R. Lohmar and W. Steglich, *Angew. Chem.*, 1978, **90**, 493; *Angew. Chem. Internat. Edn.*, 1978, **177**, 450; W. Steglich, *Chimia*, 1978, **32**, 394.
[282] G.C. Barrett, *Tetrahedron*, 1978, **34**, 611.
[283] M. Joucla and J. Hamelin, *Tetrahedron Letters*, 1978, 2885.
[284] R. Grigg and J. Kemp, *Tetrahedron Letters*, 1978, 2823.
[285] S.S. Simons and D.F. Johnson, *J. Org. Chem.*, 1978, **43**, 2886.
[286] S.S. Simons, *Analyt. Biochem.*, 1978, **90**, 705.
[287] V. Svedas, I.Y. Galaev, and I.V. Berezin, *Biorg. Khim.*, 1978, **4**, 19.
[288] V. Svedas, I.Y. Galaev, and I.V. Berezin, *Biorg. Khim.*, 1978, **4**, 130.
[289] J.V. Singh, S.K. Khanna, and G.B. Singh, *Analyt. Biochem.*, 1978, **85**, 581.
[290] S. Nanya and E. Maekawa, *Nippon Kaguku Kaishi*, 1977, 1750 (*Chem Abs.*, 1979, **90**, 105 587).
[291] U. Pindur, *Z. Lebensm.-Untersuch.*, 1978, **166**, 148.
[292] K. Nakajima, F. Takai, T. Tanaka, and K. Okawa, *Bull. Chem. Soc. Japan*, 1978, **51**, 1577.
[293] R.D. Allan, *Tetrahedron Letters*, 1978, 2199.
[294] G. Szymanovicz, O. Mercier, A. Randoux, and J.P. Borel, *Biochimie*, 1978, **60**, 499.
[295] S.H. Lipton, *J. Agric. Food Chem.*, 1978, **26**, 1406.
[296] A. Hanaki and H. Kamida, *Chem. Pharm. Bull.*, 1978, **26**, 325.

and removal of unwanted sulphone by crystallization,[297] oxidation of methionine with iodine to yield products derived from an intermediate cyclic sulphimine,[298] formation of 3-chlorotyrosine through the hydrolysis in air of glycoproteins by hot hydrochloric acid,[299] formation of 3-phenyl- and 2-amino-5-phenylpyridines by pyrolysis of phenylalanine,[300] conversion of histidine into imidazoleacetic acid and imidazole-lactic acid by peroxides and hydroperoxides derived from methyl linoleate,[301] oxidative processes with tryptophan (Cu_2Cl_2–py–O_2 causes indole cleavage;[302] photo-oxygenation yields the intramolecular addition product involving the amino and indole groups when sensitized by Rose Bengal,[303] then N^{ind}-formylkynurenine;[304] ozonolysis in methanol also yields the latter product *via* the N^{ind}-methoxyhydroperoxide[305]); formation of tryptathionine from tryptophan and cystine under conditions normally used to hydrolyse proteins (6M-HCl) and its subsequent conversion into cysteine and β-(3-oxoindolyl)alanine by hydrolysis;[306] similar involvement of C-2 of the indole moiety of tryptophan in its condensation with 4-dimethylaminobenzaldehyde to give 1 : 1- and 2 : 1-adducts;[307] in the substitution of *N*-acetyltryptophan methyl ester by 2′,3′-*O*-isopropylidene-5-bromouridine through acetone-sensitized irradiation,[308] and in the reduction of L-tryptophan derivatives with BH_3-py to 2,3-dihydro-derivatives;[309] and conversion of tryptophan into indoleacetamide *via* an electronically-excited intermediate, judging by the accompanying luminescence and formation of pyridoxoic acid, through reaction with pyridoxal phosphate, and horseradish peroxidase, in the presence of manganese(II) ions.[310]

Specific Reactions of Amino-acids Related to Biochemical Processes.—While some of the papers in preceding sections would be equally appropriate here, two papers have been selected to represent the continuing interest in model reactions and studies of properties. Duplex formation involving tryptophan methyl ester and oligodeoxyribonucleotides, studied by c.d. and fluorescence methods, does not follow an intercalation mechanism.[311] In Zn^{2+}-mediated

