1
Amino Acids

BY G. C. BARRETT

1 Introduction

The literature searched to generate this chapter continues to be centred on the chemical journals and monographs, though taking in the biochemical and biological literature where this deals with analytical advances and material relevant to chemical studies.

Textbooks and Reviews. – A comprehensive coverage is offered by a multi-author text[1] that concentrates on the advances in knowledge of the chemistry and biochemistry of the amino acid in the period following coverage given in Greenstein's and Winitz's classic treatise.[2] Several chapters of 'Methods in Enzymology'[3] will be of general interest to readers of this chapter, as will reviews of new natural amino acids,[4] uses and biochemistry of amino acids,[5,6] and methods for their synthesis.[6] More specialized reviews concern valine,[7] tryptophan,[8] biochemistry of sulphur-containing amino acids,[9] ketimine derivatives of sulphur-containing amino acids,[10] electrophoresis of amino acids,[11] motion in solid amino acids,[12] and nitrogen interconversion and amino acid metabolism in germinating seedlings.[13]

2 Naturally Occurring Amino Acids

Occurrence of Known Amino Acids. – A narrower outlook than in previous volumes can be detected in the following section, which lacks mention of amino

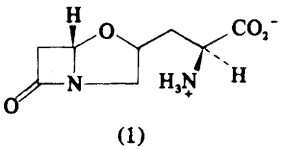
[1] 'Chemistry and Biochemistry of the Amino Acids', ed. G. C. Barrett, Chapman and Hall, London, 1984.
[2] J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids', Wiley, New York, 1961.
[3] 'Methods in Enzymology', Vol. 91, Part I (Enzyme Structure), ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, 1983: 'γ-Carboxyglutamic Acid', P. A. Price, p. 13; '*N*-Methylamino Acids', M. Elzinga and N. Alonzo, p. 8; 'Buffer Systems for the Amino Acid Analyzer', C. H. W. Hirs, p. 3 (see also refs. 426, 435, and 436).
[4] I. Wagner and H. Nusso, *Angew. Chem.*, 1983, **95**, 827.
[5] B. Hoppe and J. Martens, *Chem. Unserer Zeit*, 1983, **17**, 41.
[6] V. S. Shpak and I. Ya. Tyuryaev, *Vestn. Akad. Nauk S.S.S.R.*, 1983, 107.
[7] A. S. Polyanskaya, I. Kalnins, N. I. Aboslalova, and G. I. Prigorodov in 'Sintesy Issled. Nitrosoedin. Aminokislot', ed. G. V. Nekrasova, Leningr. Gos. Pedagog. Inst., Leningrad, 1983, p. 51.
[8] B. Witkop, *Heterocycles*, 1983, **20**, 2059.
[9] A. J. L. Cooper, *Ann. Rev. Biochem.*, 1983, **52**, 263.
[10] D. Cavallini, *Progr. Clin. Biol. Res.*, 1983, **125**, 355.
[11] Z. Deyl in 'Electrophoresis: Part B', ed. Z. Deyl, Elsevier, Amsterdam, 1983, p. 55.
[12] M. A. Keniry, R. L. Smith, H. S. Gulowsky, and E. Oldfield in 'Structure and Dynamics: Nucleic Acids and Proteins', ed E. Clementi and R. H. Sarma, Adenine Press, Guilderland, New York, 1983, p. 435.
[13] P. J. Lea and K. W. Joy, *Recent Adv. Phytochem.*, 1983, **17**, 77.

acids as constituents of meteorites or Earthly geological sources but otherwise covers non-routine work with uncommon amino acids in plant and animal sources. *cis*-4-Hydroxy- and 2,4-*cis*-4,5-*trans*-dihydroxy-pipecolic acids are present in leaves of *Calliandra pittieri*,[14] and isoasparagine makes its first natural appearance in *Chara corallina*.[15] 2,6-Diamino-7-hydroxyazelaic acid, a constituent of the edeine antibiotics, has the (2*R*,6*S*,7*R*) configuration.[16] N^{ϵ}-Methyl-lysine is a constituent of flagellins from *Proteus morganii*,[17] and *N*-trimethylalanine appears at the N-terminus of myosin light chains.[18] The structure of the cross-linking residue of collagen, pyridinoline (see Vol. 14, p. 3), has been confirmed.[19]

A crop of papers describes *N*-substituted amino acids of particular interest either in themselves or in their location: L-γ-glutamyl-*cis*-3-aminoproline in *Morchella esculenta*,[20] 4′-*O*-diacylglyceryl-*N*-trimethylhomoserine from fronds of the fern *Adiantum capillus veneris* (the first report of this amino acid in a vascular plant, previously detected in algae),[21] and a role of *N*-acetyl-L-aspartic acid as one heat-stable factor essential for the conversion of lignoceric acid to cerebronic acid and glutamic acid, catalysed by a rat-brain particulate preparation.[22]

New Natural Amino Acids. – *Streptomyces clavuligerus* produces the new clavam $R_0$22-5417 (1), as well as other β-lactams.[23] New kainoids [acromelic acids A and B, *i.e.* (2) and the corresponding 5′-pyridin-2′-one-6′-carboxylic acid, respectively] have been isolated from *Clitocybe acromelalga*.[24]

Mutant strains of *Neurospora crassa* that accumulate prephenic and arogenic acids (see Vol. 13, p. 3) also produce spiro-L-arogenate, the previously unknown lactam of arogenic acid.[25]



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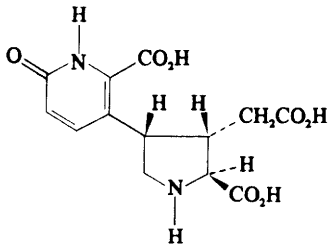
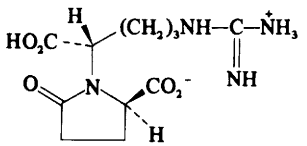


