1
Amino-acids

BY G. C. BARRETT

1 Introduction

The main emphasis in this Chapter is on the chemical and biochemical literature on α-amino-acids, and readers seeking references to biological aspects, such as the distribution of the common amino-acids, amino-acid metabolism, or biosynthesis, will find only scant coverage.

Textbooks and Reviews.—Distribution of non-protein amino-acids in plants[1] and amino-acids in marine organisms,[2] metabolism of aromatic amino-acids,[3] and biosynthesis of unusual amino-acids[4] are topics among areas largely excluded from this Chapter which have been reviewed recently. Fermentative[5] and chemical synthesis[6] of α-amino-acids has been reviewed, and a thorough coverage of dehydro-amino-acids and α-hydroxy- and -mercapto-α-amino-acids has appeared.[7] Specific chemical topics (non-enzymatic racemization of amino-acids,[8] N^{ω}-alkyl di- amino-acids,[9] and the conformation of γ-aminobutyric acid[10]) have been reviewed.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—The Mighei Type II carbonaceous chondrite which fell in Russia in 1889 contains alanine, α- and β-aminobutyric acids, δ-aminoadipic acid, norvaline, and aspartic acid; since these are all racemic, it is concluded that they are indigenous to the meteorite,[11] and the same reasoning has been applied to the range of protein and non-protein amino-acids present in the Allan Hills meteorite 77306.[12] This topic has been reviewed.[13]

[1] L. Fowden, P. J. Lea, and E. A. Bell, *Adv. Enzymol.*, 1979, **50**, 117.
[2] T. Shiba, *Kagaku Sosetsu*, 1979, **25**, 246 (*Chem. Abstr.*, 1980, **92**, 71099).
[3] C.-K. Wat and G. H. N. Towers, *Recent Adv. Phytochem.*, 1979, **12**, 371.
[4] P. J. Lea, *Int. Rev. Biochem.*, 1978, **18**, 79.
[5] S. Kinoshita and K. Nadayama, *Econ. Microbiol.*, 1978, **2**, 209; O. Tajima, *Hakko Kogaku Kaishi*, 1979, **57**, 232; Y. Hirose and H. Okada, in 'Microbiological Technology', 2nd edn. 1979, Vol. 1, p. 241.
[6] K. Matsumoto and K. Harada, *Kagaku no Ryoiki*, 1979, **33**, 207.
[7] U. Schmidt, J. Hausler, E. Oehler, and H. Poisel, *Fortschr. Chem. Forsch.*, 1979, **37**, 251.
[8] J. H. McKerrow, *Mech. Ageing Dev.*, 1979, **10**, 371 (*Chem. Abstr.*, 1979, **91**, 85466).
[9] N. L. Benoiton, in 'Chemistry and Biochemistry of Amino-acids, Peptides, and Proteins', ed. B. Weinstein, Dekker, New York, 1979, Vol. 5, p. 163.
[10] I. A. Sytinskii and A. T. Soldatenkov, *Usp. Khim.*, 1979, **48**, 1256.
[11] P. H. Buhl, in 'Trace Organic Analysis: New Frontiers in Analytical Chemistry', NBS Spec. Publ., Washington, D.C., 1979, pp. 519, 771 (*Chem. Abstr.*, 1979, **91**, 104069).
[12] R. K. Kotra, A. Shimoyama, C. Ponnamperuma, and P. E. Hare, *J. Mol. Evol.*, 1979, **13**, 179.
[13] G. P. Vdovykin, *Usp. Sovrem. Biol.*, 1979, **87**, 49 (*Chem. Abstr.*, 1979, **90**, 163363).

Unusual amino-acids in fungal sources include *cis*-3-amino-L-proline in growth media and cultured mycelia of *Morchella esculanta*,[14] and L-saccharopine together with γ-L-glutamyglycine in the mushroom *Agaricus bisporus*.[15]

Plant sources and amino-acids found therein are: *Halopytis incurvus* (*N*-methyl-L-aspartic acid),[16] *Sagittaria pygmaea* (N^{ε}-carboxymethyl-L-lysine),[17] *Crotalaria juncea* (δ-hydroxynorleucine but not β-hydroxy-*N*-methyl-DL-norvaline, clearing up an earlier uncertainty),[18] seeds of *Neonotonia wightii* [3-carboxy-L-tyrosine, *alias* 3-(3′-carboxy-4′-hydroxyphenyl)-L-alanine],[19] species of fern (*Filicinae*)[20] and *Reseda luteola*[21] (4*R*-hydroxy-2*S*-aminopimelic acid and its 4*S*-diastereoisomer), *Filicinae*[20] (4*S*-hydroxy-4-methyl-*S*-glutamic acid and its 4*R*-diastereoisomer), leaves of *Acacia* (homoarginine, 4-hydroxypipecolic acid),[22] and the red seaweed *Palmaria palmata* (D-homocysteic acid).[23] The possibility of the formation of artefacts during the isolation of sensitive compounds from natural sources must always be taken into account, but an additional problem, the possibility that pesticides or herbicides are sources of unusual amino-acids found in plants, has been mentioned in connection with the isolation of N^{ε}-carboxymethyl-L-lysine.[17]

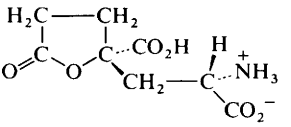
Reports describing the involvement of amino-acids as intermediates on biosynthetic pathways occasionally include information of more general importance, and, although this topic is largely excluded from this Chapter, the discussion of the natural occurrence of α-aminomalonic acid derivatives[24] (*cf.* arcamine[25]) is particularly interesting. However, at the top of the list in the 1979 literature is the splendid work[26–29] describing the establishment of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the biosynthesis of ethylene. Systems discussed in this work include *Pisum sativum* homogenates[27] and apple tissue,[28] the latter being used in a study of the pathway from methionine to ethylene *via* *S*-adenosylmethionine and 1-aminocyclopropane-1-carboxylic acid.[28]

New Natural Amino-acids.—There have been several near relatives of known amino-acids described in the recent literature. (2,3-Dihydroxyphenyl)-L-alanine, together with small amounts of *o*- and *m*-hydroxy-L-phenylalanines, has been isolated from chloridazone-degrading bacteria grown on a medium containing L-phenylalanine.[30] Aminoxyalkanoic acids have become of interest as enzyme

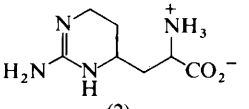
[14] M. Moriguchi, S. Sada, and S. Hatanaka, *Appl. Environ. Microbiol.*, 1979, **38**, 1018.
[15] Y. Oka, T. Ogawa, and K. Sasaoka, *Agric. Biol. Chem.*, 1979, **43**, 1995.
[16] S. Sciuto, M. Piatelli, and R. Chillemi, *Phytochemistry*, 1979, **18**, 1058.
[17] H. Matsutani, S. Kusumoto, R. Koizumi, and T. Shiba, *Phytochemistry*, 1979, **18**, 661.
[18] D. J. Pilbeam and E. A. Bell, *Phytochemistry*, 1979, **18**, 320, 973.
[19] M. F. Wilson, M. A. Bholah, G. S. Morris, and E. A. Bell, *Phytochemistry*, 1979, **18**, 1391.
[20] L. K. Meier and H. Sorensen, *Phytochemistry*, 1979, **18**, 1173.
[21] L. K. Meier, O. Olsen, and H. Sorensen, *Phytochemistry*, 1979, **18**, 1505.
[22] C. S. Evans and E. A. Bell, *Phytochemistry*, 1979, **18**, 1807.
[23] M. V. Laycock, A. G. McInnes, and K. C. Morgan, *Phytochemistry*, 1979, **18**, 1220.
[24] D. Schipper, J. L. van der Baan, and F. Bickelhaupt, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2017.
[25] A. W. Sangster, S. E. Thomas, and N. L. Tingling, *Tetrahedron*, 1975, **31**, 1135.
[26] K. Luerssen, K. Naumann, and R. Schroeder, *Naturwissenschaften*, 1979, **66**, 264; K. Luerssen, K. Naumann, and R. Schroeder, *Z. Pflanzenphysiol.*, 1979, **92**, 285.
[27] J. R. Konze and H. Kende, *Planta*, 1979, **146**, 293; T. Boller, R. C. Herner, and H. Kende, *Planta*, **145**, 293.
[28] D. O. Adams and S. F. Yang, *Proc. Natl. Acad. Sci. USA.*, 1979, **76**, 170.
[29] M. C. C. Lizada and S. F. Yang, *Anal. Biochem.*, 1979, **100**, 140.
[30] R. Buck, J. Eberspaecher, and F. Lingens, *Liebigs Ann. Chem.* 1979, 564.

inhibitors (*e.g.* L-2-aminoxy-3-phenylpropionic acid against phenylalanine ammonia-lyase [31]), and the co-occurrence of β-aminoxy-D-alanine and cycloserine, both possessing antimicrobial properties, has been established in *Streptomyces.*[32]

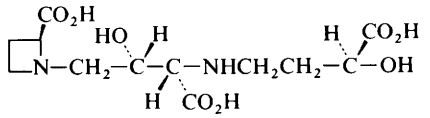
Lycoperdic acid [1; 3-(5*S*-carboxy-2-oxotetrahydrofuran-5-yl)-*S*-alanine] has been isolated from the mushroom *Lycoperdon perlatum,*[33] tetrahydrolathyrine [2; 3-(2-amino-3,4,5,6-tetrahydropyrimidin-4-yl)-*S*-alanine] from seeds of *Lanchocarpus costaricensis* (configuration at C-4 of tetrahydropyrimidinyl moiety not established),[34] mugeneic acid (3) from root washings of water-cultured barley (*Hordeum vulgare*),[35] and 2*S*-acetylamino-4*R*-carboxypiperidine (4) from leaves of *Calliandra haematocephala.*[36] Higher sources are the mussel *Mytilus galloprovincialis* (mytilins A and B, 5 and 6 respectively)[37] and the toad *Bufo americanus,* from whose skin 3-suberoyl-L-glutamine esters have been isolated.[38]



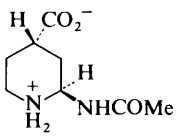
(1)



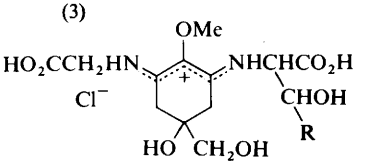
(2)



(3)



(4)



(5) R = H
(6) R = Me

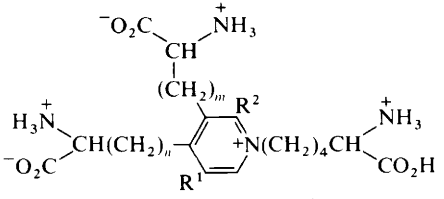
An area of viral chemistry whose importance has now been realized is represented this year (see also Vol. 10, p. 3, Vol. 11, p. 3) by the report of the existence of large amounts of a new metabolite, agropine, in crown gall tumours of tobacco. The compound ($C_{11}H_{17}NO_7$) is considered to be a condensation product of a sugar with an amino-acid.[39]

New Amino-acids from Hydrolysates.—A topic which is not covered as a rule in this Chapter in more than a brief fashion is the area of amino-acid–nucleoside

[31] N. Amrhein and J. Gerhardt, *Biochim. Biophys. Acta*, 1979, **583**, 434.
[32] K. Yagishita, J. Hideaki, Y. Haron, and M. Kiruko, *Nippon Daigaku Nojuigakubu Gakujutsu Kenkyu* Hokoku 1979, **36**, 1 (*Chem. Abstr.*, 1979, **90**, 199 159).
[33] N. Rhugenda-Banga, A. Welter, J. Jadot, and J. Casimir, *Phytochemistry*, 1979, **18**, 482.
[34] L. Fellows, E. A. Bell, T. S. Lee, and D. H. Janzen, *Phytochemistry*, 1979, **18**, 1333.
[35] T. Takemoto, K. Nomoto, S. Fushiya, R. Ouchi, G. Kusano, H. Hikino, S. Takagi, Y. Matusuura, and M. Kakudo, *Proc. Jpn. Acad., Ser. B*, 1978, **54**, 469.
[36] M. Marlier, G. Dardenne, and J. Casimir, *Phytochemistry*, 1979, **18**, 479.
[37] F. Chioccara, G. Misuraca, E. Novellino, and G. Prota, *Tetrahedron Lett.*, 1979, 3181.
[38] K. Shimada and T. Nambara, *Tetrahedron Lett.*, 1979, 163.
[39] J. L. Firmin and G. R. Fenwick, *Nature* (*London*), 1978, **276**, 842.

derivatives. *N*-[(9-β-D-ribofuranosyl-2-methylthiopurin-6-yl)carbamoyl] threonine is not only a new nucleoside (rabbit liver transfer RNA) but also represents the first example of a mammalian tRNA carrying a methylthio-substituent.[40]

Recurring topics in this section in previous volumes have been novel cross-links in proteins and novel amino-acids found in peptide antibiotics. In the former category, the structure determination and synthesis of anabilysine, previously reported in preliminary communication form (Vol. 10, p. 5), have been fully described,[41] and the existence of dityrosine cross-links in elastin and structural glycoproteins from young chicks[42-44] has been reported. A simple synthesis of this bis(amino-acid) involves the oxidation of L-tyrosine with hydrogen peroxide in the presence of horseradish peroxidase.[44] Desmosine cross-linked elastin carries a second cross-link, possibly lysinonorleucine, about 35 amino-acid residues removed from the desmosine cross-link;[45] a new desmosine isomer, photodesmosine (7) is formed (*via* pyridinium ring-opening) through irradiation of desmosine at



(7) $R^1 = H$, $R^2 = (CH_2)_2CH(\overset{+}{N}H_3)CO_2^-$
$n = 2$, $m = 3$

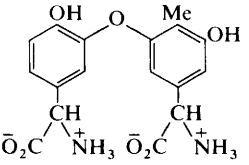
254 nm.[46] Ovamucoid proteins treated with alkali and by cyanolysis contain lysinoalanine cross-links, demonstrated through the isolation of N^{ε}-(DL-2-amino-2-carboxyethyl)-L-lysine from hydrolysates.[47] Lanthionine is also present.[47]

The *C*-terminal residue of the lipophilic undecapeptide rhodotorucine A is *S-trans, trans*-farnesyl-L-cysteine.[48]

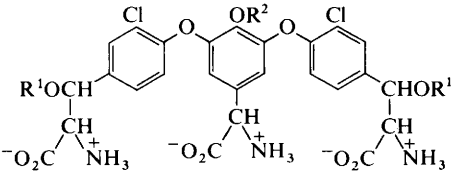
Further reports from research groups who have found themselves studying the same antibiotic (ristocetin is identical with ristomycin A) have settled the structure of the bis(phenylglycine) moiety (8) (see Vol. 11, p. 5) of this condensed peptide antibiotic[49,50] and of the biphenyl homologue actinoidinoic acid (2′,4,6-trihydroxybiphenyl-2,5′-diyl)diglycinate,[50,51] which is also present. This latter component has the *S*-configuration and is at the *N*-terminus of the antibiotic.[52] A

[40] Z. Yamaizumi, S. Nishimura, K. Limburg, M. Raba, H. J. Gross, P. F. Crain, and J. A. McCloskey, *J. Am. Chem. Soc.*, 1979, **101**, 2224.
[41] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2282.
[42] V. Malanik and M. Ledvina, *Connect. Tissue Res.*, 1979, **6**, 235 (*Chem. Abstr.*, 1979, **91**, 118996).
[43] V. Malanik and M. Ledvina, *J. Chromatogr.*, 1979, **170**, 254.
[44] V. Malanik and M. Ledvina, *Prep. Biochem.*, 1979, **9**, 273.
[45] R. P. Mecham and J. A. Foster, *Biochem. J.*, 1978, **173**, 617.
[46] J. F. Larochelle and F. Lamy, *Biochem. Biophys. Acta*, 1979, **584**, 327.
[47] R. G. Walsh, A. S. Nashef, and R. E. Feeney, *Int. J. Pept. Protein Res.*, 1979, **14**, 290.
[48] Y. Kamiya, A. Sakurai, S. Tamura, and N. Takahashi, *Agric. Biol. Chem.*, 1979, **43**, 1049.
[49] T. M. Harris, C. M. Harris, J. R. Fehlner, R. Bognar, and F. Sztaricskai, *J. Org. Chem.*, 1979, **44**, 1009.
[50] C. M. Harris, J. J. Kibly, J. R. Fehlner, A. B. Raabe, T. A. Barber, and T. M. Harris, *J. Am. Chem. Soc.*, 1979, **101**, 437.
[51] D. H. Williams, V. Rajananda, and J. R. Kalman, *J. Chem. Soc., Perkin Trans. 1*, 1979, 737.
[52] D. H. Williams, V. Rajananda, G. Bojesen, and M. P. Williamson, *J. Chem. Soc., Chem. Commun.*, 1979, 906.

triaminotricarboxylic acid (9; R′ = H) from the same family of antibiotics is built up from β-hydroxychlorotyrosine units,[53, 54] and actinoidin differs from vancomycin in carrying one fewer chlorine atom in this portion of the molecule.[53]



(8)



(9)

3 Chemical Synthesis and Resolution of Amino-acids

General Methods of Synthesis of α-Amino-acids.—Established methods continue to provide reliable preparative routes to a wide variety of α-amino-acids; the acetamidomalonate route ($RX + AcNHCH(CO_2Et)_2 \rightarrow AcNHCR(CO_2Et)_2 \rightarrow H_3\overset{+}{N}CHRCO_2^-$) has been used perhaps more than any other general route, as represented in the 1979 literature. The synthesis of amino-acids by this route is mentioned elsewhere in this Chapter.[21, 55, 89, 125, 147, 162] The use of the analogue $R^1CONHCR(OAc)CO_2Et$ in the synthesis of 2-substituted α-amino-acids and dehydro-amino-acids has been described.[56] A further example of the introduction of a variation of a standard method is the conversion of an α-bromoalkanoic acid into the α-isocyanato analogue with a metal isocyanate, followed by conversion into the corresponding α-amino-acid (in yields of 91—99%) *via* the carbamates.[57]

Aldehydes offer a variety of entries to amino-acids, and as well as standard Strecker procedures[90] (*cf.* earlier volumes) the conversion of aldoximes into aminomalononitriles and thence into amino-acids by hydrolysis[58] and Zelinsky–Stadnikoff synthesis illustrated for the synthesis of DL-serine from glycollaldehyde and masked equivalents[59] have been described. Alkylation of a variety of substrates by ethyl 3-bromo-2-hydroxyiminopropanoate in the presence

[53] F. Sztaricskai, C. M. Harris, and T. M. Harris, *Tetrahedron Lett.*, 1979, 2861.
[54] G. S. Katrukha, B. Diarra, A. B. Silaev, Z. P. Trifonova, B. V. Rozynov, and O. S. Rashetova, *Antibiotiki*, 1979, **24**, 179.
[55] G. R. Pettit and T. S. Krupa, *J. Org. Chem.*, 1979, **44**, 396.
[56] Y. Ozaki, T. Iwasaki, H. Horikawa, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, 1979, **44**, 391.
[57] F. Effenberger and K. Drauz, *Angew. Chem.*, 1979, **91**, 504.
[58] S. Y. Sizov, L. V. Semenova, and N. P. Utrobin, *Prikl. Biokhim. Mikrobiol.*, 1978, **14**, 915.
[59] L. Bassagnani, B. Biancini, A. Brandt, V. Caciagli, G. E. Bianchi, L. Re, A. Rossodivita, and P. Zappelli, *Chem. Ber.*, 1979, **112**, 148.

of sodium carbonate, followed by aluminium amalgam reduction, provides a general route to α-amino-acid esters of the β-substituted alanine family:[60]

$$BrCH_2C(=NOH)CO_2Et \longrightarrow RCH_2C(=NOH)CO_2Et \longrightarrow RCH_2CH(NH_2)CO_2Et$$

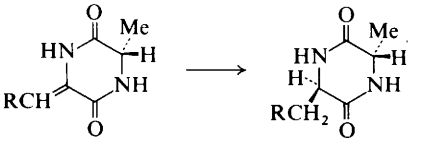
Synthesis of β-Amino-acids and Higher Homologues.—General routes to higher homologous amino-acids are not easily classified, and, as in earlier years, a crop of papers has been collected from the recent literature, each describing a specific class of ω-aminoalkanoic acid.