[297] N. Fujii, T. Sasaki, S. Funakoshi, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, 1978, **26**, 650.
[298] P.R. Young and L.-S. Hsieh, *J. Amer. Chem. Soc.*, 1978, **100**, 7121.
[299] M. Havlikova, P. Smolek, G. Entlicher, and J. Kocourek, *J. Chromatog.*, 1978, **154**, 336.
[300] K. Tsuji, T. Yamamoto, H. Zenda, and T. Kosuge, *Yakugaku Zasshi*, 1978, **98**, 910 (*Chem. Abs.*, 1978, **89**, 163 925).
[301] S.H. Yong and M. Karel, *J. Amer. Oil Chemists' Soc.*, 1978, **55**, 352.
[302] E. Balogh-Hergovich and G. Speier, *Magyar Kém. Folyóirat*, 1977, **83**, 188.
[303] M. Nakagawa and T. Hino, *Heterocycles*, 1977, **6**, 1675.
[304] M. Nakagawa, H. Watanabe, S. Kodato, H. Okajima, T. Hino, J.L. Flippen, and B. Witkop, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 4730; J.P. McCormick and T. Thomason, *J. Amer. Chem. Soc.*, 1978, **100**, 312.
[305] F. Sakiyama, N. Masuda, T. Nakazawa, and Y. Katsuragi, *Chem. Letters*, 1978, 893.
[306] T. Ohta and T. Nakai, *Biochim. Biophys. Acta*, 1978, **533**, 440.
[307] U. Pindur, *Arch. Pharm.*, 1978, **311**, 615.
[308] I. Saito, S. Ito, and T. Matsuura, *J. Amer. Chem. Soc.*, 1978, **100**, 2901.
[309] Y. Kikugawa, *J. Chem. Research (S)*, 1978, 184.
[310] O. Augusto and G. Cilento, *Biochem. Biophys. Res. Comm.*, 1977, **79**, 1238.
[311] N.G. Dolinnaya, E.S. Gromova, A.P. Razzhirin, Z.A. Shabarova, and M.A. Prokofev, *Doklady Akad. Nauk S.S.S.R.*, 1978, **239**, 603.

ternary complex formation involving ATP and aromatic amino-acids, an order Trp > Tyr > Phe has been established[312] for complex-forming efficiency.

Effects of Electromagnetic Radiation on Amino-acids.—An increasing number of papers deserves discussion, but again as in previous volumes, little more than a brief summary is possible in the space available.

The two major topic groupings are degradation of amino-acids and their derivatives under short wavelength irradiation, and electronic excitation at longer wavelengths. In the former category, γ-radiolysis of crystalline amino-acids,[313] *N*-acetyl-L-valine,[314] and solutions of sulphur-containing amino-acids[315] and e.s.r.-monitoring to assess radical-pair formation,[313] corresponding *X*-irradiation studies of crystalline hydrochlorides of L-phenylalanine and L-tryptophan,[316] and irradiation of L-tryptophan hydrochloride[317] and other amino-acids and peptides[318] with high-energy electrons, have been described. A common feature of the *X*-ray and electron-irradiation studies[316—318] is the formation of a C-2 radical by deamination, but additional radicals formed by H-abstraction and addition processes are also observed. There is a continuing interest in enantiomeric discrimination possibilities under irradiation[173—177] and further discussion of the consequences of positron-irradiation of chiral molecules has been published.[319] The category in which this type of study belongs also embraces an interesting project,[320] in which the selective photo-destruction of amino-acids has been assessed. Thirty five amino-acids have been found, so far, in extracts from the Murchison meteorite, and many more are believed to be present;[320] of the various types present, α-amino-acids are selectively destroyed as their copper complexes at 254 nm (order of stability $\alpha > \beta > \gamma = \delta = \varepsilon = 0$), while for dicarboxylic amino-acids, $\alpha > \beta \approx \gamma$. Scope for speculation that the meteorite may have originally contained a much higher α-amino-acid content arises from the finding[320] that when all the α-amino-acids in a mixture have been photodegraded, *ca.* 80% of the initial concentrations of β- and γ-amino-acids remains.

One paper,[321] describing the formation of tyrosine by the hydroxylation of phenylalanine by hydroxide radicals generated by photoreduction of anthraquinone-sulphonate, is located in this section, while others on photochemistry of amino-acids can be found in the preceding section. Amino-acid fluorescence studies described in the 1978 literature almost exclusively concern tryptophan and its derivatives,[322—328] dealing with the pH-dependence of the fluorescence intensity of the tryptophan–histidine charge-transfer complex,[322] fluorescence