A convenient general synthesis of β-amino-acids[61] employs conjugate addition of hydroxylamine to αβ-unsaturated alkanoic acids, followed by hydrogenation over a palladium catalyst. A new synthesis of L-4-amino-2-hydroxybutanoic acid[62] starts from L-asparagine, and 4-aminobut-2-enoic acids have been obtained for evaluation as analogues of γ-aminobutyric acid, using αβ-unsaturated alkanoic acids as starting materials.[63]

Asymmetric Synthesis of α-Amino-acids.—Further development of methods fully described in previous volumes has been reported. A general review[64] and a review of catalytic asymmetric hydrogenation of dehydro-amino-acids[65] have appeared.

Homogeneous asymmetric catalytic hydrogenation of 2-acylaminoalken-2-oic acids using rhodium–chiral phosphine catalysts continues to be studied in several laboratories.[65-69] High optical yields (*e.g.* 90 ± 3%[66]) are being secured. The hydrogenation of unsaturated azlactones (4-alkylidene- and 4-arylidene-oxazolin-5-ones) over palladium in the presence of *S*-1-phenylethylamine gives modest asymmetric induction,[70] L-tyrosine and its *O*-methyl derivative being obtained with enantiomeric excesses up to 60%. An alternative approach, in which a chiral derivative of the acylaminoalkenoic acid is subjected to palladium-catalysed hydrogenation, leads to extraordinarily high (99%) asymmetric induction for cyclodipeptides (10) → (11).[71] A remaining variation of the asymmetric hydrogenation route, in which palladium-catalysed hydrogenation of a Schiff base formed between an α-keto-ester and a chiral benzylamine leads to an α-amino-acid with optical purity reaching *ca.* 60%, has been illustrated further for the asymmetric synthesis of L-alanine using *R*-phenylglycine.[72] An alternative use of Schiff-base intermediates, obtained from a chiral benzylamine and an aldehyde, has been illustrated for the asymmetric synthesis of *S*-(+)-2-amino-3-(1-adamantyl) pro-

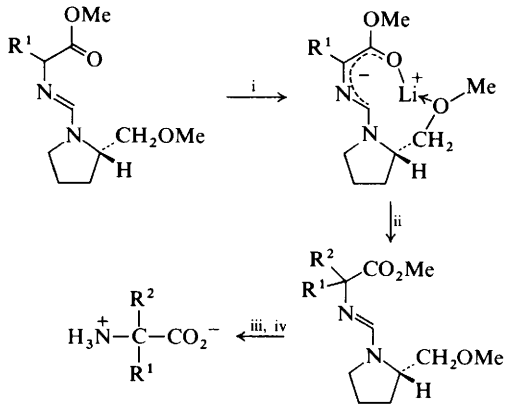
[60] T. L. Gilchrist, D. A. Lingham, and T. G. Roberts, *J. Chem. Soc., Chem. Commun.*, 1979, 1089.
[61] K. Basheeruddin, A. A. Siddiqui, N. H. Khan, and S. Saleha, *Synth. Commun.*, 1979, **9**, 705.
[62] T. Yoneta, S. Shibahara, S. Fukatsu, and S. Seki, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3296.
[63] R. D. Allan and B. Twitchin, *Aust. J. Chem.*, 1978, **31**, 2283.
[64] N. Ikota and T. Shioiri, *Kogaku no Ryoiki*, 1979, **33**, 507.
[65] Y. Sugi, *Yuki Gosei Kagaku Kyokaishi*, 1979, **37**, 71.
[66] M. D. Fryzuk and B. Bosnich, *J. Am. Chem. Soc.*, 1978, **100**, 5491.
[67] H. Brunner and W. Pieronczyk, *Angew. Chem.*, 1979, **91**, 655.
[68] K. Onuma, T. Ito, and A. Nakamura, *Tetrahedron Lett.*, 1979, 3163.
[69] D. Lafont, D. Sinou, and G. Descotes, *J. Organomet. Chem.*, 1979, **169**, 87.
[70] E. S. Neupokoeva, E. I. Karpeiskaya, L. F. Godunova, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR.*, 1979, 139; E. S. Neupokoeva, E. I. Karpeiskaya, L. F. Godunova, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR*, 146.
[71] T. Kanmera, S. Lee, H. Aoyagi, and N. Izumiya, *Tetrahedron Lett.*, 1979, 4483; S. Lee, T. Kanmera, H. Aoyagi, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1979, **13**, 207.
[72] K. Harada and M. Tamura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1227.



(10) (11)

pionic acid.[73] Asymmetric addition of HCN to the Schiff base and following stages have been established earlier (see Vol. 10, p. 5).[73]

Asymmetric alkylation of (+)-bornyl or (−)-menthyl isocyanoacetates under phase-transfer catalysis conditions, followed by conventional reactions, has been shown to give products of moderate optical purity (*e.g.* L-alanine, 48% optical purity).[74] Alkylation of Schiff bases formed between an α-amino-acid ester and *S*-(−)-1-dimethoxymethyl-2-methoxymethylpyrrolidine (Scheme 1) occurs with a moderate degree of asymmetric induction (highest enantiomeric excess 50%),[75] dependent on the size of the electrophile R^2X (enantiomeric excess only 5% with R^2 = 3,4-$(OMe)_2C_6H_3CH_2$).[75] The stereochemistry of the diastereoisomer formed predominantly implies preferential *re*-face approach of the electrophile towards the anion. An advantage of the route displayed in Scheme 1 is the recovery of the chiral reagent.[75]



Reagents: i, $LiNPr^i_2$; ii, R^2X; iii, $MeOH–H_2O$, 25 °C; iv, 3M-HCl, reflux 60 h, neutralize

Scheme 1

Prebiotic Synthesis; Model Reactions.—Nearly 20 years after the experiment performed by Urey and Miller, and with many papers of a similar theme appearing in the intervening period, the formation of glycine, alanine, serine, aspartic acid, and glutamic acid in xenon lamp-irradiated mixtures of titanium dioxide, aqueous ammonia, and NH_4Cl has been described, a novel variation on the original system.[76] Many of the current studies of the same type, while employing ammonia

[73] K. Q. Do, P. Thanei, M. Caviezel, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 956.
[74] B. Laangstroem, B. Stridsberg, and G. Bergson, *Chem. Scripta*, 1979, **13**, 49.
[75] M. Kolb and J. Barth, *Tetrahedron Lett.*, 1979, 2999.
[76] H. Reiche and A. J. Bard, *J. Am. Chem Soc.*, 1979, **101**, 3127.

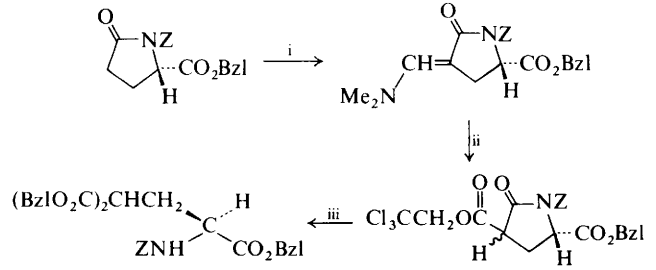
as the nitrogen source, choose some other simple organic compound and alternative energy source (ethanol subjected to neutron flux,[77] two-carbon hydrocarbons under electric discharge,[78] formaldehyde in sunlight,[79] glycollaldehyde, glyceraldehyde, or higher sugars,[80] metal salts of acetic acid [81] or citraconic, citraconamic, or itaconamic acids [82]). Further studies of the use of hydroxylamine (see Vol. 10, p. 8) and formaldehyde in the synthesis of glycine, serine, aspartic acid, and β-alanine have been reported.[83]

The other major area of chemistry, within which model reactions relevant to the prebiotic synthesis of amino-acids are being explored, is based on condensation and oligomerization of inorganic and organic cyanides. A complex, probably $H_2[(CN)_2$-$Cu_2NC\cdot CN(CN)_2]\cdot 2H_2O$, which is formed in solutions containing NaCN and $CuSO_4$, gives glycine on boiling in dilute hydrochloric acid.[84] Cyanamide–KNO_2 reaction products yield amino-acids, nucleosides, and other organic compounds on hydrolysis.[85] A polemical exchange against [86] and for [87] the oligomerization of HCN as the prebiotic origin of amino-acids and peptides highlights the main experimental facts and the possible interpretations. Earlier work on the formation of insoluble microspheres from aldehyde–cyanide reaction mixtures has been extended,[88] and the amino-acids released from this material have been shown to be formed in proportions dependent on the structures of the aldehydes.

Synthesis of Protein Amino-acids and Other Naturally Occurring Amino-acids.—General methods of synthesis of α-amino-acids, discussed in an earlier section, have been used routinely for the synthesis of natural amino-acids, further examples being a new synthesis of DL-δ-aminoadipic acid from diethyl acetamidomalonate and $I(CH_2)_3CN$[89] and Strecker synthesis of lysine and ornithine starting from 2-methoxy-*N*-acylpiperidine as a masked aldehyde.[90] The generation of an appropriate aldehyde for the synthesis of *erythro*-β-L-histidine, a component of the peptide antibiotic bleomycin, has been achieved starting from D-glucosamine.[91]

A simple synthesis of γ-carboxy-L-glutamic acid from L-pyroglutamic acid [92] (Scheme 2) is a substantial achievement. An alternative approach to this protein amino-acid starts from *N*-benzyloxycarbonyl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester, nucleophilic displacement of the toluene-*p*-sulphonyloxy group by di-

[77] K. Kawamoto, K. Kawai, H. Maki, and M. Akaboshi, *Ann. Repts. Res. React. Inst., Kyoto Univ.*, 1978, **11**, 147 (*Chem. Abstr.*, 1979, **91**, 5444).
[78] H. Ebisawa, E. Miyoshi, T. Shirai, and S. Yanagisawa, *Nippon Kagaku Kaishi*, 1979, 331 (*Chem. Abstr.*, 1979, **90**, 181958); H. Ebisawa, E. Miyoshi, T. Shirai, and S. Yanagisawa, *Nippon Kagaku Kaishi*, 1979, 1304.
[79] S. Ranganayaki and M. Srivastava, *Zh. Org. Khim.*, 1979, **15**, 1124.
[80] H. Yamagawa, Y. Kobayashi, and F. Egami, *J. Biochem. (Tokyo)*, 1980, **87**, 359.
[81] S. Ranganayaki, B. Srivastava, and U. Jyotishmati, *Anusandnan Patrika*, 1978, **21**, 185 (*Chem. Abstr.*, 1979, **90**, 181839).
[82] K. Harada and M. Matsuyama, *Biosystems*, 1979, **11**, 47.
[83] Kamaluddin, H. Yamagawa, and F. Egami, *J. Biochem. (Tokyo)*, 1979, **85**, 1503.
[84] M. Beck, V. Gaspar, and J. Ling, *Magy. Kem. Foly.*, 1979, **85**, 147.
[85] G. Wollin and W. B. F. Ryan, *Biochim. Biophys. Acta*, 1979, **584**, 493.
[86] J. P. Ferris, *Science*, 1979, **203**, 1135.
[87] C. N. Matthews, *Science*, 1979, **203**, 1136.
[88] G. E. Pollock and R. Heiderer, *J. Mol. Evol.*, 1979, **13**, 253.
[89] S. Wolfe and M. G. Jokinen, *Can. J. Chem.*, 1979, **57**, 1388.
[90] K. Warning, M. Mitzlaff, and H. Jensen, *Annalen*, 1978, 1707.
[91] S. M. Hecht, K. M. Rupprecht, and P. M. Jacobs, *J. Am. Chem. Soc.*, 1979, **101**, 3982.
[92] S. Danishefsky, E. Berman, L. A. Clizbe, and M. Hirama, *J. Am. Chem. Soc.*, 1979, **101**, 4385.



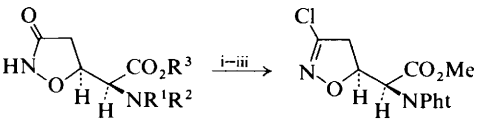
Reagents: i, $(Me_2N)_2CHOBu^t$; ii, $ClCO_2CH_2CCl_3$; iii, $PhCH_2OH$

Scheme 2

t-butyl malonate anion yielding the protected γ-carboxyglutamic acid.[93] A related side-chain modification approach has been used for the synthesis of L-(+)-discadenine and its desamino- and descarboxy-derivatives, from *N*-phthaloyl-4-bromo-2-aminobutanoic acid ethyl ester and adenines.[94]

2*S*-Amino-3*S*-phenylbutyric acid, synthesized from diethyl *R*-(1-phenylethyl) malonate *via* conventional bromination, decarboxylation, and amination stages, is identical with the amino-acid present in bottromycin, thus establishing its absolute configuration.[95]

A total synthesis of the antitumour antibiotic AT-125 (2-amino-3′-chloro-4′,5′-dihydroisoxazol-5′-ylacetic acid) has been reported (Scheme 3),[96] starting from tricholomic acid.



Tricholomic acid
$R^1 = R^2 = R^3 = H$

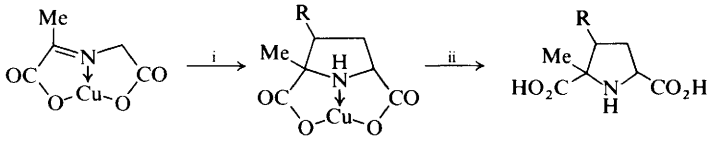
Pht = phthalimido

Reagents: i, *N*-carbethoxyphthalimide; ii, CH_2N_2; iii, $(Me_2N)_3PCl_2$–THF

Scheme 3

Further development of recently introduced synthetic routes has been described for the synthesis of L-aspartic acid, from *meso*-dibromosuccinic esters and *R*-1-phenylethylamine followed by hydrolysis and hydrogenolysis of the resulting chiral aziridinedicarboxylic esters (*cf.* Vol. 11, p. 8)[97], and a use of *N*-pyruvylideneglycinate copper(II) complexes (Scheme 4) in a new route to proline derivatives has been reported.[98]

[93] N. T. Boggs, B. Goldsmith, R. E. Gawley, K. A. Koehler, and R. G. Hiskey, *J. Org. Chem.*, 1979, **44**, 2262.
[94] F. Seela and D. Hasselmann, *Chem. Ber.*, 1979, **112**, 3072.
[95] G. Tsuchihashi, S. Mitamura, and H. Ogura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2167.
[96] R. C. Kelly, I. Schletter, S. J. Stern, and W. Wierenga, *J. Am. Chem. Soc.*, 1979, **101**, 1054.
[97] K. Harada and I. Nakamura, *Chem. Lett.*, 1978, 1171.
[98] L. Casella, M. Gullotti, A. Pasini, and R. Psaro, *Synthesis*, 1979, 150.



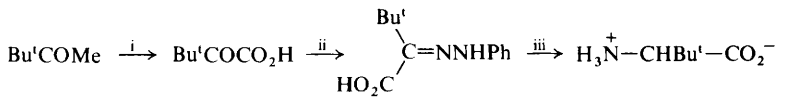
Reagents: i, $RCH{=}CH_2$, pyridine, NEt_3; ii, H_3O^+

Scheme 4

Conversion of glycine into L-serine through the use of microbial L-serine hydroxymethyltransferase[99] illustrates the possibilities for enzymic synthesis of protein amino-acids.

Synthesis of Aliphatic α-Amino-acids.—Amination of $CH_2{=}C(CO_2Et)CH(CO_2Et)_2$ with chloramine and NaH, followed by hydrolysis, yields β-methyleneaspartic acid[100] through a procedure which is only rarely brought into use in amino-acid synthesis.