[312] J.J. Toulme, *Bio-inorg. Chem.*, 1978, **8**, 319.
[313] T. Matsuyama and H. Yamaoka, *J. Phys. Soc. Japan*, 1978, **45**, 717.
[314] G.H. Schnepel, *Radiat. Res.*, 1978, **74**, 240.
[315] T. Oku, *Nippon Daigaku Nojnigakubu Gakujutsu Kenkyu Hokoku*, 1978, **35**, 163 (*Chem. Abs.*, 1978, **89**, 24 748); p. 181 (*Chem. Abs.*, 1978, **89**, 24 749).
[316] W. Flossmann and E. Westhof, *Radiat. Res.*, 1978, **73**, 75; *Internat. J. Radiat. Biol., and Related Studies in Phys., Chem., Med.*, 1978, **33**, 139.
[317] E. Sagstuen, H.G. Byrkjeland, and T. Henriksen, *Radiat. Res.*, 1978, **74**, 10.
[318] M.D. Sevilla, J.B. D'Arcy, and D. Suryanarayana, *J. Phys. Chem.*, 1978, **82**, 2589.
[319] Y.-C. Jean and H.J. Ache, *J. Phys. Chem.*, 1977, **81**, 1157.
[320] N. Levi and J.G. Lawless, *Analyt. Biochem.* 1978, **90**, 796.
[321] S.I. Skuratova and G.V. Fomin, *Zhur. fiz. Khim.*, 1978, **52**, 2971.
[322] S.V. Koner, V.P. Khrapovitskii, and S.N. Cherenkevich, *Zhur. priklad. Spektrosk.*, 1978, **29**, 37.

enhancement accompanying rapid transfer of tryptophan from ^1H_2O to ^2H_2O,[323] and more routine studies[324—326] of the amino-acid itself, including a detailed study of emission from the excited triplet state.[326] N^{α}-Acetyltryptophanamide fluorescence[327] is reduced by the addition of Eu-$(NO_3)_2$.[328] Fluorescence attributable to the tyrosinate anion, previously observed only at pH values above 10.3, can be discerned at neutral pH in concentrated buffer solutions.[329]

6 Analytical Methods

Reviews of sub-nanomole amino-acid analysis[330] and appropriate internal standards for ion-exchange analysis[331] form parts of a recent serial issue. Separations of 'uncommon' amino-acids have been reviewed.[332] The second edition of a standard orienting text has appeared.[333]

Gas–Liquid Chromatography.—There is some overlap with the material discussed in an earlier section dealing with the mass spectrometry of amino-acids. The largest body of literature dedicated to this section concerns the quantitative analysis of amino-acid mixtures, but the use of g.l.c. to identify derivatives of amino-acid residues cleaved from peptides through sequence analysis is also covered.

The conversion of amino-acids into volatile derivatives continues to offer problems of choice of method rather than inherent practical difficulties. Recent papers describe the development of established derivatization methods and routine extensions of them; *N*-trifluoroacetylamino-acid n-butyl esters have featured in several studies,[334—338] some of which deal with particular amino-acids (glutamic acid and glutamine;[335] 4-nitrohistidine[336]), *N*-heptafluorobutyroylamino-acid iso-amyl esters[338] and their isobutyl,[339,340] butyl,[341] or propyl[340] analogues providing alternative types of volatile derivatives. Bis(pentafluoropropionyl) derivatives of histidine and 1,4-dimethylhistidine

[323] M. Nakanishi and M. Tsuboi, *Chem. Phys. Letters*, 1978, **57**, 262.
[324] L.C. Pereira, I.C. Ferreira, M.P.F. Thomaz, and M.I.B.M. Jorge, *J. Photochem.*, 1978, **9**, 425.
[325] D.M. Rayner and A.G. Szabo, *Canad. J. Chem.*, 1978, **56**, 743.
[326] A.L. Kwiram, J.B.A. Ross, and D.A. Deranleau, *Chem. Phys. Letters*, 1978, **54**, 506.
[327] V.L. Zima, A.I. Dragan, and P.G. Bogach, *Dopovidi Akad. Nauk Ukrain R.S.R., Ser. B*, 1978, 1016 (*Chem. Abs.*, 1979, **90**, 68 039).
[328] J. Bello and H.R. Bello, *Internat. J. Peptide Protein Res.*, 1978, **11**, 91.
[329] D.M. Rayner, D.T. Krajcarski, and A.G. Szabo, *Canad. J. Chem.*, 1978, **56**, 1238.
[330] P.E. Hare, *Methods in Enzymol.*, 1977, **47**, 3.
[331] R.W. Geise, *Methods in Enzymol.*, 1977, **47**, 31.
[332] M. Horakova and Z. Deyl, *J. Chromatog.*, 1978, **159**, 227.
[333] 'Amino-acid Determination: Methods and Techniques', ed. S. Blackburn, Dekker, New York, 1978.
[334] S.V. Vitt, E.A. Paskonova, and V.M. Belikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 2716.
[335] F.S. Collins and G.K. Summer, *J. Chromatog.*, 1978, **145**, 456.
[336] E. Giralt, M D. Ludevid, M. Fort, and J.L. Parra, *J. Chromatog.*, 1978, **151**, 228.
[337] N. Yoshida, T. Yoneda, and Y. Ishihara, *Hokkaido Daigaku Suisan Gakubu Kenkyu Iho*, 1977, **28**, 165.
[338] T. Hisanaga, *GC–MS News*, 1978, **6**, 44 (*Chem. Abs.*, 1978, **89**, 102 886).
[339] P. Felker, *J. Chromatog.*, 1978, **153**, 259.
[340] D.J. de Lange, C.M. Potgieter, and Z.J. Bosch, *S. Afr. J. Dairy Technol.*, 1977, **9**, 81.
[341] C.F. Poole and M. Verzele, *J. Chromatog.*, 1978, **150**, 439.

have proved suitable for g.l.c.–m.s. purposes.[342] γ-Aminobutyric acid can be determined at picomole levels in tissue samples by g.l.c.–m.s. of its *N*-dinitrophenyl ethyl ester.[343] The use of 2,2-bis(chlorodifluoromethyl) 1,3-oxazolidinones, formed from amino-acids by reaction with 1,3-dichlorotetrafluoroacetone and pyridine followed by cyclization with trifluoroacetic anhydride, has been advocated further (see Vol. 10, p. 20), this time for use with glutamic and aspartic acids and their side-chain amides.[344] Homologous *N*-carboxyalkanoyl amino-acid methyl esters,[345] and *NN*-dimethylamino-acid methyl or butyl esters (prepared from the esters by treatment with HCHO and sodium cyanoborohydride),[346] are new variations of conventional structural types. Trimethylsilyl derivatives[347—349] continue to be used for g.l.c.–m.s. identification and quantitative analysis of amino-acids.

Automation of g.l.c. procedures for amino-acid analysis has been explored further.[350]

Acylation with *N*-trifluoroacetyl-L-prolyl chloride (see also Vol. 10, p. 28) yields a diastereoisomer mixture with partly-racemized amino-acids, and this general approach forms the basis of enantiomer analysis of amino-acids in fossils.[351]

Analysis at picomole levels can be achieved by g.l.c. (electron capture detection) for pentafluorophenylthiohydantoins produced by modified Edman degradation of peptides.[352]

Ion-exchange Chromatography.—The substantial literature covering the instrumental aspect of amino-acid analysis cannot be covered fully here, but topic areas are represented by citations for automated analysers[353—356] (inexpensive[353] or otherwise), mathematical modelling aimed at optimized operating conditions,[357] factors determining the effectiveness of ion-exchange resins used in amino-acid chromatography,[358] assessment of a short programme analysis procedure[359] and a single column procedure using stepwise elution[360] and a study of the use of titanous chloride as a reducing agent for ninhydrin, aimed at improving the precision of the colorimetric stage in amino-acid analysis.[361]

[342] M. Nahy and E. Gelpi, *Chromatographia*, 1978, **11**, 573.
[343] B.N. Colby and M.W. McCaman, *Biomed.Mass Spectrom.*, 1978, **5**, 215.
[344] P. Husek and V. Felt, *J. Chromatog.*, 1978, **152**, 363, 546.
[345] N. Gregersen, I. Gron, K. Rasmussen, and S. Kolvraa, *Biomed. Mass Spectrom.*, 1978, **5**, 80.
[346] T. Severin and H. Popp-Ginsbach, *J. Chromatog.*, 1978, **147**, 473.
[347] M. Takimoto, T. Takeda, S. Takahashi, and T. Murata, *Shimadzu Hyoron*, 1977, **34**, 159 (*Chem. Abs.*, 1978, **89**, 163 926).
[348] N. Heki, *Nippon Naibumpi Gakkai Zasshi*, 1978, **54**, 1157.
[349] S. Yamada, *GC–MS News*, 1978, **6**, 76 (*Chem. Abs.*, 1979, **90**, 18 417).
[350] B.M. Nair, *J. Chromatog.*, 1978, **155**, 249.
[351] E.A. Hoopes, E.T. Peltzer, and J.L. Bada, *J. Chromatog. Sci.*, 1978, **16**, 556.
[352] B.H. Robertson, R.M. Wolcutt, and J.C. Bennett, *Proc. Soc. Exp. Biol. Med.*, 1977, **155**, 287.
[353] G.R. Beecher, *Adv. Exp. Med. Biol.*, 1978, **105**, 827.
[354] V.R. Villanneva and R.C. Adlakha, *Analyt. Biochem.*, 1978, **91**, 264.
[355] R.W. Welch, *J. Chromatog.*, 1978, **152**, 528.
[356] C.St. Pierre and R. Paquin, *J. Chromatog.*, 1978, **156**, 79.
[357] V.G. Maltsev, B.G. Belenkii, and M.L. Aleksandrov, *Zhur. analit. Khim.*, 1978, **33**, 798.
[358] J. Rahm, *Chem. listy*, 1978, **72**, 381.
[359] K. Olek, S. Uhlhaas, and P. Wardenbach, *J. Clin. Chem. Clin. Biochem.*, 1978, **16**, 283.
[360] S. Thoenes, B. Furch, and R. Tutschek, *J. Chromatog.*, 1978, **153**, 536.
[361] L.B. James, *J. Chromatog.*, 1978, **152**, 298.