A variation on a standard route to t-leucine is conveniently operated on a large scale (Scheme 5).[101]



Reagents: i, alkaline $KMnO_4$; ii, $PhNHNH_2$; iii, H_2–Pd

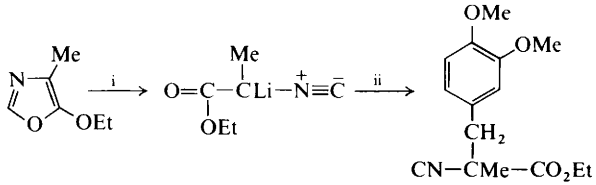
Scheme 5

Synthesis of α-Alkyl Analogues of Protein Amino-acids and of Other Natural Amino-acids.—Alkylation of 4-substituted oxazolin-5-ones, followed by hydrolysis, provides a general route to the title compounds.[102] A specific version of this route providing α-hydroxymethyl analogues through alkylation by formaldehyde has been used for the synthesis of *N*-benzoyl-*S*-benzyl-α-hydroxymethyl-L-cysteine (racemization ensues in this route if an optically active amino-acid is used as starting material, and the optically active product was obtained after resolution by quinine of the hydrolysed oxazolin-5-one).[103]

The dimethoxy analogue of α-methyldopa (α-methyl-3,4-dihydroxyphenylalanine) has been obtained from 4-methyl-5-ethoxyoxazole through alkylation of the lithiated α-isocyanopropionate formed on ring opening (Scheme 6).[104]

The carbon–carbon bond-forming potential of organocuprate reagents has now been applied to the synthesis of optically active α-alkyl- or α-aryl-amino-acids from *N*-toluene-*p*-sulphonyl-L-amino-acids.[105] Inversion of configuration accompanies the introduction of the substituent by a lithium dialkyl- or lithium diaryl-cuprate.

[99] M. Ema, T. Kakimoto, and I. Chibata, *Appl. Environ. Microbiol.*, 1979, **37**, 1053.
[100] P. Dowd and C. Kaufman, *J. Org. Chem.*, 1979, **44**, 3956.
[101] T. Miyazawa, K. Takashima, Y. Mitsuda, T. Yamada, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1539.
[102] B. Kuebel, P. Gruber, R. Hurnaus, and W. Steglich, *Chem. Ber.*, 1979, **112**, 128.
[103] M. T. Leplawy and A. Olma, *Pol. J. Chem.*, 1979, **53**, 353 (*Chem. Abstr.*, 1979, **91**, 39815); Z. Kaminski, M. Leplawy, and J. Zobrocki, Pol. Patent 86403 (*Chem. Abstr.*, 1979, **91**, 21107).
[104] P. A. Jacobi, S.-N. Ueng, and D. Carr, *J. Org. Chem.*, 1979, **44**, 2042.
[105] S. Terashima, C. C. Tseng, and K. Koga, *Chem. Pharm. Bull.*, 1979, **27**, 747.



Reagents: i, BuLi; ii, 3,4-$(MeO)_2C_6H_3CH_2Br$

Scheme 6

Synthesis of α-Heteroatom-substituted α-Amino-acids.—Less new material has been reported recently in this area, perhaps because one major research group has invested its efforts into compiling an extensive review.[7] α-Alkoxy-α-amino-acid esters, formed by electrochemical alkoxylation of 2-acetamido-2-alkylmalonic half-esters (see Vol. 10, p. 11), are useful precursors of dehydro-amino-acids and of α-benzylthio-α-amino-acids,[106] and *N*-pyrimidinyl-amino-acids.[131]

Synthesis of Halogenoalkyl Amino-acids.—With the exception of a full account [107] of results published last year in preliminary form (Vol. 11, p. 11), describing the preparation of α-halogenomethyl-α-amino-acids from Schiff bases, all the work currently published concerns fluorine analogues of protein amino-acids. These have acquired special interest as potential enzyme inhibitors. A new synthesis of β-fluoroaspartic acid involves the diazotization of *meso*-diaminosuccinic acid by $NaNO_2$ in liquid HF.[108] A hydroxy-amino-acid (serine, threonine, or *threo*-β-hydroxyphenylalanine) yields the corresponding fluoro-amino-acid on treatment with SF_4 in liquid HF at −78 °C.[109] A novel route to β-fluoro-α-amino-acids [110] and ββ-difluoro-analogues [111] is based on the opening by HF of 3-carbethoxy-2-phenylazirine [110] or its 2-methyl analogue.[111] The azirines are conveniently prepared from β-azido-αβ-unsaturated esters.

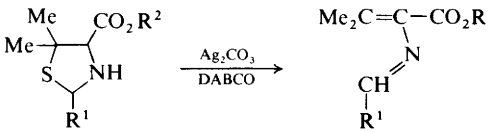
Synthesis of Aliphatic Amino-acids Carrying Hydroxy-groups in Side Chains.—A crop of papers has appeared dealing with the synthesis of β-hydroxy-α-amino-acids. *erythro*-Diastereoisomers are obtained through the reaction of a ketone with *NN*-bis(trimethylsilyl)glycine trimethylsilylester carbanion,[112] while *threo*-diastereoisomers are formed from *N*-benzyloxycarbonylglycine ethyl ester on treatment with $LiNPr^i_2$ and a ketone *via* the derived *trans*-oxazolidin-2-one [112] or from the *trans*-oxazoline formed between an aldehyde and isocyanoacetamide.[113] An asymmetric synthesis of L-threonine uses *erythro*-2,3-dibromobutanoic acid and *S*-1-phenylethylamine, cleavage of the resulting *N*-1-phenylethylaziridine with aqueous acid, and hydrogenolysis (H_2–Pd) of the *N*-substituent (see also Vol. 11, p. 8);[114] the required stereoisomer is accompanied by *ca.* 15% of the *erythro*-

[106] T. Iwasaki, H. Horikawa, K. Matsumoto, and M. Miyoshi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 826.
[107] P. Bey, J. P. Vevert, V. Van Dorsselaer, and M. Kolb, *J. Org. Chem.*, 1979, **44**, 2732.
[108] K. Matsumoto, Y. Ozaki, T. Iwasaki, H. Horikawa, and M. Miyoshi, *Experientia*, 1979, **35**, 850.
[109] J. Kollonitsch, S. Marburg, and L. M. Perkins, *J. Org. Chem.*, 1979, **44**, 771.
[110] T. N. Wade, F. Gaymard, and R. Guedj, *Tetrahedron Lett.*, 1979, 2681.
[111] T. N. Wade and R. Guedj, *Tetrahedron Lett.*, 1979, 3953.
[112] A. Shanzer, L. Somekh, and D. Butina, *J. Org. Chem.*, 1979, **44**, 3967.
[113] Y. Ozaki, S. Maeda, M. Miyoshi, and K. Matsumoto, *Synthesis*, 1979, 216.
[114] I. Nakamura and K. Harada, *Chem. Lett.*, 1979, 313.

diastereoisomer (L-isothreonine).[114] A synthesis of D-isothreonine from L-threonine follows conventional steps (replacement of NH_2 by Br with retention of configuration using NOBr, and amination with NH_4OH) but the stereospecificity is ascribed to the intermediacy of the 2*R*,3*R*-oxiran.[115] Diastereoisomers of β-hydroxy-L-aspartic acid formed from cysteine sulphinate and dihydroxyfumaric acid mediated by aspartate aminotransferase can be separated without difficulty by ion-exchange chromatography.[116]

Borane reduction of *N*-phthaloyl-γ-carboxy-DL-glutamic acid α-methyl ester, followed by de-protection, gives 5,5′-dihydroxy-DL-leucine.[117]

Synthesis of α-Amino-acids with Unsaturated Side Chains.—The so-called 'dehydro-amino-acids', *alias* 2-amino alken-2-oic acids, continue to receive more attention than their alkenyl isomers, or alkynyl analogues, even though the latter classes are important as potential enzyme inhibitors. Some particularly interesting synthetic work has been described in the recent literature, concerning dehydro-amino-acids, notably the first synthesis of an *N*-arylidene derivative (12) from a thiazolidine-5-carboxylic-ester[118] and the condensation of ethyl cyanoformate with an active methylene compound in the presence of $ZnCl_2$ and triethylamine [$EtO_2CCN + XCH_2Y \rightarrow H_2NC(=CXY)CO_2Et$].[119]



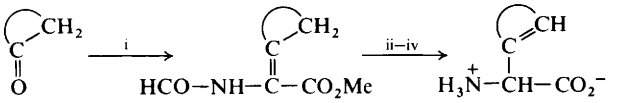
(12)

A variation of the synthesis of dehydro-amino-acids *via* 4-alkylidene- or 4-arylidene-oxazolin-5-ones has been described,[120] in which the intermediates are obtained from 2-phenyl-5-trimethylsilyloxyoxazole by condensation with an aldehyde or ketone in CH_2Cl_2 in the presence of $SnCl_4$, or with the corresponding acetal under similarly mild conditions. This procedure will be useful with base-sensitive substrates for which the classical route *via* 2-phenyloxazolin-5-one would be unusable.

A route to dehydro-amino-acids from 2-azidoalken-2-oate esters,[121] and another from α-keto-esters involving condensation with benzyl carbamate[122] ($R^1CH_2COCO_2Et + PhCH_2OCONH_2 \rightarrow ZNHC(=CHR^1)CO_2Et$), have been explored; the latter appears particularly promising but will depend on suitable methods for selective removal of protecting groups. Double-bond migration also occurs in a condensation of cyclic ketones with methyl isocyanoacetate (Scheme 7),[123] possibly at the saponification stage, where proton abstraction by the

[115] Y. Shimohigashi, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 949.
[116] W. T. Jenkins, *Anal. Biochem.*, 1979, **93**, 134.
[117] S. Bory, M. Gaudry, A. Marquet, and R. Azerad, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 85.
[118] E. Oehler and U. Schmidt, *Chem. Ber.*, 1979, **112**, 107.
[119] T. Iimori, Y. Nii, T. Izawa, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, 1979, 2525.
[120] H. Takagaki, S. Tanabe, M. Asaoka, and H. Takei, *Chem. Lett.*, 1979, 347.
[121] C. G. Shin, Y. Yonezawa, K. Unoki, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1657.
[122] C. Shin, Y. Yonezawa, K. Unoki, and J. Yoshimura, *Tetrahedron Lett.*, 1979, 1049.
[123] K. Nunami, M. Suzuki, and N. Yoneda, *J. Chem. Soc. Perkin Trans. 1*, 1979, 2224.

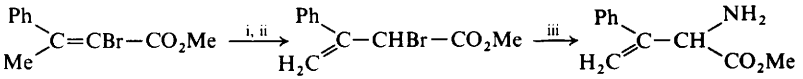
carboxylate anion could explain the production of $\beta\gamma$-unsaturated α-amino-acids.[123]



Reagents: i, $CNCH_2CO_2Me$; ii, 2M-KOH in MeOH, 50 °C; iii, HCl (aq) in THF; iv, neutralization

Scheme 7

Another new approach to this class of amino-acid depends on bond migration in 2-bromoalk-2-enoate esters (Scheme 8).[124]



Reagents: i, $LiNPr^i_2$–THF, −78 °C; ii, 2.5% HCl (aq); iii, NH_3–DMSO, 24 °C

Scheme 8

Alkylation of diethylacetamidomalonate with a non-conjugated diene, using palladium(II) acetate with PPh_3 and sodium phenoxide as catalyst system, gives alka-4,ω-dienyl-amino-acids;[125] alkylation by allylic alcohols or chlorides can also be achieved with this system.[125]

'α-Acetylenic' α-amino-acids can be obtained by carboxylation of the di-anion of t-butyl *N*-trimethylsilyl prop-2-ynylcarbamate, followed by methylation (CH_2N_2), anion formation, and alkylation with an alkyl halide.[126] L-Propargylglycine has been prepared from diethyl acetamidomalonate.[127]

Synthesis of Aromatic and Heterocyclic α-Amino-acids.—This section excludes compounds in which amino- and carboxy-groups are substituted directly on aromatic or heteroaromatic systems, but covers arylglycines ($H_3N^+CHArCO_2^-$) and relatives of the naturally occurring aromatic and heterocyclic amino-acids.

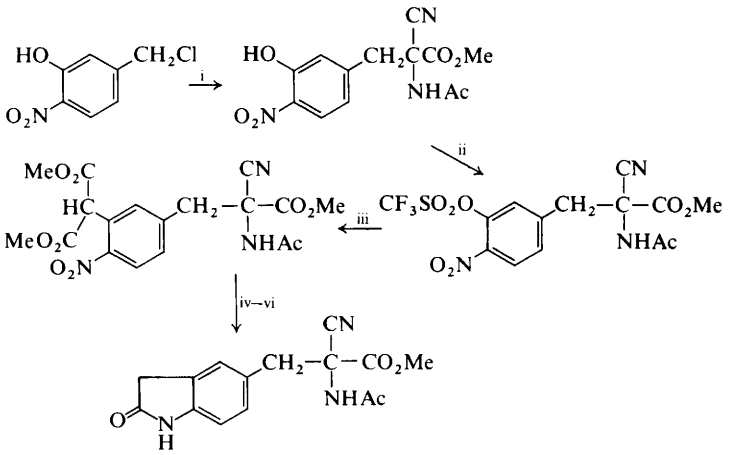
Phase-transfer catalysed conversion of benzaldehydes RC_6H_4CHO into arylglycines (R = H, 4-Cl, 4-F, 3-F, 4-MeO, 4-Me) by reaction with NH_3 and $CHCl_3$[128] competes with well established routes to these compounds.

An example of microbial synthesis relevant to this section is the conversion of L-tyrosine into L-dopa by *Actinomycetes*.[129]

The simplest heterocyclic amino-acid for which new work has been published in 1979 is pyrrol-1-ine-5-carboxylic acid, the form in which L-glutamic acid γ-semialdehyde is isolated after its formation from L-ornithine by enzyme-catalysed oxidation.[130] A range of α-pyrimidinyl-amino-acids has been obtained through

[124] R. V. J. Chari and J. Wemple, *Tetrahedron Lett.*, 1979, 111.
[125] J. P. Haudegoud, Y. Chauvin, and D. Commerenc, *J. Org. Chem.*, 1979, **44**, 3063.
[126] B. W. Metcalf and E. Bonilavri, *J. Chem. Soc., Chem. Commun.*, 1978, 915 (α-vinyl-α-amino-acids); B. W. Metcalf and P. Sasara, *J. Chem. Soc., Chem. Commun.*, 1979, 119.
[127] J. L. Fauchere, O. Leukart, A. Eberle, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 1385.
[128] D. Landini, F. Montanari, and F. Rolla, *Synthesis*, 1979, 26.
[129] C. P. Sukumaran, D. V. Singh, P. D. Khedkar, and P. R. Mahadevan, *J. Bioscience*, 1979, **1**, 235.
[130] D. J. Hayzer, R. V. Kirshna, and R. Margraff, *Anal. Biochem.*, 1979, **96**, 94.

reaction of *N*-chloropyrimidines with *N*-acyl-α-acetoxy-α-amino-acid esters,[131] and several β-heteroaryl-α-methylalanines have been prepared through alkylation of the potassium salt of *N*-benzylidenealanine methyl ester with chloromethyl-heterocycles.[132] A different approach has been used for the synthesis of 2-selenienylalanine [2-amino-3-(selenophen-2-yl)propanoic acid], in which the heterocyclic moiety is built on to *N*-acetylpropargylglycine ethyl ester;[133] the same starting material is used in an improved synthesis of L-*o*-carboranylalanine.[127] The heterocyclic side chain is constructed through novel transformation of the *o*-nitrophenol group of 3′-hydroxy-4′-nitrophenylalanine, in a synthesis of 5-(oxindolyl)alanine in improved (25%) yield.[134] The route (Scheme 9) has also been used for the synthesis of the α-methyl analogues of this amino-



Reagents: i, $MeCONHCH(CN)CO_2Me$; ii, $(CF_3SO_2)_2O$; iii, dimethyl malonate; iv, NaOH (aq); v, H_3O^+; vi, $SnCl_2$–HCl

Scheme 9

acid,[134] using methyl 2-isocyanopropionate as reagent (i) in the synthesis in place of acetamidocyano-acetate. A superficially similar approach is used in the synthesis of tryptophans from nitrotoluenes, through Michael addition of diethyl formamido-malonate to the α-nitroarylacrolein followed by reduction of the nitro-group and indolization.[135] A modified procedure using methyl nitroacetate gives *R*-6-methyltryptophan in 82% enantiomeric excess *via* the heteroarylidene-glycine ester and hydrogenation catalysed by a Rh-chiral phosphine catalyst.[135]

Full details have been published of the synthesis of anabilysine [1-(5-amino-5-carboxypentyl)pyridinium chloride][136] (see Vol. 10, p. 5).

[131] T. Nishitani, T. Iwasaki, Y. Mushika, and M. Miyoshi, *J. Org. Chem.*, 1979, **44**, 2019.
[132] J. W. Tilley, P. Levitan, and R. W. Kierstead, *J. Heterocycl. Chem.*, 1979, **16**, 333.
[133] P. M. Jacobs and M. A. Davis, *J. Org. Chem.*, 1979, **44**, 178.
[134] J. G. Atkinson, B. K. Watson, J. J. Fuenles, Y. Girard, and C. S. Rooney, *Tetrahedron Lett.*, 1979, 2857.
[135] U. Hengartner, A. D. Batcho, J. F. Blount, W. Leimgruber, M. E. Larscheid, and J. W. Scott, *J. Org. Chem.*, 1979, **44**, 3748.
[136] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2282.

Synthesis of *N*-Substituted α-Amino-acids.—As in previous volumes, this section deals with compounds of potential biological interest, but excludes *N*-protected amino-acids prepared for peptide synthesis. *N*-Nitroso-amines can be converted into *N*-nitroso-amino-acids through successive anion formation ($LiNPr^i_2$) and reaction with methyl chloroformate.[137]

Synthesis of Aminoxy-acids.—These compounds are being used as enzyme inhibitors[138] and for the synthesis of analogues of physiologically active peptides,[139] and can be obtained in optically active form from D- or L-amino-acids *via* the corresponding α-bromo-acids, using an *N*-alkoxycarbonylhydroxylamine and NaH.[139]

Synthesis of α-Amino-acids Containing Sulphur, Selenium, or Tellurium.—Whereas the lighter chalcogens have been represented in earlier volumes, and examples are included elsewhere in this Chapter, this section is reserved this year for the reports on synthesis of DL-telluromethionine, $MeTe(CH_2)_2CH(NH_3^+)CO_2^-$,[140] and its phenyl analogue,[141] formed from 2-bromoethylhydantoin and methaneteullurol and tellurophenol, respectively.