Precautions to be taken to obtain reliable analysis of arginine, using the amino-acid analyser, have been described.[362] The behaviour in the amino-acid analyser of side-chain *N*-methyl arginines,[363] γ-carboxyglutamic acid (present to the extent of 9.6 % in prothrombin),[364] cystine (lower detection limit 1 nmol),[365] common aromatic amino-acids, together with 3-iodo-, 3,5-di-iodo-, 3,5-dibromo-3-nitro, 3,5-dinitro-, and 3-amino-tyrosines and 4-nitrophenylalanine,[366] and αε-di-aminopimelic acid,[367] has been reported. The separation of the last-named amino-acid from a mixture by anion-exchange chromatography presents no difficulties.[368] β-Alanine and β-aminoisobutyric acid assays based on ion-exchange separation have been developed with special relevance to the analysis of physiological fluids.[369]

The amino-acid analyser equivalent of the enantiomeric analysis of a partly-racemic amino-acid by g.l.c.[351] is similarly based on diastereoisomeric peptide formation; coupling with Boc-L-leucine *N*-hydroxysuccinimide ester (a procedure involving no detectable racemization, *i.e.* < 0.1 %) followed by de-protection gives a DL–LL peptide pair, which through quantitative analysis provides an accurate D–L-assay (less than one part of D-amino-acid in the presence of 1000 parts of the L-enantiomer can be detected).[370]

Further development of the chromatofuge principle, in which centrifugal force replaces hydraulic pressure, has been reported for preparative ion-exchange separation of amino-acids.[371]

Thin-layer and Paper Chromatography.—Papers of a routine nature have been excluded, which amounts to the majority of published work on this well-established technique. Analytical separations of amino-acids by t.l.c. (leucine from isoleucine,[372] ^{125}I-labelled thyroxine, tri-iodothyronine, and iodide ion[373]), by concurrent two-dimensional electrophoresis and paper- or t.l.c.,[374] and by ascending ion-exchange paper chromatography on carboxymethylcellulose (for N^{τ}-methylhistidine)[375] have been reported. Colour reactions of particular importance are *o*-phthalaldehyde and ninhydrin, of which the former is preferable for the visualization of ^{14}C-labelled amino-acids on t.l.c. since it does not cause loss of carbon.[376] Tryptophan can be detected on t.l.c. at 40–850 pmol levels through the yellow-orange fluorescence caused by spraying with 70 % $HClO_4$ before [377] or after[378] a fluorescamine spray.

[362] J.B. Lewis, *J. Chromatog.*, 1978, **166,** 333.
[363] C.G. Zarkadas, *Canad. J. Biochem.*, 1978, **56,** 952.
[364] F. Yamagata, H. Kasai, and T. Okuyama, *Bunseki Kagaku,* 1977, **26,** 819 (*Chem. Abs.*, 1978, **88,** 85 398).
[365] D. Allard, P. Parvy, J.C. Castets, Y. Huang, and P. Kamoun, *Ann. Clin. Biochem.*, 1978, **15,** 161.
[366] K. Ohtsuki and H. Hatano, *J. Chromatog.*, 1978, **148,** 536.
[367] J. Mzik, J.P. Hogan, J.R. Lindsay, and P. Davis, *J. Chromatog.*, 1978, **152,** 269.
[368] U. Chetal, U.R. Mehra, B.P. Singh, and Y.R. Saxena, *Sep. Sci. Technol.*, 1978, **13,** 831.
[369] K.C. Kuo, T.F. Cole, C.W. Gehrke, T.P. Waalkes, and E. Borek, *Clin. Chem.*, 1978, **24,** 1373.
[370] A.R. Mitchell, S.B.H. Kent, I.C. Chu, and R.B. Merrifield, *Analyt. Chem.*, 1978, **50,** 637.
[371] J.W. Finley, J.M. Krochta, and E. Heftmann, *J. Chromatog.*, 1978, **157,** 435.
[372] E.V. Degterev and G.P. Podgornova, *Khim.-Farm. Zhur.*, 1978, **12,** 133.
[373] M. Dobias, J. Mucha, and P. Talan, *Radiochem. Radioanayt. Letters,* 1978, **33,** 179.
[374] R.L. Munier and S. Meunier, *Chromatographia,* 1978, **11,** 508.
[375] N. Nishizawa, T. Noguchi, S. Hareyama, and R. Funabiki, *J. Chromatog.*, 1978, **151,** 424.
[376] H.M. Davies and B.J. Miflin, *J. Chromatog.*, 1978, **153,** 284.
[377] H. Nakamura and J.J. Pisano, *J. Chromatog.*, 1978, **152,** 167.
[378] H. Nakamura and J.J. Pisano, *J. Chromatog.*, 1978, **152,** 153.

Dansylamino-acids detected[379—381] at very low levels[379,390] (< 1 pmol in nanolitre samples of solutions using the ^{14}C variation[379]), and procedures for the analysis of PTH's[382] and diphenylindenonylthiohydantoins,[383] illustrate development of techniques for the identification of amino-acid derivatives.

High Performance Liquid Chromatography.—Although much more recent in its origins, h.p.l.c., like t.l.c., has spawned a routine literature, some of which is included in the present survey. Sophisticated procedures, such as the automated procedure employing *o*-phthalaldehyde colorimetry for the analysis of γ-aminobutyric acid in cerebrospinal fluid,[384] have been developed for the support of particularly active areas of research, but off-the-shelf equipment is used more generally, with *o*-phthalaldehyde detection for dopa (to 4.6 ng levels),[385] and 5-hydroxytryptophan,[386] and with electrochemical detection for cysteine and homocysteine[387] and for dopa and *S*-cysteinyl-dopa.[388] Cation exchange separation of tryptophan and its metabolites[389] and separation of thyroidal amino-acids[390] have been described, and h.p.l.c. determination of valine as its dansyl derivative (down to 100 fmol levels) has been reported in a new Journal devoted to this technique.[391]

H.p.l.c. analysis of PTH's is being studied in many laboratories,[392—395] some points of interest arising in the lower detection limit set at 10 pmol,[393] and in the possibility for a short programme (20 min).[394] Effects of pH and eluent composition have been determined for the liquid chromatographic separation of PTH's on a stationary phase carrying L-valyl-L-valyl-L-proline linked through its carboxy group.[395] H.p.l.c. effects the separation of dimethyl-

[379] M. Weise and D.E. Oken, *J. Chromatog.*, 1978, **152**, 175.
[380] D. Kocmierska-Grodzka, A. Mazanowska, and G.B. Gerber, *Pol. J. Pharmacol. Pharm.*, 1978, **30**, 17.
[381] R.M. Metrione, *J. Chromatog.*, 1978, **154**, 247.
[382] R.L. Munier and A.M. Drapier, *Compt. rend.*, 1978, **286**, 433. E. Soczewinski, J. Iskierbo, and B. Kanadys, *Chem. Anal.*, 1978, **23**, 533 (*Chem. Abs.*, 1979, **90**, 2531).
[383] I. Mancheva and Y. Vladovska-Yukhnovska, *J. Chromatog.*, 1978, **151**, 207.
[384] P. Boehlen, P.J. Schechter, W. Van Damme, G. Coquillat, J.C. Dorsch, and J. Koch-Weser, *Clin. Chem.*, 1978, **24**, 256.
[385] P.M. Froehlich and T.D. Cunningham, *Analyt. Chim. Acta*, 1978, **97**, 357.
[386] F. Engbaek and I. Magnussen, *Clin. Chem.*, 1978, **24**, 376.
[387] R. Saetre and D.L. Rabenstein, *Analyt. Biochem.*, 1978, **90**, 684.
[388] C. Hansson, L.E. Edholm, G. Agrup, H. Rorsman, A.M. Rosengren, and E. Rosengren, *Clin. Chim. Acta*, 1978, **88**, 419.
[389] A. Saito, K. Maeda, K. Kobayashi, F. Yazawa, and Y. Yamamoto, *Igaku No Ayumi*, 1978, **106**, 37 (*Chem. Abs.*, 1978, **89**, 142 736).
[390] M.T.W. Hearn, W.S. Hancock, and C.A. Bishop, *J. Chromatog.*, 1978, **157**, 337.
[391] R. Bongiovanni and W. Dutton, *J. Liq. Chromatog.*, 1978, **1**, 617.
[392] A. Devillers-Thiery and G. Blobel, *Analyt. Biochem.*, 1978, **87**, 66; C.L. Zimmermann and J.J. Pisano, *Methods in Enzymol.*, 1977, **47**, 45; A.S. Bhown, J.E. Mole, W.L. Hollaway, and J.C. Bennett, *J. Chromatog.*, 1978, **156**, 35; R. Zeeuws and A.D. Strosberg, *F.E.B.S. Letters*, 1978, **85**, 68; J. Elion, M. Downing, and K. Mann, *J. Chromatog.*, 1978, **155**, 436; J. Van Beeumen, J. Van Damme, and J. De Ley, *F.E.B.S. Letters*, 1978, **93**, 373; M. Abrahamsson, K. Groningsson, and S. Castensson, *J. Chromatog.*, 1978, **154**, 313; D.J.P. Raulais, P.C. Rivaille, B. Daguilhanes, G.J.G. Lefevre, and G. Milhaud, *J. Chem. Research (S)*, 1978, 11; G. Oestvold, E. Jensen, and T. Greibrokk, *Medd. Nor. Farm. Selsk.*, 1978, **40**, 173.
[393] M.N. Margolies and A. Brauer, *J. Chromatog.*, 1978, **148**, 429.
[394] A.S. Bhown, J.E. Mole, A. Weissinger, and J.C. Bennett, *J. Chromatog.*, 1978, **148**, 532.
[395] G.W.K. Fong and E. Grushka, *Analyt. Chem.*, 1978, **50**, 1154.