Synthesis of Phosphorus-containing α-Amino-acids.—DL-Phosphinothricin, $MeP(O)(OH)(CH_2)_2CH(NH_3^+)CO_2^-$, has been prepared through Strecker synthesis using $MeP(O)(OEt)CH_2CHO$.[142]

Amino-acids Synthesized for the First Time.—New amino-acids not mentioned elsewhere in this Chapter are collected here:

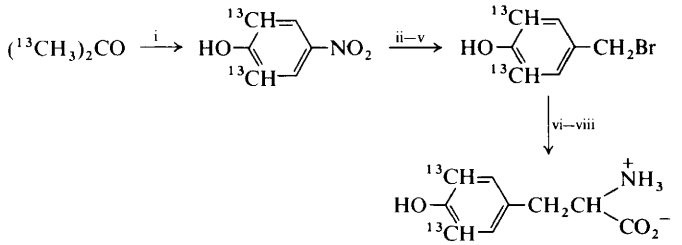
Compound	*Ref.*
DL-2-Amino-3-carboxymethylbutyric acid	143
DL- and *meso*-β-Aminoaspartic acid	144
DL-β-(Diazoacetyl)alanine	145
DL-β-(*O*-DL-Serylacetyl)alanine	145
DL-β-(*O*-DL-Threonylacetyl)alanine	145
DL-*S*-(Trialkylstannyl)cysteines	146
DL-*S*-(Trialkylstannyl)homocysteines	146
DL-*p*-Benzylphenylalanine	147
L-Tryptophan analogue with O in place of indole NH	148
L-Tryptophan analogue with S in place of indole NH	148
6-Fluoro-L-tryptophan	148
5-Fluoro-L-tryptophan	148
Tropolone analogue of DL-mimosine [DL-α-amino-β-(2-hydroxy-5-methyl-3-oxo-cyclohepta-1,4,6-trienyl)propanoic acid]	149
Tropolone analogue of DL-mimosine [DL-α-amino-β-(3,5-disubstituted 4-oxo-cyclohepta-1,4,6-trienyl propanoic acid]	150

[137] K. Piotrowska, *Synthetic Commun.*, 1979, **9**, 765.
[138] N. Amrhein and H. Hollaender, *Planta*, 1979, **144**, 385.
[139] M. T. Briggs and J. S. Morley, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2138.
[140] F. F. Knapp, *J. Org. Chem.*, 1979, **44**, 1007.
[141] F. F. Knapp, K. R. Ambrose, and A. P. Callahan, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 157.
[142] E. Gruszecka, M. Soroka, and P. Mastalerz, *Pol. J. Chem.*, 1979, **53**, 937.
[143] S. Mishima and K. Sakai, *Nippon Kagaku Kaishi*, 1978, 1675 (*Chem. Abstr.*, 1979, **90**, 120958).
[144] Y. Ozaki, T. Iwasaki, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, 1979, **44**, 1714.
[145] P. K. Chang, L. B. Lachman, and R. E. Handschumacher, *Int. J. Pept. Protein Res.*, 1979, **14**, 27.
[146] P. J. Smith, R. L. Hyams, J. S. Brooks, and R. W. Clarkson, *J. Organomet. Chem.*, 1979, **171**, C29.
[147] W. Podkoscielny, M. Podgorski, and E. Smulkowska, *Pol. J. Chem.*, 1978, **52**, 2455.
[148] H. M. Rajh, J. H. Uitzetter, L. W. Westerhuis, C. L. van den Dries, and G. I. Tesser, *Int. J. Pept. Protein Res.*, 1979, **14**, 68.
[149] T. Teitei, *Aust. J. Chem.*, 1979, **32**, 1631.
[150] T. Teitei and R. L. N. Harris, *Aust. J. Chem.*, 1979, **32**, 1329.

Synthesis of Labelled Amino-acids.—A larger number of papers than usual have been sieved from the literature on which this Chapter is based, partly because the proceedings of two major conferences have been published in 1979.

Raney nickel-catalysed deuteriation of phenylalanine and tyrosine brings about total exchange of aromatic protons after extended reaction periods, except the protons *ortho* to CH_2; while the α-CH proton exchanges slowly, the β-CH_2 protons exchange rapidly.[151] Specific labelling leading to *R*-4-[4-^{2}H]aminobutyric acid through decarboxylation of L-glutamic acid in ^2H_2O implies retention of configuration.[152] The ^{3}H-analogue is obtained similarly, from L-glutamic acid in ^3H_2O in the presence of L-glutamate decarboxylase,[153] whereas *S*-[^{3}H]-γ-aminobutyric acid is prepared from L-alanine with L-alanine transaminase in ^3H_2O.[154] The lability of protons α to sulphimide sulphur has been exploited in a conversion of L-methionine into its C^2H_3 analogue *via* 'dehydro-L-methionine' in MeO^2H–MeONa.[155]

The whole range of carbon isotopic labelling has been represented. ^{11}C-Carboxy-labelled hippuric acid,[156] L-alanine,[157] DL-alanine,[158] DL-tryptophan,[159] and a series of amino-acids[160] have been prepared from $^{11}CO_2$ through various strategies. $^{13}CH_3{}^{13}CO_2H$ and $(^{13}CH_3)_2CO$ have been employed in syntheses of [2,3-$^{13}C_2$]-D- and L-alanines,[161] and [3,5-$^{13}C_2$]-DL-tyrosine (Scheme 10),[162]



Reagents: i, $NaC(CHO)_2NO_2$; ii, $SnCl_2$, HCl; iii, diazotization, $Cu(CN)_2$; iv, H_3O^+; v, PBr_3; vi, $MeCONHCNa(CO_2Et)_2$; vii, NaOH (aq); viii, H_3O^+

Scheme 10

respectively. Alkylation of diethylphthalimidomalonate with $PhtNCH_2CH_2O$-$^{14}CH_2CH_2Cl$ and subsequent conventional steps leads to ^{14}C-labelled DL-2-amino-4-(2-aminoethoxy)butanoic acid.[163]

[151] R. C. Woodworth and C. M. Dobson, *FEBS Lett.*, 1979, **101**, 329.
[152] M. Kauska and S. Drabarek, *Radiochem. Radioanal. Lett.*, 1979, **38**, 155.
[153] E. Santaniello, M. G. Kienle, A. Manzocchi, and E. Bosisio, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1677.
[154] G. Burnett, C. Walsh, K. Yonaha, S. Toyama, and K. Soda, *J. Chem. Soc., Chem. Commun.*, 1979, 826.
[155] D. C. Billington and B. T. Golding, *Tetrahedron Lett.*, 1979, 2937.
[156] S. J. Gatley, J. S. Crawford, J. R. Halama, R. D. Hichwa, M. T. Madsen, J. L. Martin, R. J. Nickles, and D. J. Simpkin, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 182.
[157] M. B. Cohen, L. Spolter, C. C. Chang, and N. S. MacDonald, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 63.
[158] B. Langstrom and B. Stridsberg, *Int. J. Appl. Radiat. Isotopes*, 1979, **30**, 151.
[159] L. C. Washburn, T. T. Sun, B. L. Byrd, R. L. Hayes, and T. A. Butler, *J. Nuclear Med.*, 1979, **20**, 857.
[160] L. C. Washburn, T. T. Sun, B. L. Byrd, R. L. Hayes, T. A. Butler, and A. P. Callahan, *Report 1979* abstracted in *Chem. Abstr.*, 1979, **91**, 170853.
[161] V. N. Kerr and D. G. Ott, *J. Labelled Comp. Radiopharm.*, 1978, **15**, 503.
[162] V. Viswanatha and V. J. Hruby, *J. Org. Chem.*, 1979, **44**, 2892.
[163] Y. Y. Liu, E. Thom, and A. A. Liebman, *Can. J. Chem.*, 1978, **56**, 2853.

Double labelling achieved through relatively intricate synthetic routes, to provide amino-acids for use in biosynthetic investigations, has featured less in the 1979 literature than in preceding years; the examples which have been reported, 4*S*-[5-^{3}H, 5-^{14}C]-L-leucine and its [2-^{14}C]-isomer,[164] were prepared by a modification of a previously described route,[165] starting from 2*R*-[1-^{3}H]-2-methyl-4-phenylbutan-1-ol.

[β-^{13}N]-L-Asparagine[166] and [β-^{15}N]-L-leucine[167] have been prepared by conventional methods, and one standard procedure is illustrated[168] in the formation of [^{15}N]-L-glutamic acid from 2-ketoglutaric acid, $^{15}NH_4Cl$, and glutamate dehydrogenase–alcohol dehydrogenase.

Both oxygen atoms of the carboxy-group of an α-amino-acid can be exchanged, to an extent >90%, with $H_2{}^{18}O$ at elevated temperatures.[169]

^{76}Br- and ^{77}Br-labelled bromo-L-dopa and bromo-5-hydroxytryptophan have been prepared from the amino-acids and metal bromides, using chloroperoxidase.[170]

Resolution of Amino-acids.—Diastereoisomeric 2,5-dioxomorpholines formed between D-$HOCH_2CMe_2CH(OH)CO_2H$ and DL-proline can be separated and hydrolysed to yield the two enantiomers, with return of chiral reagent.[171] A similar example of this familiar general approach is the acylation of a DL-amino-acid with (+)-*trans*-1-cyclohexanedicarboxylic anhydride, followed by fractional crystallization and hydrolysis.[172] Further examples of techniques which have been represented regularly in this section in previous volumes are preferential crystallization [the D-isomer from aqueous solutions of DL-*p*-$HOC_6H_4CH(NH_3{}^+)CO_2HArSO_3{}^-$][173] and ligand-exchange chromatography (in which chloromethylated polystyrene is treated with a chiral amine, complexed with Cu^{2+} or Ni^{2+} ions, and used in the conventional column chromatography mode).[174,175] A novel variation of the ligand-exchange technique[176] employs an aqueous eluant containing a chiral copper(II)–proline complex for resolving DL-amino-acids by ion exchange and depends on the different stabilities of the diastereoisomeric amino-acid–copper(II) complexes. Partial asymmetric transformations of DL-α-amino-acids can be effected through reversible Schiff-base complex formation with cobalt(III) [1-(−)-menthyl β-(2-hydroxybenzoyl)propionate].[177]

[164] C. Fuganti, P. Grasselli, and G. Pedrocchi-Fantoni, *Tetrahedron Lett.*, 1979, 2453.
[165] R. Cardillo, C. Fuganti, D. Ghiringhelli, P. Graselli, and G. Gatti, *J. Chem. Soc., Chem. Commun.*, 1977, 474.
[166] D. R. Elmaleh, D. H. Hnatowich, and S. Kulprathipanja, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 92.
[167] R. A. Asaturyan, *Biol. Zh. Arm.*, 1978, **31**, 720 (*Chem. Abstr.*, 1979, **90**, 99 297).
[168] O. Bojan, M. Bologa, G. Niac, N. Palibroda, E. Vargha, and O. Barzu, *Anal. Biochem.*, 1979, **101**, 23.
[169] R. C. Murphy, F. A. Anderson, and K. L. Clay, in 'Stable Isotopes: Proceedings of the Third International Conference', ed. E. R. Klein and P. D. Klein, Academic Press, New York, 1979.
[170] A. M. Friedman, J. Cheronis, M. Zalutsky, M. Cooper, P. Hoffman, A. Heller, D. Israelstam, P. Herper, and T. Hill, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 66.
[171] T. D. Marieva, V. M. Kopelovich, and V. I. Gunar, *Khim. Prir. Soedin.*, 1979, 106.
[172] K. Murakami, N. Katsuka, K. Takano, Y. Yamamoto, T. Kakegawa, K. Saigo, and H. Nohira, *Nippon Kagaku Kaishi*, 1979, 765.
[173] S. Yamada, C. Hongo, and I. Chibata, *Agric. Biol. Chem.*, 1978, **42**, 1521.
[174] I. A. Yamskov, B. B. Berezin, V. E. Tikhonov, and V. A. Davankov, *Biorg. Khim.*, 1979, **5**, 492.
[175] A. A. Kurganov, L. Y. Zhuchkova, and V. A. Davankov, *Makromol. Chem.*, 1979, **180**, 2101.
[176] P. E. Hare and E. Gil-Av, *Science*, 1979, **204**, 1226.
[177] Y. Numata, H. Okawa, and S. Kida, *Chem. Lett.*, 1979, 293.

Further results from Cram's group on the differential complexation of D-and L-amino-acid ester salts by macrocyclic chiral polyethers have been reported,[178] and the demonstration of preferential complexation of an amino-acid ester in the presence of simple amines by an acyclic phosphine oxide ligand[179] opens up the possibility of resolution by chiral ligands of this family.

Explanations for the enantioselection which favoured the L-enantiomers of amino-acids in prebiotic times continue to be sought. The rare occurrence of a constructive outcome to a polemical debate can be reported; stereospecific binding of L-leucine, L-aspartic acid, and D-glucose to colloidal clay (bentonite)[180] has been noted earlier,[181] and this correction of a claim for a first finding has also led to the reasoning that now the implicit chirality of clay should be verified by other means.[182,183] An important observation,[184] that left circularly polarized light (284 nm) is absorbed and re-emitted by D-tryptophan in MeOH, with appreciably higher fluorescence efficiency than is shown by the L-enantiomer, may imply that enhanced stereoselectivity should be observed in photoreactions of these enantiomers, where intramolecular energy transfer is involved. A role for chiral electromagnetic radiation in enantioselection has long been sought (see previous volumes) and there should be scope for further deductions and associated experimentation based on this result.

A theoretical discourse on those weak neutral current effects that are capable of ensuring that only one enantiomer of an amino-acid is selected in the formation of self-replicating molecules has been published.[185]

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—Routine *X*-ray crystal-structure determinations, on which no further description is offered here, have been reported for γ-carboxy-L-glutamic acid,[186] α-methyl-DL-glutamic acid hemihydrate,[187] two crystal modifications of 3,5-dinitro-L-tyrosine,[188] *S*-carboxymethyl-L-cysteine,[189] sarcosine hydrochloride,[190] thienyl-DL-serine hydrate,[191] *N*-acetyl-L-4-hydroxyproline hydrate,[192] *N*-acetyl-DL-methionine diethyl- and *N*-acetyl-DL-ethylmethyl-amide,[193] *N*-acetyldehydroalanine,[194] *N*-t-butoxycarbonyldehydro-

[178] S. C. Peacock, L. A. Domeier, F. C. A. Gaeta, R. C. Helgeson, J. M. Timko, and D. J. Cram, *J. Am. Chem. Soc.*, 1978, **100**, 8190.
[179] A. H. Alberts, K. Timmer, J. G. Noltes, and A. L. Spek, *J. Am. Chem. Soc.*, 1979, **101**, 3375.
[180] S. C. Bondy and M. E. Harrington, *Science*, 1979, **203**, 1243.
[181] T. A. Jackson, *Science*, 1979, **206**, 483; *Science, Chem. Geol.*, 1971, **7**, 295.
[182] S. C. Bondy, *Science*, 1979, **206**, 484.
[183] D. Wellner, *Science*; 1979, **206**, 484.
[184] C. D. Tran and J. H. Fendler, *J. Am. Chem. Soc.*, 1979, **101**, 1285.
[185] K. Tennakone, *Prog. Theor. Phys.*, 1979, **62**, 841.
[186] K. A. Satyshur and S. T. Rao, *Acta Crystallogr.*, 1979, **B35**, 2260.
[187] C. Derricott and J. Trotter, *Acta Crystallogr.*, 1979, **B35**, 2230.
[188] V. Cody, D. A. Langs, and J. P. Hazel, *Acta Crystallogr.*, 1979, **B35**, 1829.
[189] A. D. Mighell, C. R. Hubbard, J. Harris, J. A. Staffa, and C. Zervis, *Acta Crystallogr.*, 1979, **B35**, 1258.
[190] S. C. Bhattacharyya and N. N. Saha, *J. Cryst. Mol. Struct.*, 1979, **8**, 105.
[191] S. Swaminathan and K. K. Chacko, *Acta Crystallogr.*, 1979, **B35**, 208.
[192] M. Hospital, C. Courseille, F. Leroy, and B. P. Roques, *Biopolymers*, 1979, **18**, 1141.
[193] A. Aubry, J. Protas, M. T. Cung, and M. Marraud, *Acta Crystallogr.*, 1979, **B35**, 2634.
[194] D. Ajo, G. Granozzi, E. Tondello, A. Del Pra, and G. Zanotti, *J. Chem. Soc., Perkin Trans. 2*, 1979, 927.

leucine (the *Z*-configuration is adopted),[195] creatine monohydrate,[196] and L-pyroglutamyl-*NN'*-dicyclohexylurea.[197]

The absolute configuration of (+)-coronamic acid is incorporated in its systematic name (+)-(1*S*,2*S*)-1-amino-2-ethylcyclopropanecarboxylic acid, and this result from *X*-ray crystal analysis corrects an earlier assignment (Vol. 10, p. 10) based on circular dichroism (c.d.) data.[198] A combined *X*-ray–i.r.–c.d. study of *N*-t-amyloxycarbonyl-L-proline,[199] and an *X*-ray–c.d. study of the four stereoisomers of *N*-acetyl-S-(2-nitro-1-phenylethyl)cysteine,[200] have been reported.

X-Ray analysis of the inclusion complex which separates from supersaturated solutions of *NN*-diethyl-β-alanine in benzene reveals the conformational features of the amino-acid derivative which facilitate complex formation.[201]

Recently reported *X*-ray and neutron-diffraction crystal structures of amino-acids have been studied [202] to determine the range of hydrogen-bonding distances involved.

N.M.R. Spectroscopy.—The continuing development of instrumental techniques is represented in the recent literature, which also contains reports of new results obtained with routine laboratory spectrometers.