aminoazobenzenesulphonyl amino-acids,[396] and resolves (–)-2-methoxy-α-methyl-1-naphthaleneacetyl-DL-amino-acid ester pairs.[397]

Fluorimetry.—Detection of amino-acids and their derivatives after ion-exchange or t.l.c. separation can be achieved by their conversion into fluorescent derivatives, and reviews of the use of fluorescamine[398] or *o*-phthalaldehyde[399] as reagents for this purpose have appeared. There have been suggestions that the fluorescamine reaction does not go to completion, and that substantial differences exist between the different amino-acids in their respective yields in this reaction.[400] Greater conversions in the fluorescamine reaction were achieved by sequential addition of fluorescamine, indicating that a competitive reaction causing inactivation of the reagent was in operation.[400] Analysis of amino-acid mixtures at picomole levels has been advocated for the fluorescamine procedure, but care must be taken to exclude amine contaminants from buffers, since they have been shown to yield non-fluorescent products at low pH.[401]

The procedure involving the conversion of amino-acids into 1-alkylthio-2-alkylisoindoles with *o*-phthalaldehyde and an alkanethiol[285—288] continues to gain adherents, and picomole level analysis of amino-acids,[402,403] including a fluorimetric procedure for estimating transmitter amino-acids (taurine, aspartic acid, glutamic acid, glycine, and γ-aminobutyric acid) in rat-brain tissue,[403] has been described.

Alternative general fluorimetric procedures based on dansylation[404] and reaction with 7-chloro-4-nitrobenzofurazan[405] have been developed further, the latter providing a more sensitive assay for hydroxyproline in blood plasma than colorimetric procedures employing ninhydrin or *p*-dimethylaminobenzaldehyde.[405] Specific fluorimetric assays for cysteine [conversion into the N^{α}-acetyl-*S*-(*N*-acridinylmaleimidyl) derivative][406] and arginine (sub-μg assay after reaction with 9,10-phenanthrenequinone at high pH)[407] have been described.

Other Separation Methods.—Established principles are exploited in separations of amino-acid mixtures by ligand exchange chromatography on Cu^{2+}-loaded silylated glass,[408] and by gel-electrofocusing.[409] Electrophoretic separation of hydroxylysine from its glycosides has been described.[410]

[396] J. Lammens and M. Verzele, *Chromatographia*, 1978, **11**, 376.
[397] J. Goto, M. Hasegawa, S. Nakamura, K. Shimada, and T. Nambara, *J. Chromatog.*, 1978, **152**, 413.
[398] S. Udenfriend and S. Stein, *Proc. Fifth Amer. Peptide Symp.*, ed. M. Goodman and J. Meienhofer, Wiley, New York, 1977, p. 14.
[399] K.S. Lee and D.G. Drescher, *Internat. J. Biochem.*, 1978, **9**, 457.
[400] R.F. Chen, P.D. Smith, and M. Maly, *Arch. Biochem. Biophys.*, 1978, **189**, 241.
[401] N.S. Nadi and F.L. Margolis, *Analyt. Biochem.*, 1978, **91**, 180.
[402] D.J. Reeder, L.T. Sniegoski, and R. Schaffer, *Analyt. Biochem.*, 1978, **86**, 490.
[403] K. Aoki and Y. Kuroiwa, *Chem. Pharm. Bull.*, 1978, **26**, 2684.
[404] P.K. Macnicol, *Analyt. Biochem.*, 1978, **85**, 71.
[405] M. Roth, *Clin. Chim. Acta*, 1978, **83**, 273.
[406] M. Machida, T. Takahashi, K. Itoh, T. Sekine, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1978, **26**, 596.
[407] R.E. Smith and R. MacQuarrie, *Analyt. Biochem.*, 1978, **90**, 246.
[408] R.G. Masters and D.E. Leyden, *Analyt. Chim. Acta*, 1978, **98**, 9.
[409] P.G. Righetti and A. Chrambach, *Analyt. Biochem.*, 1978, **90**, 633.
[410] C. Cintrou, B.D. Peczon, and C.L. Kublin, *Analyt. Biochem.*, 1978, **87**, 622.

Determination of Specific Amino-acids.—This section may give the impression that specific colorimetric assays are represented to a small extent, relative to specific enzyme-based procedures. Several papers in the former category, however, have been cited in preceding sections, and are not repeated here; but nevertheless, enzymatic analyses are being taken up more widely. Estimation of tyrosine and dopa (tyrosinase),[411] branched chain aliphatic amino-acids (leucine dehydrogenase),[412] lysine or ornithine (L-lysine: α-ketoglutarate ε-aminotransferase),[413] are representative. Electrochemical assays of L-amino-acids using the appropriate oxidase and an iodide-selective electrode to determine the concentration of H_2O_2 released in the enzymatic degradation,[414] and for lysine or arginine using membrane-immobilized decarboxylase and diamine oxidase against an oxygen electrode,[415] represent continuing development of well-established principles. Radioenzymic methods for dopa (conversion into [^{3}H]-3-*O*-methyldopamine)[416] and for *S*-adenosyl-L-methionine[417] have been described.

In addition to alternative methods cited elsewhere in this Chapter, γ-aminobutyric acid is amenable to assay by a sensitive radioreceptor method exploiting its natural transmitter function.[418]

Colorimetric assays of protein amino-acids have been developed for glycine (formation of the azlactone with *p*-dimethylaminobenzaldehyde after conversion into hippuric acid),[419] hydroxyproline (development of photometric[420] and colorimetric[421] methods), methionine[422,423] (decolourization of halide platinates[422]) and cystine,[423] tyrosine (coupling with 1-nitroso-2-naphthol),[424] and tryptophan (fluorimetry after conversion into norharman;[425] condensation with α-ketoglutaric acid[426]). Colorimetric procedures with non-protein amino-acids have been described for hydroxylysine and its glycosides,[427] and N^{τ}-methylhistidine.[428]

[411] Y. Menezo, C. Voulot, J.P. Ortonne, and C. Khatchadourian, *Arch. Dermatol. Res.*, 1978, **263**, 267.
[412] T. Ohshima, H. Misono, and K. Soda, *Agric. Biol. Chem.*, 1978, **42**, 1919.
[413] K. Soda, T. Hirasawa, and T. Fukumura, *Analyt. Biochem.*, 1978, **87**, 283.
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[417] P.H. Yu, *Analyt. Biochem.*, 1978, **86**, 498.
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[419] S. Ohmori, M. Ikeda, Y. Watanabe, and K. Hirota, *Analyt. Biochem.*, 1978, **90**, 662.
[420] K. Lindenhayn, H. Kretschmer, and R. Rahn, *Z. Med. Laboratoriumsdiagn.*, 1978, **19**, 246.
[421] J.O. Gordeladze, J. Halse, O. Djoeseland, and H.N. Haugen, *Biochem. Med.*, 1978, **20**, 23.
[422] D.J. Collins, R. Robinson, and A.J. Knell, *Clin. Chim. Acta*, 1978, **88**, 277.
[423] C.O. Moorhouse, A.R. Law, and C. Maddix, in *Technicon Int. Congress on Advances in Automated Analysis*, ed. E.C. Barton, Mediad Inc., Tarrytown, N.J., U.S.A., 1977.
[424] T. Forgach and S.A. Balakrishnan, *Proc. Hung. Ann. Meeting Biochem.*, 1978, **18**, 223.
[425] C. Escande, B. Bousquet, and C. Dreux, *Ann. Biol. Clin.*, 1977, **35**, 387.
[426] N.E. Anderson and F.M. Clydesdale, *J. Food Sci.*, 1978, **43**, 1595.
[427] W.C. Bisbee and P.C. Kelleher, *Clin. Chim. Acta*, 1978, **90**, 29.
[428] L.C. Ward, *Analyt. Biochem.*, 1978, **88**, 598.