Conformational studies dominate this section, with studies of the amino-acids themselves being outnumbered by studies of *N*-acyl amino-acid esters or amides to simulate the behaviour of side chains in peptides. ^{1}H-N.m.r. data of deuteriated DL-histidine at $p^2H = 8.2$ in ^2H_2O compared with data for the L-enantiomer permit the assignment of the lower and higher field resonances associated with the β-protons to the pro-*R*- and pro-*S*-protons, respectively.[203] DL-Carnitine studied under the same conditions has been shown [204] to adopt the *gauche* conformation about the C-3—C-4 bond and to undergo rapid rotation about the C-2—C-3 bond. *cis*-D-*allo*-Hydroxyproline [205] and *cis*-4-fluoro-L-proline,[206] the latter as its diketopiperazine derivative with D- or L-phenylalanine, have been studied; this project amounts to a re-investigation of ring conformations of the pyrrolidine amino-acids and reveals a γ-C-*endo*–δ-C-*exo*-envelope conformation for the former amino-acid.[205] ^{13}C-N.m.r. studies of glycine, alanine, and lysine in H_2O and ^2H_2O as a function of pH reveal ^{2}H isotope effects of up to 0.9 p.p.m.[207]

A comparison of vicinal proton coupling constants between α- and β-protons in amino-acids, with vicinal carboxylate ^{13}C–β-H coupling constants, has been made,

[195] V. S. Chauhan, C. H. Stammer, L. Norskov-Lauritzen, and M. G. Newton, *J. Chem. Soc., Chem. Commun.*, 1979, 412.
[196] Y. Kato, Y. Haimoto, and K. Sakurai, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 233.
[197] F. Bechtel, J. P. Bideau, and M. Cotrait, *Cryst. Struct. Commun.*, 1979, **8**, 815.
[198] A. Ichihara, K. Shiraishi, S. Sakamura, A. Furusaki, N. Hashiba, and T. Matsumoto, *Tetrahedron Lett.*, 1979, 365.
[199] E. Benedetti, A. Ciajolo, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, and C. M. Bonora, *Int. J. Pept. Protein Res.*, 1979, **14**, 130.
[200] W. Winter, G. Heusel, H. Fouad, and G. Jung, *Chem. Ber.*, 1979, **112**, 3171.
[201] M. A. Peterson, H. Hope, and C. P. Nash, *J. Am. Chem. Soc.*, 1979, **101**, 946.
[202] S. N. Vinogradov, *Biopolymers*, 1979, **18**, 1559.
[203] M. Kainosho, K. Ajisaka, S. Sawada, M. Tanokura, and T. Miyazawa, *Chem. Lett.*, 1979, 395.
[204] G. Agostini, F. Coletta, A. Gambaro, and S. Castellano, *Spectrochim. Acta, Part A*, 1979, **35**, 733.
[205] M. J. O. Anteunis, P. Cuypers, and M. Budesinsky, *Bull. Soc. Chim. Belg.*, 1979, **88**, 147.
[206] M. J. O. Anteunis, P. Cuypers, B. Liberek, and A. Kolodziejczyk, *Bull. Soc. Chim. Belg.*, 1978, **87**, 877.
[207] J. J. Led and S. B. Petersen, *J. Magn. Reson.*, 1979, **33**, 603.

with the objective of improved conformational diagnosis in the amino-acid series.[208] Wide-line n.m.r. of amino-acids has received further attention with particular reference to their dynamic properties in solution.[209]

Conformational studies for *N*-acetyl-L-phenylalanine methyl ester[210] and *N*-acetyl- or *N*-benzoyl-L-proline methyl ester[211] have sought similar information in terms of side-chain structure and mobility. The use of lanthanide shift reagents shows[211] that the pyrrolidine ring in the proline derivatives in C^2HCl_3 is mainly (60%) in a half-chair conformation (C-γ up, C-β down, CO_2R up) and 40% in an envelope conformation (C-γ down). Self-association of *N*-acetyl-L-alanine methyl ester or methylamide[212] in solution takes the form of dimerization, with the N–H proton bonded intermolecularly to the carboxyl oxygen atom in the case of the ester, and the amide N–H proton bonded to the acetyl carbonyl oxygen atom in the case of the amide.[212] Self-association is also observed with *N*-acetyl-amino-acid diethylamides in CCl_4.[213]

The chemical shift of the amide proton in an α-acyl-amino-acid is in a linear relationship with the electronegativity of the acyl group [δ (p.p.m.) = $-0.22\,pK_a + 9.18$].[214] The comparable relationship between C=O stretching frequency and electron release by the acyl group was established several years ago.[215]

Significant deductions for other structural features from n.m.r. data have been reported for histidine,[216] where the small red shift in the visible absorption spectrum and associated ^{1}H-n.m.r. changes in the presence of the dye Rose Bengal suggest an interaction in solution between the imidazole ring and the dye, and for N^G-nitroarginine methyl ester hydrochloride, where ^{15}N- and ^{1}H-n.m.r. data reveal the adoption of the nitrimine tautomeric structure $H_2NC(=NNO_2)$-$NH(CH_2)_3$ $CH(NH_3^+)CO_2Me\ Cl^-$.[217]

O.R.D. and C.D. Spectra.—Routine uses of c.d. are represented in studies combining several spectroscopic techniques, *viz.* *X*-ray, i.r., and c.d. studies of *N*-t-amyloxycarbonyl-L-proline revealing solvation effects and solute–solute association,[199] and in the assignment of absolute configuration to (+)-coronamic acid.[198] There are lessons to be learned from these studies; c.d. data taken alone can lead to incorrect information on solution conformations of amino-acid derivatives,[199] and use of an empirical sector rule led to the wrong absolute configuration in the latter case.[198] Although a chirality rule has been proposed[218] to relate the sign of the Cotton effect near 330 nm with absolute configuration for the 1-pyrrolinones obtained from α-amino-acid esters with fluorescamine, a major

[208] R. B. Martin, *J. Phys. Chem.*, 1979, **33**, 2404.
[209] S. Ganapathy and R. Srinivasan, *Ind. J. Biochem. Biophys.*, 1979, **16**, 310.
[210] N. Nicolai and E. Tiezzi, *J. Phys. Chem.*, 1979, **83**, 3249.
[211] D. F. DeTar and N. P. Luthra, *J. Org. Chem.*, 1979, **44**, 3299.
[212] T. Asukura, M. Kamio, and A. Nishioka, *Biopolymers*, 1979, **18**, 467.
[213] K. Mizuno, S. Nishio, and Y. Shindo, *Biopolymers*, 1979, **18**, 693.
[214] Y. Shimohigashi, T. Kato, S. Kang, Y. Minematsu, M. Waki, and N. Izumiya, *Tetrahedron Lett.*, 1979, 1327.
[215] H. Determann, J. Heuer, P. Pfaender, and M.-L. Reinartz, *Liebigs Ann. Chem.*, 1966, **94**, 190.
[216] A. Sidorowicz, *Stud. Biophys.*, 1978, **73**, 185.
[217] D. Gust, G. Dirks, and G. R. Pettit, *J. Org. Chem.*, 1979, **44**, 314.
[218] V. Toome and B. Wegrzynski, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1496.

revision of this rule is needed since examples of false conclusions have been uncovered.[219]

Further examples of chromophoric derivatives of amino-acids have been reported, namely *N*-(2-pyrazinoyl)-α-amino-acid esters,[220] stereoisomers of *N*-acetyl-*S*-(2-nitro-1-phenylethyl)cysteine,[200] and the 2,4-dinitrophenyl *p*-methoxyanilides of the iturinic acids, $MeCHR(CH_2)_8CH(NH_2)CH_2CO_2H$ (R = Me, Et), released from iturin A on hydrolysis (c.d. data permit the assignment of the *R*-configuration at C-3).[221]

An analysis of the c.d. and magnetic c.d. of phenylalanine and its derivatives has been reported,[222] and in a different region of the electromagnetic spectrum an extension of work reported last year (Vol. 11, p. 20) on vibrational c.d. involves studies of L-alanine and L-serine in both solid and solution states.[223]

Mass Spectrometry.—The non-routine literature has moved entirely across to the applications of sophisticated instrumentation for gathering structural and analytical information. In the former category, developments of earlier studies have been reported on the determination of proton affinities of amino-acids [224] and of gas-phase acidity and basicity of glycine by ion cyclotron resonance.[225] In the latter category, all the papers cited here are based on chemical ionization mass spectrometry data for *N*-trifluoroacetyl amino-acid carboxybutyl esters,[226] protonated di-amino-acids,[227] trimethylsilylated amino-acids,[228] and thiohydantoins.[229] Further references to mass spectrometric analysis of amino-acids can be found in the later section covering gas–liquid chromatography.

Other Physical and Theoretical Studies.—Infrared and Raman spectrometric studies seem to have returned to greater favour in the amino-acid field, with studies of alanine in solid and solution states,[230] of *NN*-dibutylglycine in solution and vapour states,[231] of serine in solid [232] and solution states,[233] and *N*-acetyl methylamides of glycine, alanine, and leucine in $CHCl_3$ or CCl_4.[234] Points of interest from these studies include evidence from Raman spectra that L-serine exists in at least two different conformations in aqueous solutions,[233] and clear evidence for strong NH hydrogen bonding was not obtained for the C-7 (eq) conformation of an *N*-acetyl-amino-acid methylamide.[234]

[219] K. L. Kovacs, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 995.
[220] M. M. El-Abadelah, S. S. Sabri, A. A. Jarrar, and M. H. Abu Zarga, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2881.
[221] U. Nagai, F. Besson, and F. Peypoux, *Tetrahedron Lett.*, 1979, 2359.
[222] T. Komiyama and M. Miwa, *Chem. Phys. Lett.*, 1979, **65**, 136.
[223] M. Diem, E. Photos, H. Khouri, and L. A. Nafie, *J. Am. Chem. Soc.*, 1979, **101**, 6829.
[224] M. Meot-Ner, E. P. Hunter, and F. H. Field, *J. Am. Chem. Soc.*, 1979, **101**, 686.
[225] M. J. Locke, R. L. Hunter, and R. T. McIver, *J. Am. Chem. Soc.*, 1979, **101**, 272.
[226] E. E. Kingston and A. M. Duffield, *Biomed. Mass Spectrom.*, 1978, **5**, 621.
[227] R. J. Weinkam, *J. Org. Chem.*, 1978, **43**, 2531.
[228] H. Budzikiewicz and G. Meissner, *Org. Mass Spectrom.*, 1978, **13**, 608.
[229] K. Okada and A. Sakuno, *Org. Mass Spectrom.*, 1978, **13**, 535.
[230] M. Castineira and J. Herranz, *An. Quim.*, 1979, **75**, 40, 250.
[231] G. S. Denisov, I. G. Rumynskaya, and V. M. Shraiber, *Zh. Prikl. Spektrosk.*, 1979, **31**, 275.
[232] C. Madec, J. Lauransan, and C. Garrigou-Lagrange, *Can. J. Spectrosc.*, 1978, **23**, 166.
[233] C. Madec, J. Lauransan, and C. Garrigou-Lagrange, *C. R. Hebd. Seances Acad. Sci., ser. B*, 1979, **288**, 69.
[234] F. R. Maxfield, S. J. Leach, E. R. Stimson, S. P. Powers, and H. A. Scheraga, *Biopolymers*, 1979, **18**, 2507.

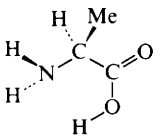
Polarized Raman spectra of a single crystal of α-glycine-C-$^2H_2$ and DL-alanine-α,β-$^2H_4$ have been determined.[235]

Photoelectron spectra of amino-acids have been surveyed,[236] and a new application of this technique to gaseous L-tryptophan has been reported.[237] *X*-Ray electron and Auger spectroscopic studies of glycine, alanine, and serine deposited on silver sheets from aqueous solutions have been described.[238]

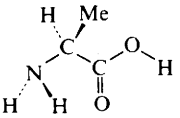
Solute–solvent interactions are open to study by ultrasonic absorption methods, and the pH dependence of the interaction of amino-acids with water has been studied.[239]

A range of titration studies have been reported, covering first and second dissociation constants of glycine in THF–H_2O mixtures,[240] p*K*–structure relationships for *N*-substituted α,ω-di-amino-acids,[241] self-association constants of L-amino-acids in aqueous solutions,[242] and demonstration of the effect of a 4-nitro-group on enhancing the acidity of histidine.[243]

Molecular orbital calculations include a topic related to the experimental studies mentioned in the preceding paragraph, a ranking of side-chain proton affinities Arg > His > Ser ≈ Tyr > Lys.[244] Apart from a study in which experimental data on geminal carboxyl-^{13}C–H coupling constants $^3J(C^1H)$ are compared with calculations for α-amino-acids,[245] the other theoretical papers collected here are concerned with conformational assignments. *N*-Acetyl-amino-acid methylamides have available to them a number of minimum-energy conformations when varying hydration patterns are considered, and molecular orbital calculations have been employed to define the different forms.[246] Minimum-energy conformations of L-glutamine and of its metabolic antagonist *S*-carbamoyl-L-cysteine show that these two amino-acids have complementary conformational features, which may be a major factor in accounting for their functions.[247] The conformations (13) and (14) emerge from molecular orbital calculations for alanine as the most stable forms of



(13)



(14)

[235] K. Machida, A. Kagayama, and Y. Saito, *J. Raman Spectrosc.*, 1979, **8**, 133.
[236] P. H. Carrington and N. S. Ham, *J. Electron Spectrosc. Rel. Phenom.*, 1979, **15**, 79.
[237] K. Seki and H. Inokuchi, *Chem. Phys. Lett.*, 1979, **65**, 158.
[238] R. J. Cotton, J. S. Munday, J. R. Wyatt, and J. J. DeCorpo, *Surface Sci.*, 1979, **84**, 235.
[239] A. P. Sarvazyan, D. P. Kharakoz, and P. Hemmes, *J. Phys. Chem.*, 1979, **83**, 1796.
[240] R. N. Roy, J. J. Gibbons, J. L. Padron, K. Buechter, and S. Faszholz, in 'Thermodynamic Behaviour of Electrolytes in Mixed Solvents', Advances in Chemistry Series, American Chemical Society, 1979, No. 177, p. 277.
[241] J. T. Edward, P. G. Farrell, J. C. Halle, J. Kirchnerova, R. Schaal, and F. Terrier, *J. Org. Chem.*, 1979, **44**, 615.
[242] J. Vliegen and L. C. Van Poucke, *Bull. Soc. Chim. Belg.*, 1978, **87**, 837.
[243] E. Gitalt, M. D. Ludevid, F. Albericio, and M. Bassedas, *Bioorg. Chem.*, 1979, **8**, 59.
[244] P. G. Mezey, J. J. Ladik, and S. Suhai, *Theor. Chim. Acta*, 1979, **51**, 323.
[245] P. Mohanakrishnan and K. R. K. Easwaran, *Org. Magn. Reson.*, 1979, **12**, 196.
[246] Z. I. Hodes, G. Nernethy, and H. A. Scheraga, *Biopolymers*, 1979, **18**, 1565.
[247] A. U. De and A. K. Ghose, *Ind. J. Chem.*, 1978, **16B**, 717.

the amino-acid;[248] these are closely similar to the preferred conformations established by various methods for glycine.

5 Chemical Studies of Amino-acids

Racemization.—The use of racemization kinetic data of amino-acids to provide estimates of the age of recent fossils, average temperatures at sites where these fossils are found, and other geochronological information has been a feature of this section in each of the preceding volumes, and Bada's pioneering work has been augmented by studies by other research groups. There is once again no shortfall in the chemical and intellectual interest to be found in the work reported this year, a spectacular example[249] being the use of the degree of conversion of isoleucine to alloisoleucine in fossil bones of 'Peking man' to provide a date 3.7—4.6 $\times$ 10^5 years for this specimen. Data for 'Lantian man' and 'Yuanmo man' are also included in this study.[249] The dangers of false conclusions arising from such factors as catalysis of the racemization process, temperature variations at the site over the years, and differential leaching of amino-acid enantiomers from the specimen have all been well appreciated; one cautionary result[250] is the finding that the rate of epimerization of isoleucine in peptides depends on the structure of the peptide, and another is the fact that the racemization of L-aspartic acid in aqueous solution is accelerated when D-glucose is added, as a result of Schiff-base formation.[251] The analytical method based on g.l.c. separation of diastereoisomeric derivatives (see later section) must also be reliable, and comparisons of data obtained in different laboratories for fossil bones show high reproducibility for the D : L ratio for aspartic acid (the amino-acid best suited for dating purposes since it racemizes more rapidly than other protein amino-acids).[252] However, it is not clear why comparable reproducibility for the D : L ratios for glutamic acid and for alanine from the same sources in different laboratories could not be secured.[252] Further work (see Vol. 11, p. 23) on the use of the γ-carboxyglutamic acid content of a fossil bone as a quantitative index of leaching suggests that there should be no significant error introduced in this way.[253]

Dating a femur from a Yuha skeleton at 23 600 years, using a measured D : L ratio for aspartic acid of 0.52—0.56 and taking into account the known temperature variation of racemization for this amino-acid, gives a result which is consistent with both ^{14}C and ^{230}Th dates for the fossil, based on sampling of the calcrete coating on the bone.[254]

Ongoing studies of amino-acid racemization, which are aimed at the implications of the findings that amino-acids present in meteorites are invariably racemic, involve 'radioracemization'.[255, 256] Optically pure protein amino-acids suffer

[248] H. L. Sellers and L. Schaefer, *Chem. Phys. Lett.*, 1979, **63**, 609.
[249] R.-W. Li and D.-X. Lin, *Ti Chih K'o Hsueh*, 1979, 56 (*Chem. Abstr.*, 1979, **90**, 167 295).
[250] N. Kriausakal and R. M. Mitterer, *Science*, 1978, **201**, 1011.
[251] J. E. Zumberge, *Geochim. Cosmochim. Acta*, 1979, **43**, 1443.
[252] J. L. Bada, E. Hoopes, D. Darling, G. Dangworth, H. J. Kessels, K. A. Kvenvolden, and D. J. Blunt, *Earth Planet. Sci. Lett.*, 1979, **43**, 265.
[253] K. King and J. L. Bada, *Nature (London)*, 1979, **281**, 135.
[254] J. L. Bischoff and W. M. Childers, *Earth Planet. Sci. Lett.*, 1979, **45**, 172.
[255] W. A. Bonner, N. E. Blair, and R. M. Lemmon, *Origins Life*, 1979, **9**, 279.
[256] W. A. Bonner, N. E. Blair, and R. M. Lemmon, *J. Am. Chem. Soc.*, 1979, **101**, 1049.

racemization in neutral (but not in acid) solutions, as well as decomposition under ^{60}Co γ-ray irradiation, and even isovaline (present, like certain protein amino-acids, in the Murchison meteorite) undergoes 4.8% racemization as well as *ca.* 79% destruction under these conditions.[256]

Racemization of amino-acids *via* Schiff-base formation in the presence of metal ions is a well known phenomenon. The role of Zn^{2+} ions in the racemization of L-alanine in media containing pyruvic acid is to stabilize the Schiff base since Zn^{2+} ions alone do not promote racemization of this amino-acid at pH $\geqslant$10 and at $\leqslant$80 °C.[257] Poly(acylamidosalicylaldehyde)s have been employed in the racemization of L-amino-acids.[258] α-Amino-ε-caprolactam is rapidly racemized as its nickel(II) chloride complex $(DL\text{-aa})_3NiCl_2$ in refluxing EtOH containing catalytic amounts of EtONa.[259]

The mechanism of the direct exchange racemization of cysteine derivatives, involving the β-heteroatom, has been investigated further,[260] supporting the view that electronic factors determine the rate of base-catalysed racemization through a series of cysteine analogues.

General Reactions.—Sensitized photo-oxidation [261] and ultrasonic degradation [262] are not responded to equally by the common protein amino-acids. Glycine is unaffected by the former treatment,[261] while serine, threonine, proline, and valine are not degraded during ultrasonication.[262] Slight losses of aspartic acid, alanine, and alloisoleucine must be expected during ultrasonic treatment of geological samples in preparation for amino-acid analysis, and this must be borne in mind as a source of error in amino-acid dating studies. The slight *increases* in the amounts of glycine, glutamic acid, leucine, and especially isoleucine which result from ultrasonic treatment of amino-acid mixtures [262] must mean that these amino-acids are formed from the amino-acids which are 'lost' from the mixtures.

More drastic degradation is brought about by pyrolysis; crystalline anhydrous L-leucine-^{14}C has been subjected to treatment at 451—578 K in order to check the likely survival of amino-acids in ancient (*i.e.* pre-Cambrian) geological samples.[263] A result with frightening dietary implications has been described; amino-acids heated with benzaldehyde or carbohydrates at 40—80 °C in a buffer at pH = 5.4 generate HCN as long as oxygen is not excluded.[264] The kinetics of $KMnO_4$ oxidation of amino-acids in moderately concentrated H_2SO_4 in the presence of a silver(I) salt represents another area of study in which a wide range of variations of the available parameters of this well studied reaction continues to be investigated.[265] Complex formation rather than oxidation is the result of the interaction of amino-acids with osmium(IV) oxide [266] (see also ref. 319).

Reduction of amino-acids to aldehydes is not a well established preparative procedure, and the problem has been tackled by two groups in different

[257] A. Dempsey and S. A. Phipps, *Inorg. Chim. Acta,* 1979, **36**, L425.
[258] I. A. Yamskov, V. E. Tikhonov, and V. A. Davankov, *Vysokomol. Soedin.*, 1979, **21A**, 1838.
[259] W. J. Boyle, S. Sifniades, and J. F. Van Peppen, *J. Org. Chem.*, 1979, **44**, 4841.
[260] M. Barber, J. H. Jones, and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1,* 1979, 2425.
[261] R. Straight and J. D. Spikes, *Photochem. Photobiol.*, 1978, **27**, 565.
[262] B. J. Katz and E. H. Man, *Geochim. Cosmochim. Acta,* 1979, **43**, 1567.
[263] G. A. Lavrentev, T. F. Strigunkova, and I. A. Egorov, *Dokl. Akad. Nauk. SSSR,* 1979, **248**, 1474.
[264] G. Lehmann and H. D. Zinsmeister, *Z. Lebensm.-Unters. -Forsch.*, 1979, **169**, 357.
[265] V. S. Rao, B. Sethuram, and T. N. Rao, *Int. J. Chem. Kinet.*, 1979, **11**, 165.
[266] A. J. Nielson and W. P. Griffith, *J. Chem. Soc., Dalton Trans.*, 1979, 1084.

ways;[267, 268] a reliable, though long, procedure[267] involves conversion of the amino-acid into the corresponding diazomethylketone (*via* the mixed anhydride), thence to the trifluoroacetoxymethyl ketone and the derived hemiacetal ($RCO_2H \rightarrow RCOCHN_2 \rightarrow RCOCH_2OCOCF_3 \rightarrow RCH(OH)CH_2OH \rightarrow RCHO$). Alternatively,[268] the amino-acid may be converted into an *N*-protected amino-acid imidazolide, which is then subjected to reduction by di-isobutyl aluminium hydride.

A mechanistic study of the decomposition of *N*-chloro-amino-acids into aldehydes, NH_3, CO_2, and α-keto-acid has been undertaken, starting with *N*-chloroalanine.[269]

Dehydroascorbic acid reacts with an α-amino-acid to give several products, one of which is the tri(2-deoxy-2-L-ascorbyl)amine, the source of the relatively stable blue radical formed by air oxidation.[270] The converse process, the reactions of α-amino-acids with radicals, is represented by reactions with chlorine radical anions involving H-abstraction,[271] and with azide radicals.[272] Kinetic studies of reactions of α-amino-acids with 4-dimethylamino-1-methoxycarbonylpyridinium chloride,[273] with 1,2-diketo-compounds,[274] and with *o*-phthalaldehyde and mercaptoethanol[275] have been described. Points of interest arising from these studies are the wide variations in reaction rates with 1,2-diketones, the highest rates being for the basic and the hydroxy-amino-acids,[274] the fact that the *o*-phthalaldehyde system is now widely used for fluorimetric analysis of amino-acids, and rate differences between the different amines can be exploited in novel ways (*e.g.* the assay of α-amino-acid esters in the presence of amino-acids).[275]

Amino-acid derivatives have a number of uses in synthesis, not only in areas of heterocyclic chemistry; but their reactions are also of interest as models of the behaviour of amino-acid residues in peptides and proteins. Hydrolysis of amino-acid esters catalysed by copper or nickel complexes of polystyrene-bound L-cysteine proceeds at different rates for D- and L-enantiomers.[276] Similar rate differences are found in the hydrolysis of alkoxycarbonyl-L-amino-acid *p*-nitrophenyl esters catalysed by *N*-lauroyl-D- or *N*-lauroyl-L-histidine in the presence of a cationic micellar compound.[277] Conversion of α-amino-acid esters into α-keto-esters through reaction with toluene-*p*-sulphenyl chloride followed by treatment with PPh_3 and silica also constitutes a synthesis of α-(toluene-*p*-sulphenylimino)esters.[278] Further intramolecular thermal cycloaddition reactions of Schiff bases derived from α-amino-acid esters have been described (see also Vol. 11, p. 25).[279]

[267] R. P. Sharma, M. G. Gore, and M. Akhtar, *J. Chem. Soc., Chem. Commun.*, 1979, 875.
[268] H. Khatri and C. H. Stammer, *J. Chem. Soc., Chem. Commun.*, 1979, 79.
[269] W. D. Stanbro and W. D. Smith, *Environ. Sci. Technol.*, 1979, **13**, 446.
[270] T. Hayashi and M. Namiki, *Tetrahedron Lett.*, 1979, 4467.
[271] T. Masuda, S. Shimada, and M. Kondo, *J. Radiat. Res.*, 1979, **20**, 209.
[272] E. J. Land and W. A. Pruetz, *Int. J. Radiat. Biol. Relat. Stud. Phys., Chem., Med.*, 1979, **36**, 75.
[273] G. Guillot-Edelheit, M. Laloi-Diard, E. Guibe-Jampel, and M. Wakselman, *J. Chem. Soc., Perkin Trans. 2*, 1979, 1123.
[274] M. Piloty and W. Baltes, *Z. Lebensm.-Unters. -Forsch.*, 1979, **168**, 368, 374.
[275] V. J. K. Svedas, I. J. Galaev, I. L. Borisov, and I. V. Berezin, *Anal. Biochem.*, 1979, **101**, 188.
[276] I. A. Yamskov, B. B. Berezin, L. A. Belchich, and V. A. Davankov, *Makromol. Chem.*, 1979, **180**, 799.
[277] K. Yamada, H. Shosenji, and H. Ihara, *Chem. Lett.*, 1979, 491; K. Yamada, H. Shosenji, H. Ihara, and Y. Otsubo, *Tetrahedron Lett.*, 1979, 2529.
[278] E. M. Gordon and J. Pluscec, *J. Org. Chem.*, 1979, **44**, 1218.
[279] R. Grigg, M. Jordan, and J. F. Malone, *Tetrahedron Lett.*, 1979, 3877.

Reactions of *N*-substituted α-amino-acids described in the recent literature include Kolbe electrolysis of *p*-nitrobenzoylglycine in MeOH to give the acetal *p*-$NO_2C_6H_4CONHCH_2OMe$[280] and the synthesis of symmetrical anhydrides from *N*-alkoxycarbonyl-α-amino-acids using 0.5 equiv. *N*-ethyl-*N'*-3-dimethylaminopropylcarbodi-imide hydrochloride;[281] a full account has been published [282] of the alternative course for this reaction, the cyclization of *N*-benzyloxycarbonyl-L-amino-acids into 2-benzyloxy-4-substituted L-oxazolin-5-ones (see Vol. 10, p. 23).[282] An interesting result, established by the isolation of $HO_2CNHCH_2CONH_2$ from the hydrogenolysis of *N*-benzyloxycarbonylglycinamide, confirms that carbamic acids are intermediates in this widely used *N*-protection–deprotection technique.[283]

A useful paper defining the preparation of L-amino-acid 2,2,2-trichloroethyl esters has been published.[284] A mild methyl esterification procedure for amino-acids has been uncovered[285] during studies of the four-component condensation reaction; an *N*-substituted amino-acid and the enamine $Me_2C{=}CNR^1R^2$ [$R^1R^2 = (CH_2)_2O(CH_2)_2$] yield the methyl ester with Bu^tNC and MeOH.

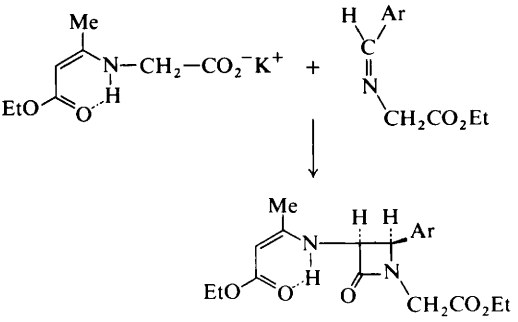
A study of the nitrosation of functional groups in the protein amino-acids, other than the α-amino-group, has been fully described;[286] aqueous acetic acid solutions of *N*-acetylcysteine methyl ester to which $NaNO_2$ is added yield the corresponding cystine derivative *via* the thionitrite, while the tyrosine analogue gives a mixture of 3-nitro- and 3-nitroso-derivatives. *N*-Acetyl-histidine methyl ester and the methionine analogue are not nitrosated under these conditions, but only in more highly acidic media. Methionine gives a mixture of *N*-nitroso-amide and *N*-nitrosamine-*S*-oxide, while nitrosation of tryptophan derivatives at the indole nitrogen atom gives a derivative easily solvolysed in aqueous MeOH.[286] Two reports have appeared on the decarboxylation of *N*-nitroso-α-amino-acids, one dealing with the reaction of the sodium salts in refluxing diglyme,[287] the other employing lead tetra-acetate as reagent.[288]

Specific Reactions of Natural Amino-acids.—Papers dealing with reactions of side-chain functional groups are collected in this section, together with some uses in synthesis of natural amino-acids (other than use for the synthesis of peptides).

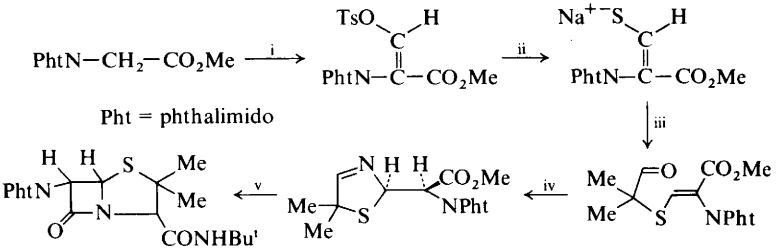
Formaldehyde reacts with L-lysine to give N^{ε}-formyl-lysine as well as N^{ε}-methyl-lysine already reported as reaction product,[289] but mono- and di-methyl-lysines are the only derivatives so far isolated from formaldehyde-treated proteins, *e.g.* casein.[290] Reversible reductive alkylation of N^{α}-acetyl-L-lysine with an α-hydroxyaldehyde or ketone in the presence of $NaBH_4$ has been demonstrated.[291]

[280] U. Hess, T. Gross, and R. Thiele, *Z. Chem.*, 1979, **19**, 195.
[281] F. M. F. Chen and N. L. Benoiton, *Synthesis*, 1978, 928.
[282] J. H. Jones and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1*, 1979, 3203.
[283] I. Schon and L. Kisfaludy, *Z. Naturforsch., Teil B*, 1978, **33**, 1196.
[284] J. F. Carson, *Synthesis*, 1979, 24.
[285] G. Skorna and I. Ugi, *Chem. Ber.*, 1979, **112**, 776.
[286] R. Bonnett and P. Nicolaidou, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1969.
[287] M. Nakajima and J.-P. Anselme, *Tetrahedron Lett.*, 1979, 4037.
[288] J. E. Saavedra, *J. Org. Chem.*, 1979, **44**, 4511.
[289] L. Treszd, I. Rusznak, E. Tyihak, T. Szarvas, I. Markus, and T. Muller, *Proc. Hung. Ann. Meetings Biochem.*, 1979, 33 (*Chem. Abstr.*, 1980, **92**, 36 263).
[290] T. Nakai, T. Ohta, and N. Hayase, *Agric. Biol. Chem.*, 1979, **43**, 1779.
[291] K. F. Geoghegan, D. M. Ybarra, and R. E. Feeney, *Biochemistry*, 1979, **18**, 5392.

Glycine provides the starting material for both components in a synthesis of β-lactams (Scheme 11),[292] and is used in a synthesis of penicillins employing the four-component condensation (Scheme 12).[293]



Scheme 11



Reagents: i, HCO_2Me + Na, then TsCl; ii, NaSH; iii, $BrCMe_2CHO$; iv, NH_4OAc; v, LiI, dmf–py, heat, then Bu^tNC

Scheme 12

Serine and threonine are degraded in aqueous HCO_2H at 10 °C during 16 h, to alanine and α-aminobutyric acid respectively.[294] Reactions at the hydroxy-group in these amino-acids include a convenient methylation procedure using MeI and NaOMe,[295] and condensation of a Schiff base obtained by reaction with formaldehyde to give the corresponding oxazolidinecarboxylic acids.[296] The analogous reaction with cysteine has been known for many years; a recent report[297] that unequal amounts of diastereoisomers are formed in the condensation of *p*-tolualdehyde with L-cysteine has been shown to be incorrect, since epimerization occurs readily.[298] On a preparative scale, epimerization of thiazolidin-4-carboxylic acids (at the C-4 chiral centre) is effected without ring opening in Ac_2O at 100 °C.[298] The SH group reacts more readily than the NH_2

[292] S. D. Sharma, M. Sunita, and P. K. Gupta, *Tetrahedron Lett.*, 1979, 1265.
[293] A. Schutz and I. Ugi, *J. Chem. Res.* (*S*), 1979, 157.
[294] A. S. Subbaraman, Z. A. Kazi, and A. S. U. Choughuley, *Ind. J. Biochem. Biophys.*, 1979, **16**, 253.
[295] F. M. F. Chen and N. L. Benoiton, *J. Org. Chem.*, 1979, **44**, 2299.
[296] S. Wolfe, G. Militello, C. Ferrari, S. K. Hasan, and S. L. Lee, *Tetrahedron Lett.*, 1979, 3913.
[297] R. Parthasarathy, B. Paul, and W. Korytuyk, *J. Am. Chem. Soc.*, 1976, **98**, 6634.
[298] L. Szilagyi and Z. Gyorgydeak, *J. Am. Chem. Soc.*, 1979, **101**, 427.

group in the reaction of cysteine with isothiocyanates.[299] *S*-Alkylation of cysteine can be achieved by reaction with thiols and sulphides under the conditions normally used for protein hydrolysis,[300] revealing a source of potential artefacts in this process. *S*-Alkylation has, however, not been observed in the reaction of alanine-3-sulphinic acid with 2-mercaptoethanol at 110 °C at pH = 15, (cysteine, cystine, and L-2-amino-7-hydroxy-4,5-dithiaheptanoic acid are formed) or at pH = 4, when the mixed cysteine–mercaptoethanol disulphide is formed.[301]

A reaction which is well known as a general procedure, the conversion of α-amino-acids into hydroxy analogues, involves retention of configuration when used with D-methionine.[302]

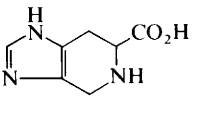
Optically pure (*N*-tetrafluoropropionyl-L-prolyl)anhydride can be obtained from the optically pure acid by reaction with the corresponding acid chloride, even though the latter is partially racemized whatever method is used for its preparation.[303]

The chemistry of the phenyl and heteroaromatic moieties of phenylalanine, tyrosine, histidine, and tryptophan is discussed in several recent papers. These are largely unrepresentative of current trends in aromatic and heteroaromatic chemistry since the objectives of work with these amino-acids are frequently in response to observations of unusual reactions occurring during peptide synthesis, or in response to problems arising from side-chain protection. Indoles are particularly susceptible to substitution by t-butyl carbonium ions, and tryptophan yields the 2,5,7-tri-t-butyl derivative with Bu^{t}OH–TFA;[304] its *N*-benzyloxycarbonyl benzyl ester undergoes substitution at positions 1, 3, and 5 of the indole moiety through reaction with t-butyl acetate and TFA,[305] while its *N*-(*p*-methoxybenzyl)oxycarbonyl derivative suffers *p*-methoxybenzylation at positions 1, 2, and 5 on acidolysis in TFA.[306] Tyrosine gives 3′-t-butyl tyrosine with t-butyl trifluoroacetate.[307]

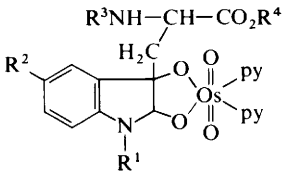
Protection of the imidazole moiety of histidine, and reactions of this grouping, are represented in several papers; *N*-acetyl histidine methyl ester gives equal amounts of N^τ- and N^π-hydroxyethyl-L-histidines after reaction with ethylene oxide followed by hydrolysis,[308] N^τ-phenacyl histidine derivatives predominate in the reaction product from the silver(I) salt of *N*-benzyloxycarbonyl histidine methyl ester and phenacyl bromide,[309] while the N^π-phenacyl isomers can best be made *via* the N^τ-trityl analogues.[309] N^τ-Methyl- or ethyl-L-histidines are prepared by cyclization of histidine methyl ester to the imidazo[1,5-*c*]pyrimidine derivative

[299] D. Podhradsky, L. Drobnica, and P. Kristian, *Experientia*, 1979, **35**, 154.
[300] C. J. Calleman, J. Ehrenberg, G. Osterman-Golkar, and D. Segerbaeck, *Acta Chem. Scand.*, 1979, **33B**, 488.
[301] A. J. Finlayson, S. L. Mackenzie, and J. W. Finlay, *Can. J. Chem.*, 1979, **57**, 2073.
[302] A. Kleemann, B. Lehmann, and J. Martens, *Angew. Chem.*, 1979, **91**, 858.
[303] I. Tomida and M. Matsuzaki, *Agric. Biol. Chem.*, 1979, **43**, 925.
[304] M. Low, L. Kisfaludy, E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *Z. Physiol. Chem.*, 1978, **359**, 1637.
[305] E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *Z. Physiol. Chem.*, 1978, **359**, 1629.
[306] H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, 1978, **26**, 3144.
[307] B. F. Lundt, N. L. Johansen, and J. Markussen, *Int. J. Pept. Protein Res.*, 1979, **14**, 344.
[308] C. J. Calleman and C. A. Wachtmeister, *Acta Chem. Scand.*, 1979, **33B**, 277.
[309] A. R. Fletcher, J. H. Jones, W. I. Ramage, and A. V. Stachulski, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2261.

using *NN'*-carbonyldi-imidazole, followed by alkylation and hydrolysis with 6M hydrochloric acid.[310] Attack at the side chain is not significant when N^{im}-*p*-methoxybenzenesulphonylhistidine is deprotected with TFA at room temperature during 1 h, in the presence of Me_2S,[311] and the toluene-*p*-sulphonyl analogue is also cleaved in this way.[311] Observations of less direct relevance to peptide work are the demonstration that some racemization accompanies nitration of L-histidine,[312] a study of attack by aryldiazonium cations (also capable of substituting tyrosine and lysine, the latter through the ε-amino-group),[313] the demonstration by low-temperature n.m.r. that dye-sensitized photo-oxidation of histidine involves an unstable 2,5-*endo*-peroxide,[314] and the astonishing conclusion that the imidazo-tetrahydropyridine (15) discovered as an impurity during the preparation of ^{14}C-labelled histidine is formed through reaction of the amino-acid with formaldehyde present in the atmosphere of the laboratory.[315]



(15)



(16)

Mercuration of C-4 of the imidazole moiety of histidine, and one of the imidazole nitrogen atoms, also C-3 and C-5 of the tyrosine phenolate moiety, occurs by contact with mercuric acetate.[316] Catalysis by a copper(II) salt facilitates hydroxylation of phenylalanine by H_2O_2;[317] hydroxylated phenylalanines have been subjected to ^1H–^2H exchange, a rapid process studied kinetically by stopped-flow fluorimetry.[318]

Adduct formation involving osmium(IV) tetroxide, pyridine, and tryptophan [*e.g.* (16); $R^1 = R^2 = R^3 = R^4 = H$] has been fully explored;[319] this behaviour is in contrast with the complex formation observed in this system with other amino-acids.[266]

Specific Reactions and Properties of Amino-acids Related to Biochemical Processes.—A number of processes referred to in preceding and later sections might have been located here, and the small scope of this section is therefore an artificial indication of the amount of work which has been carried out under this category. Binding studies (tryptophan to reptilian plasma proteins,[320] *N*-acetyl-L-

[310] A. Noordam, L. Maat, and H. C. Beyerman, *Recl. Trav. Chim. Pays-Bas*, 1978, **97**, 293.
[311] K. Kitagawa, K. Kitade, Y. Kito, T. Akita, S. Fumakoshi, N. Fujii, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1979, 955.
[312] E. Giralt and M. D. Ludevid, *An. Quim.*, 1979, **75**, 331.
[313] C. H. Paik, W. C. Eckelman, and R. C. Reba, *Bioorg. Chem.*, 1979, **8**, 25.
[314] H. S. Ryang and C. S. Foote, *J. Am. Chem. Soc.*, 1979, **101**, 6683.
[315] S. Hrncir, J. Kopoldova, K. Veres, V. Dedkova, V. Hanus, and P. Sedmera, *J. Labelled Comp. Radiopharm.*, 1978, **15**, 47.
[316] A. P. Korn, F. P. Ottensmeyer, and T. R. Jack, *J. Inorg. Biochem.*, 1979, **10**, 235.
[317] S. Ishimitsu, S. Fujimoto, and A. Ohara, *Chem. Pharm. Bull.*, 1979, **27**, 2286.
[318] M. Nakanishi and M. Tsuboi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1337.
[319] J. S. Deetz and E. J. Behrman, *J. Org. Chem.*, 1980, **45**, 135.
[320] J. Hattingh and D. C. Klein, *S. Afr. J. Zool.*, 1979, **14**, 109.

tryptophan to monomeric and dimeric forms of chymotrypsin,[321] branched side-chain amino-acids to *E. coli* periplasmic protein,[322] and phenylalanine or tryptophan to β-nicotinamide adenine dinucleoside[323]) have been reported. The last two results derive from n.m.r. data, T_2-relaxation times for different protons being used to show the existence of two types of bond, one from the amino-group and the other from the hydrocarbon moiety, in interactions of branched side-chain amino-acids with proteins.[322]

Effects of Electromagnetic Radiation on Amino-acids.—Well defined categories have been established for this section by a continuing series of papers over recent years, with more laboratories entering the field.

Fluorescence studies of tryptophan and its N^{α}-acetyl amide[324–327] deal with quenching by H_2O_2[324] and *N*-bromosuccinimide;[325,326] the effects of KH_2PO_4 on tyrosine fluorescence have been described.[328] Salts can often greatly enhance the phosphorescence[329] and delayed luminescence[330] of tryptophan[329] and tyrosine;[330] light emission from photo-excited tryptophan[331,332] and tyrosine[333] is influenced by several parameters.

Photochemical studies supporting the intermediacy of the tricyclic hydroperoxide in the photodegradation of tryptophan to formylkynurenine in aqueous solutions have been reported.[334,335] Nakagawa's earlier formulation of this reaction pathway has been verified in the most direct way, the isolation of the hydroperoxide.[334,335] Flash photolysis of *N*-acetyl-L-tryptophanamide[336] and photoionization of tryptophan[337] have been described. Reaction products resulting from hydrated electrons liberated from irradiated aqueous solutions of tyrosine have been analysed,[338] and degradation rates for amino-acids subjected to laser-generated singlet oxygenation in ^2H_2O have been reported.[339]

Higher-energy radiation generates radicals in samples of amino-acids, both in solution and in the solid state. Short-lived radicals formed by γ-irradiation of aqueous DL-methionine[340] have been spin-trapped and studied. Dityrosine is formed in γ- or *X*-irradiated aqueous solutions of tyrosine even after careful deoxygenation;[341] the same process is readily brought about by H_2O_2 oxidation.[44]

[321] R. Tellam, J. De Jersey, and D. J. Winzor, *Biochemistry*, 1979, **18**, 5316.
[322] A. M. Chavret-Monges, J. P. Monti, A. Crevat, C. Gaudin, J. C. Sari, and J. P. Belaich, *J. Chim. Phys. Phys.-Chim., Biol.* 1979, **76**, 714.
[323] K. J. Neurohr and H. H. Mantsch, *Can. J. Chem.*, 1979, **57**, 2297.
[324] P. Cavatorta, R. Favilla, and A. Mazzini, *Biochim. Biophys. Acta*, 1979, **578**, 541.
[325] B. F. Peterman and K. J. Laidler, *Can. J. Chem.*, 1979, **57**, 1471.
[326] B. F. Peterman and K. J. Laidler, *Biochim. Biophys. Acta*, 1979, **577**, 314.
[327] J. P. Privat, P. Wahl, and J. C. Auchet, *Biophys. Chem.*, 1979, **9**, 223.
[328] O. Shimizu, J. Watanabe, and K. Imakule, *Photochem. Photobiol.*, 1979, **29**, 915.
[329] M. L. Meyers and P. G. Seybold, *Anal. Chem.*, 1979, **51**, 1609.
[330] S. Yameshita and G. Tomita, *Z. Naturforsch., Teil B*, 1979, **34**, 344.
[331] B. T. Thu, *J. Chem. Phys.*, 1979, **70**, 3544.
[332] B. T. Thu, *J. Chem. Phys.*, 1979, **70**, 3536.
[333] B. T. Thu and A. Petit, *J. Phys. Chem.*, 1979, **83**, 1300.
[334] M. Sun and S. Zigman, *Photochem. Photobiol.*, 1979, **29**, 893.
[335] M. Nakagawa, S. Kato, S. Kataoka, and T. Hino, *J. Am. Chem. Soc.*, 1979, **101**, 3136.
[336] R. F. Evans, R. R. Kuntz, W. A. Volkert, and C. A. Ghiron, *Photochem. Photobiol.*, 1978, **27**, 511.
[337] E. Amouyal, A. Bernas, and D. Grand, *Photochem. Photobiol.*, 1979, **29**, 1071.
[338] J. F. Baugher and L. I. Grossweiner, *Photochem. Photobiol.*, 1978, **28**, 175.
[339] I. B. C. Matheson and J. Lee, *Photochem. Photobiol.*, 1979, **29**, 879.
[340] K. Makino and H. Hatano, *Chem. Lett.*, 1979, 119; K. Makino, *J. Phys. Chem.*, 1979, **83**, 2520.
[341] G. Boguta and A. M. Dancewicz, *Stud. Biophys.*, 1978, **73**, 149.

Similar studies have been reported for crystalline L-arginine hydrochloride, L-asparagine hydrate, and L-histidine hydrochloride,[342] and for *N*-acetyl-alanine and glutamic acid at 77 K;[343] e.s.r. monitoring is both routine and mandatory in all these projects.

Pulse radiolysis of ^{2}H-exchanged glycine yields a mixture of the radical cation $^2H_2NCH_2CO_2^-$ and radical anion $^2H_3N^+CH_2CO_2{}^2H$.[344]

An important extension of earlier results dealing with the interaction of chiral radiation with racemic amino-acids is the report[345] that 13.9—21–5% higher concentrations of radicals are formed in ^{90}Y-β-irradiated crystalline D-alanine than in the L-enantiomer.

6 Analytical Methods

Gas–Liquid Chromatography.—The quantitative estimation of amino-acids by g.l.c. methods is an undisputed rival to the ion-exchange amino-acid analyser technique, and g.l.c. is more compatible with mass spectrometric instrumentation and with optical purity determination. All these aspects are represented in the recent literature.

A review of the g.l.c. and h.p.l.c. analysis of amino-acids and peptides has appeared.[346] The conversion of amino-acids into volatile derivatives, a prerequisite for g.l.c. analysis, continues to provide a topic for study, and *N*-trifluoroacetyl amino-acid n-butyl esters,[347–351] pentafluoropropionyl analogues[352–354] (in one case as hexafluoroisopropyl esters),[354] and *N*-heptafluorobutyryl amino-acid isobutyl esters[355–358] are widely used. *N*-Acetyl amino-acid n-propyl esters[359, 360] and *N*-trifluoroacetyl analogues,[360] *N*-trimethylsilyl amino-acid trimethylsilyl esters,[361, 362] in one case[362] a particularly extensive study (58 compounds, including assessment of *N*-trimethylsilyl amino-acid n-butyl or (−)-methyl esters), and also condensation products of amino-acids with 1,3-dichlorotetrafluoroacetone[363, 364] provide suitable samples for g.l.c. analysis. As is so often the

[342] J. J. Tria, D. Hoel, and R. H. Johnsen, *J. Phys. Chem.*, 1979, **83**, 3174.
[343] M. D. Sevilla, J. B. D'Arcy, and K. M. Morehouse, *J. Phys. Chem.*, 1979, **83**, 2893.
[344] P. O. Samskog, T. Gillbro, and G. Nilsson, *Chem. Phys. Lett.*, 1979, **64**, 162.
[345] M. Akaboshi, M. Noda, K. Kawai, H. Maki, and K. Kawamoto, *Origins Life*, 1979, **9**, 181 (*Chem. Abstr.*, 1979, **91**, 170929).
[346] R. F. Adams, *Chromatogr. Sci.*, 1979, **9**, 1273.
[347] M. Matucha and E. Smolkova, *J. Chromatogr.*, 1979, **168**, 255.
[348] J. J. Rafter, M. Ingelman-Sundberg, and J. A. Gustafsson, *Acta Biol. Med. Ger.*, 1979, **38**, 321.
[349] S. Nagy and N. T. Hall, *J. Chromatogr.*, 1979, **177**, 141.
[350] N. T. Hall and S. Nagy, *J. Chromatogr.*, 1979, **171**, 392.
[351] C. W. Gehrke, D. R. Younker, K. O. Gerhardt, and K. C. Kuo, *J. Chromatogr. Sci.*, 1979, **17**, 301.
[352] N. Mahy, J. Tussell, and E. Gelpi, *Agents Actions*, 1978, **8**, 399 (*Chem. Abstr.*, 1979, **90**, 83 118).
[353] F. Artigas and E. Gelpi, *Analyt. Biochem.*, 1979, **92**, 233.
[354] M. Wolfensberger and H. C. Curtius, *J. Chromatogr.*, 1979, **172**, 471.
[355] S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 1979, **171**, 195.
[356] S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 1979, **173**, 53.
[357] G. Bengtsson and G. Odham, *Anal. Biochem.*, 1979, **92**, 426.
[358] J. Desgres, D. Boisson, and P. Padieu, *J. Chromatogr.*, 1979, **162**, 133.
[359] W. P. Leighton, S. Rosenblatt, and J. D. Chanley, *J. Chromatogr.*, 1979, **164**, 427.
[360] D. Lecavalier and J. C. Crawhall, *Union Med. Can.*, 1979, **108**, 566.
[361] K. L. Clay and R. C. Murphy, *J. Chromatogr.*, 1979, **164**, 417.
[362] H. Iwase, Y. Takeuchi, and A. Murai, *Chem. Pharm. Bull.*, 1979, **27**, 1307.
[363] P. Husek, V. Felt, and M. Matucha, *J. Chromatogr.*, 1979, **180**, 53.
[364] P. Husek, *J. Chromatogr.*, 1979, **172**, 468.

case when competing with well established alternatives, a promising one-step derivatization procedure (the use of 1,3-dichlorotetrafluoroacetone) is used only in the laboratory of its inventor (see Vol. 7, p. 26). Extensions of these techniques to the assay of steric purity of amino-acid samples are also well established, and are represented in derivatization of amino-acids with *N*-trifluoroacetyl-L-alanine or its chloride,[365] or with *N*-trifluoroacetyl-L-prolyl chloride;[366] the latter study includes an interesting extension of the diastereoisomer quantification technique, since if derivatization is performed using a mixture of *N*-trifluoroacetyl-L-prolyl chloride and *N*-trifluoroacetyl-D-prolyl-1-^{2}H chloride, and the relative amounts of the resulting four diastereoisomers are determined by c.i.m.s., then a source of error, which can be introduced in the simpler method if diastereoisomer formation does not go to completion, is avoided.[366] G.c.–m.s. studies are implied in many of the references cited here [348, 352–354, 357, 361, 362, 366] and in the earlier section on mass spectrometry.[226–229] Quantitative analysis of certain unusual amino-acids in physiological samples can be achieved by methylation followed by g.c.–m.s., illustrated this year for N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine[367] and *N*-(phosphonoacetyl)-L-aspartic acid.[368]

Established chiral stationary phases have been used in the determination of steric purity of amino-acids as their perfluoroalkanoyl ester derivatives,[369–372] including g.c.–m.s. isotope-labelling (hydrolysis of peptides with ^{2}HCl–^2H_2O followed by exchange of labile ^{2}H with ^{1}H [369]) and use of the 'non-natural' enantiomer of an amino-acid as internal standard, avoiding errors arising through incomplete recovery, hydrolysis, and derivatization,[370] as discussed in the preceding paragraph.[366] A novel chiral stationary phase, formed by grafting L-valine-t-butylamide on to a silicone, has been advocated for this work.[373]

Ion-exchange Chromatography.—As in the preceding section, discussion of the papers appearing under this heading is very brief where development of existing techniques is concerned. This applies to nearly all published work in this area. Instrumental innovations are excluded.

Ion-exchange chromatographic identification of proline,[374] 3-methylhistidine,[375] and *S*-adenosyl-L-methionine,[376] the last-mentioned amino-acid on sulphopropyl-Sephadex, has been reported and the simultaneous determinations of amino-acids, amino-sugars, and carbohydrates have been described.[377] γ-Carboxy-L-glutamic acid content of proteins has been determined by alkaline

[365] K. Kruse, W. Francke, and W. A. König, *J. Chromatogr.*, 1979, **170**, 423.
[366] C. Wiecek, B. Halpern, A. M. Sargeson, and A. M. Duffield, *Org. Mass Spectrom.*, 1979, **14**, 281.
[367] P. Maury, *J. Lab. Clin. Med.*, 1979, **93**, 718.
[368] J. M. Strong, Y. E. Kinney, A. R. Branfman, and K. L. Cysyk, *Cancer Treat. Rep.*, 1979, **63**, 775 (*Chem. Abstr.*, 1979, **91**, 71 142).
[369] H. Frank, W. Woiwode, G. J. Nicholson, and E. Bayer, in 'Stable Isotopes: Proceedings of the Third International Conference', ed. E. R. Klein and P. D. Klein, Academic Press, New York, 1979, p. 165.
[370] H. Frank, G. J. Nicholson, and E. Bayer, *J. Chromatogr.*, 1978, **167**, 187.
[371] G. J. Nicholson, H. Frank, and E. Bayer, *J. High Resolution Chromatogr.*, 1979, **2**, 411.
[372] W. Woiwode, D. List, and H. Weichardt, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 251.
[373] T. Saeed, P. Sandra, and M. Verzele, *Adv. Chromatogr.*, 1979, **14**, 699.
[374] R. Paquin and P. Lechasseur, *Can. J. Bot.*, 1979, **57**, 1851.
[375] M. Neuhauser and P. Furst, *Anal. Biochem.*, 1979, **92**, 294.
[376] R. I. Glazer and A. L. Peale, *Anal. Biochem.*, 1978, **91**, 516.
[377] M. M. Tikhomirov, A. Y. Khorlin, W. Voelter, and H. Bauer, *J. Chromatogr.*, 1978, **167**, 197.

hydrolysis followed by ion exchange,[378] and by using the anion-exchange auto-analyser;[379] a problem in the confluence of this amino-acid with taurine has been noted.[379] Other amino-acid analyser studies (selected from a larger field) concern the use of 3-nitro-L-arginine as internal standard,[380] studies of sulphur-containing amino-acids with particular reference to variable colour yields and proposals for ninhydrin colour factors,[381] dithiasuccinoyl-amino-acids,[382] and 3-hydroxy-L-proline assays.[383] Reports have been published on the use of a micro-column amino-acid analyser [384] and microbore analyser,[385] the latter study being one of a crop of papers on *o*-phthalaldehyde [385–387] or fluorescamine detection.[386] There is good agreement between two groups [385, 387] that high precision is possible with *o*-phthalaldehyde–2-mercaptoethanol fluorimetric detection (see also the later section on fluorimetry) at the 10 picomole level.

Thin-layer Chromatography.—Studies range from a claim that leucine, isoleucine, and phenylalanine, whose separation is a test piece for t.l.c. methodology, can be resolved by one-dimensional t.l.c.,[388] separation of (2*S*,3*R*,4*R*)-4-hydroxyisoleucine (the major free amino-acid of fenugreek seed), whose separation by ion-exchange is complicated by lactonization,[389] and ion-exchange t.l.c. of methionine, cysteine, cystine, and cysteic acid in plant extracts.[390] 'Three-dimensional t.l.c.' turns out to be a variation of two-dimensional t.l.c. in which a square plate is used in the normal way, with one side for sample, the other half for standards, then successively developed in the second dimension from one side and from the other;[391] this gives a better basis for visual comparison.

Dansyl derivatives of more than 90 'uncommon' amino-acids have been studied by two-dimensional polyamide t.l.c.,[392] and the dansylation–fluorimetry technique has been used for the assay of amino-acids in blood samples.[393] The relatively rarely used combination of t.l.c. with m.s. has been applied for the estimation of γ-aminobutyric acid, as dansyl-γ-butyrolactam.[394] Dabsyl chloride (4-dimethylaminoazobenzene-4′-sulphonyl chloride) gives photostable coloured derivatives which, although detectable on t.l.c. plates at some 60-fold lower levels than 2,4-dinitrophenyl analogues, are less satisfactory for trace analysis of amino-acids than dansyl derivatives.[395]

[378] D. Madar, R. A. Willis, K. A. Koehler, and R. G. Hiskey, *Anal. Biochem.*, 1979, **92**, 466.
[379] L. B. James, *J. Chromatogr.*, 1979, **175**, 211.
[380] R. C. White and T. E. Nelson, *J. Chromatogr.*, 1979, **176**, 430.
[381] M. Friedman, A. T. Noma, and J. R. Wagner, *Anal. Biochem.*, 1979, **98**, 293.
[382] G. Baremy and R. B. Merrifield, *Anal. Biochem.*, 1979, **95**, 160.
[383] A. Szymanovicz, G. Poulin, A. Randoux, and J. P. Borel, *Clin. Chim. Acta*, 1979, **91**, 141.
[384] J. P. Vergues and I. L. Freeman, *Anal. Biochem.*, 1979, **99**, 427.
[385] H.-W. Lee, M. D. Forde, M. C. Lee, and D. J. Bucher, *Anal. Biochem.*, 1979, **96**, 298.
[386] H.-W. Lee, D. J. Bucher, and R. C. Seid, *Ind. Eng. Chem. Prod. Res. Dev.*, 1979, **18**, 122.
[387] H. Nakamura, C. L. Zimmerman, and J. J. Pisano, *Anal. Biochem.*, 1979, **93**, 423.
[388] R. W. McBride, D. W. Jolly, B. M. Kadis, and T. E. Nelson, *J. Chromatogr.*, 1979, **168**, 290.
[389] R. Hardman and I. M. Abu-Al-Futuh, *Planta Med.*, 1979, **36**, 79.
[390] A. Varadi and S. Pongor, *J. Chromatogr.*, 1979, **173**, 419.
[391] M. Messripour, S. Naderi, and A. Wise, *Anal. Biochem.*, 1979, **97**, 328.
[392] H. Laatsch, *J. Chromatogr.*, 1979, **173**, 398.
[393] B. M. Fetterroll and J. Sander, *Monatsschr. Kinderheilkd.*, 1979, **127**, 411.
[394] P. H. Wu, D. A. Durden, and L. Hertz, *J. Neurochem.*, 1979, **32**, 379.
[395] J.-K. Lin, C.-A. Chen, and C.-H. Wang, *Proc. Nat. Sci. Council, Republic of China*, 1979, **3**, 158 (*Chem. Abstr.*, 1979, **91**, 136402).

N-Acetyl-amino-acids can be detected on t.l.c. plates by successively spraying with 12M hydrochloric acid, heating at 120 °C during 10—15 min, and then by ninhydrin colour formation.[396]

Routine t.l.c. studies with 3-phenylthiohydantoins[397] and 4-[4-(dimethylaminophenyl)azophenyl] analogues[398] have been described; in one of these,[397] detection at the 5 nanomole level was established.

High-performance Liquid Chromatography.—The volume of published work in this area continues to increase, and a number of reviews have appeared, covering h.p.l.c. of amino-acids and peptides,[346, 399] and resolution of amino-acids either through conversion into diastereoisomeric derivatives[400] or on chiral stationary phases.[401]

Separation of underivatized amino-acids has been reported in several papers: tyrosine and tryptophan in physiological fluids,[402-407] including examples of ion-pair h.p.l.c.[404, 405] in which sodium dodecyl sulphate or other surfactant is involved, tyrosine and its analogues iodotyrosine and iodothyronines,[407] dopa and its 2- and 5-(*S*-cysteinyl) derivatives,[408] ergothioneine,[409] and *S*-adenosylmethionine and -homocysteine[410] have been under scrutiny. A mixture of amino-acids can be separated adequately within 14 min by reversed-phase h.p.l.c.[411]

Several laboratories have reported work on pre-column derivatization of amino-acid mixtures with *o*-phthalaldehyde and ethanethiol,[412] allowing analysis at even sub-picomole levels. Derivatization with 2,4-dinitrofluorobenzene[413] or through dansylation[414] has served to provide quantitative data on particular amino-acids in physiological samples, and several reports have appeared on h.p.l.c. characteristics of dansyl-amino-acids.[415] Yet more papers on h.p.l.c. of 3-phenylthiohydantoins have been published;[416, 417] a reasoned assessment of the rating of h.p.l.c. for these compounds is that this separation method is some 10—50-fold

[396] R. C. Pandey, R. Misra, and K. L. Rinehart, *J. Chromatogr.*, 1979, **170**, 498.
[397] R. L. Munier and A. M. Drapier, *Chromatographia*, 1979, **12**, 548.
[398] C.-Y. Yang, *Z. Physiol. Chem.*, 1979, **360**, 1673.
[399] M. T. W. Hearn and W. S. Hancock, *Chromatogr. Sci.*, 1979, **12**, 243.
[400] R. Audebert, *J. Liq. Chromatogr.*, 1979, **2**, 1063.
[401] T. Tamegai, M. Ohmae, K. Kawabe, and M. Tomoeda, *J. Liq. Chromatogr.*, 1979, **2**, 1229.
[402] G. M. Anderson, J. G. Young, and D. J. Cohen, *J. Chromatogr.*, 1979, **164**, 501.
[403] A. M. Krstulovic, S. Ciriello, L. Bertani-Dziedzic, and S. E. Gitlow, *J. Chromatogr.*, 1979, **164**, 495.
[404] C. M. Riley, E. Tomlinson, T. M. Jeffries, and P. H. Redfern, *J. Chromatogr.*, 1979, **162**, 153.
[405] W. S. Hancock, C. A. Bishop, and M. T. W. Hearn, *Chem. N.Z.*, 1979, **43**, 17.
[406] D. D. Koch and P. T. Kissinger, *J. Chromatogr.*, 1979, **164**, 441.
[407] N. M. Alexander and M. Nishimoto, *Clin. Chem.*, 1979, **25**, 1757.
[408] C. Hansson, G. Agrup, H. Rorsman, A. M. Rosengren, E. Rosengren, and L. E. Edholm, *J. Chromatogr.*, 1979, **162**, 7.
[409] T. Mayumi, H. Kawano, Y. Sakamoto, E. Suehisa, Y. Kawai, and T. Hama, *Chem. Pharm. Bull.*, 1978, **26**, 3772.
[410] A. Floridi, C. Fini, C. A. Palmerini, R. Mozzi, and G. Porcellati, *J. Liq. Chromatogr.*, 1979, **2**, 1003.
[411] W. S. Hancock, C. A. Bishop, and M. T. W. Hearn, *Anal. Biochem.*, 1979, **92**, 170.
[412] J. C. Hodgkin, *J. Liq. Chromatogr.*, 1979, **2**, 1047; D. W. Hill, F. H. Walters, T. D. Wilson, and J. D. Stuart, *Anal. Chem.*, 1979, **51**, 1338; P. Lindroth and K. Mopper, *Anal. Chem.*, 1979, **51**, 1667; W. S. Gardner and W. H. Miller, *Anal. Biochem.*, 1979, **101**, 61.
[413] E. W. Bachmann, J. Frei, and M. Muehlemann, *Chromatographia*, 1979, **12**, 345.
[414] G. J. Schmidt, D. C. Olson, and W. Slavin, *J. Chromatogr.*, 1979, **164**, 355.
[415] J. M. Wilkinson, *J. Chromatogr. Sci.*, 1978, **16**, 547; G. J. Schmidt, D. C. Olson, and W. Slavin, *J. Liq. Chromatogr.*, 1979, **2**, 1031.
[416] W. D. Annan, *J. Chromatogr.*, 1979, **173**, 194; P. W. Moser and E. E. Rickli, *J. Chromatogr.*, 1979, **176**, 451; W. F. MooPen, M. H. Johnson, K. C. Bechtel, and D. L. Jue, *J. Chromatogr.*, 1979, **172**, 476.
[417] M. N. Margolies and A. W. Brauer, *Chromatogr. Sci.*, 1979, **10**, 177.

more sensitive than g.l.c.,[417] and easily permits the differentiation of leucine from isoleucine, and of aspartic acid from glutamic acid.

New approaches to the resolution of partly racemized amino-acids on chiral stationary phases[418-422] include reversed-phase h.p.l.c. of Cu^{2+} or Zn^{2+} complexes with L-aspartyl-L-phenylalanine methyl ester in the mobile phase,[418] L-amino-acids grafted on to poly(acrylamide)s, and complexed with Cu^{2+} ions,[419] and a similar grafting of *N*-acetyl-L-valine to 3-aminopropylsilanized silica[420] or of (+)-9-bromomethyl-10-(1-hydroxy-2,2,2-trifluoroethyl)anthracene to silica,[421] as novel stationary phases.

Fluorimetry.—The conversion of amino-acids into fluorescent derivatives continues to attract new users, particularly the use of *o*-phthalaldehyde with 2-mercaptoethanol[423,424] or with ethanethiol[412,425] or methanethiol.[425] This system has been used recently for analysis of γ-aminobutyric acid in brain tissue,[423] cysteine and cystine,[424] and γ-carboxyglutamic acid,[426] and although it is specifically applicable only to primary amines both proline and hydroxyproline can be assayed by prior oxidation with alkaline sodium hypochlorite, down to 10 picomole and 20 picomole levels, respectively.[427] Problems exist with the use of this method with lysine, where quantum yields are reduced by intramolecular quenching of the fluorescence of the substituted isoindole which is the product of the reaction;[428] fluorescence yields are low also with cysteine and cystine but prior conversion of these amino-acids into cysteic acid or into *S*-(3-sulphopropyl) derivatives is advocated to overcome this difficulty.[424] There is a need to lay down very specific details for the use of the *o*-phthalaldehyde–thiol system, since both reaction temperature and the structure of the thiol affect fluorescence yields.[425] For fluorimetric analysis of α-branched α-amino-acids, more reliable fluorescence behaviour is achieved using higher reaction temperatures and using methanethiol or ethanethiol instead of the more commonly used 2-mercaptoethanol.[425]

The major alternative reagent for the conversion of amino-acids into fluorescent derivatives is fluorescamine, which has been involved in a tyrosine assay procedure, with special reference to sources of interference when used for rat brain or human plasma samples.[429] A tryptophan fluorimetric assay has been illustrated, again using rat brain samples.[430] Fluorescent derivatives obtained from proline and its 3- or 4-hydroxy-analogues using 7-chloro-4-nitrofurazan permit the visualization of these imino-acids on t.l.c. plates at *ca.* 10 picomole levels.[431]

[418] C. Gilon, R. Leshem, Y. Tapuhi, and E. Grushka, *J. Am. Chem. Soc.*, 1979, **101**, 7612.
[419] B. Lefebvre, R. Audebert, and C. Quiviron, *J. Liq. Chromatogr.*, 1978, **1**, 761.
[420] S. Hara and A. Dobashi, *J. Liq. Chromatogr.*, 1979, **2**, 883.
[421] W. H. Pirkle and D. W. Hause, *J. Org. Chem.*, 1979, **44**, 1957.
[422] G. Guebitz, W. Jellenz, G. Loefler, and W. Santi, *J. High Resolution Chromatogr., Chromatogr. Commun.*, 1979, **2**, 145.
[423] J. A. M. Van der Heyden and J. Korf, *J. Neurochem.*, 1978, **31**, 197.
[424] K. S. Lee and D. G. Drescher, *J. Biol. Chem.*, 1979, **254**, 6248.
[425] J. R. Cronin, S. Pizzarello, and W. E. Gandy, *Anal. Biochem.*, 1979, **93**, 174.
[426] C. M. Gundberg, J. B. Lian, and P. M. Gallop, *Anal. Biochem.*, 1979, **98**, 219.
[427] P. Bohlen and M. Mellet, *Anal. Biochem.*, 1979, **94**, 313.
[428] R. F. Chen, C. Scott, and E. Trepmann, *Biochim. Biophys. Acta*, 1979, **576**, 440.
[429] G. Evans, M. K. Hartmann, L. Wood, and M. K. Gaitonde, *J. Neurochem.*, 1979, **32**, 1303.
[430] H. D. Dell and J. Fiedler, *Z. Anal. Chem.*, 1978, **293**, 407.
[431] A. Szymanowicz, C. Cheron, and J. P. Borel, *Biochimie*, 1979, **61**, 425.

Other Separation Methods.—Thin-layer electrophoresis has been used for the micro-assay of γ-aminobutyric acid, glutamic acid, and aspartic acid in tissue samples,[432] and for γ-amino-β-hydroxybutyric acid.[433]

Swollen cyclodextrin beads have been used for inclusion chromatography separation of aromatic amino-acids from non-aromatic amino-acids and from each other.[434]

Determination of Specific Amino-acids.—This section is shorter than in previous volumes, mainly as a result of inclusion of material in earlier sections of this Chapter wherever the generality of the analytical content makes this possible.

Specific colour-forming reactions have been explored for the estimation of methionine (use of Chloramine-T, quantification at 246 nm),[435] and hydroxylysine (further studies of Ehrlich reagent colorimetry).[436] New radiometric assays for L-canaline (using ^{14}C-labelled acetone)[437] and 3′-mono-iodo-L-thyronine[438] have been reported. Bioautography of L-carnitine[439] and an enzyme assay for glycine, based on spectrophotometric quantification of the proportion of SH groups exposed by the cleavage of benzoyl–coenzyme A catalysed by glycine *N*-acyltransferase,[440] provide specific examples of methods whose principles are generally applicable.

[432] S. Sarhan, N. Seiler, J. Grove, and G. Bink, *J. Chromatogr.*, 1979, **162**, 561.
[433] K. Matsushita and L. R. Gjessing, *J. Chromatogr.*, 1979, **162**, 427.
[434] B. Zsadon, M. Szilasi, F. Tudos, E. Fenyvesi, and J. Szejtli, *Starck und Staerke*, 1979, **31**, 11.
[435] G. E. Trout, *Anal. Biochem.*, 1979, **93**, 419.
[436] N. Blumenkrantz and G. Asboe-Hansen, *Clin. Biochem.*, 1979, **12**, 157.
[437] M. Sugii, H. Miura, and K. Nagata, *Anal. Biochem.*, 1979, **92**, 265.
[438] R. C. Smallridge, L. Wartofsky, B. J. Green, F. C. Miller, and K. D. Burman, *J. Clin. Endocrinol. Metab.*, 1979, **48**, 32.
[439] C. M. Lewin and L. C. Bieber, *Anal. Biochem.*, 1979, **96**, 322.
[440] S. J. Gatley, *Anal. Lett.*, 1979, **12**, 415.