(2)

[14] J. T. Romeo, L. A. Swain, and A. B. Bleecker, *Phytochemistry*, 1983, **22**, 1615.
[15] K. Sakano, T. Shimmen, S. Hatanaka, and M. Tazawa, *Phytochemistry*, 1983, **22**, 2313.
[16] J. Gumieniak, H. Wojciechowska, and E. Borowski, *Pol. J. Chem.*, 1981, **55**, 1519.
[17] B. S. Baker, S. E. Smith, and M. W. McDonough, *Microbios Lett.*, 1983, **23**, 7.
[18] G. D. Henry, D. C. Dalgarno, B. A. Levine, and I. P. Trayer, *Biochem. Soc. Trans.*, 1982, **10**, 362.
[19] S. P. Robins and A. Duncan, *Biochem. J.*, 1983, **215**, 175; S. P. Robins, *ibid.*, p. 167.
[20] M. Moriguchi, K. Kimura, and S. Hatanaka, *Nippon Kingakkai Kaiho*, 1983, **24**, 191.
[21] N. Sato and M. Furuya, *Plant Cell Physiol.*, 1983, **24**, 1113.
[22] H. Shigematsu, N. Okamura, H. Shimeno, Y. Kishimoto, L. S. Kan, and C. Fenselau, *J. Neurochem.*, 1983, **40**, 814.
[23] D. L. Pruess and M. Kellett, *J. Antibiot.*, 1983, **36**, 208; R. H. Evans, H. Ax, A. Jacoby, T. H. Williams, E. Jenkins, and J. P. Scannell, *ibid.*, p. 213.
[24] K. Konno, H. Shirahama, and T. Matsumoto, *Tetrahedron Lett.*, 1983, **24**, 939.
[25] L. O. Zamir, E. Jung, and R. A. Jensen, *J. Biol. Chem.*, 1983, **258**, 6492.

Metabolites from crown-gall tumours induced by *Agrobacterium tumefaciens* continue to provide new amino acids, helping to fuel the interest in this topic, which is clearly of broader fundamental importance. Strains that utilize neither octopine nor norpaline produce tumours that contain the lactam of N^2-(1,3-dicarboxypropyl)-L-leucine, for which the name leucinopine is suggested.[26] Similar studies have revealed the presence of pyronopaline (3) in crown-gall tumour tissue.[27]



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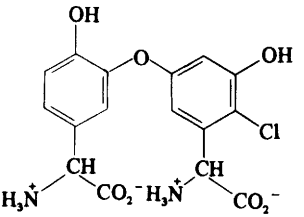

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New Amino Acids from Hydrolysates. – This section encompasses the literature that describes new amino acids found as constituents of peptides, peptidoglycans, and related natural products. A new type of carbohydrate linkage is seen in N^{β}-asparaginylglucose, a constituent of *Halobacterium* cell-wall glycoprotein[28] (a related amino acid is found in hen ovalbumin[29]). *threo*-β-Hydroxyornithine (probably the D-enantiomer[30]) is a constituent of a peptidoglycan from *Corynebacterium Co112*.[30] *erythro*-β-Hydroxyaspartic acid is present in a light chain of bovine protein C, an anticoagulant Vitamin K-dependent plasma protein[31] (eleven γ-carboxyglutamic acid residues are also present[32]).

Isolation and identification of hypusine [N'-(4-amino-2-hydroxybutyl)lysine] and its 2-deoxy analogue from brain tissue have been fully documented.[33] In another continuing study of novel amino acids as constituents of larger structures, actinoidinic acid and a related arylglycine (4) are formed by hydrolysis of the aglycone component of the glycopeptide antibiotic A35512B, the absolute configuration of (4) being (*R*) for the *p*-hydroxyphenylglycine moiety and (*S*) for the *m*-hydroxylated moiety.[34] Rhizonin A, from *Rhizopus microsporus*, contains *N*-methyl-3-(3-furyl)-L-alanine.[35]

[26] C. C. Chang, C. M. Chen, B. R. Adams, and B. M. Trost, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3573.
[27] L. M. Hall, J. L. Schrimsher, and K. B. Taylor, *J. Biol. Chem.*, 1983, **258**, 7276.
[28] F. Wieland, R. Heitzer, and W. Schaefer, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 5470.
[29] H. Nomoto and Y. Inoue, *Eur. J. Biochem.*, 1983, **135**, 243.
[30] K. H. Schleifer, I. Hayn, H. P. Seidl, and J. Firl, *Arch. Microbiol.*, 1983, **134**, 243.
[31] T. Drakenberg, P. Fernlund, P. Roepstorff, and J. Stenflo, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1802.
[32] B. A. McMullen, K. Fujikawa, W. Kisiel, T. Sasagawa, W. N. Howald, E. Y. Kwa, and B. Weinstein, *Biochemistry*, 1983, **22**, 2875.
[33] M. H. Park, H. L. Cooper, and J. E. Folk, *Methods Enzymol.*, 1983, **94**, 458.
[34] C. M. Harris and T. M. Harris, *Tetrahedron*, 1983, **39**, 1661.
[35] P. S. Steyn, A. A. Tuinman, F. R. van Heerden, P. H. van Rooyen, P. L. Wessels, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1983, 47.

Crosslinking amino acid residues in proteins having important physiological functions continue to stimulate interest. Both known[19] and novel[36] crosslinking residues feature in the 1983 literature, the latter represented in the presence of N^{π}-histidino-alanine (as well as the previously known N^{τ}-isomer) in calcium-binding phosphoprotein particles from the clam *Rangia cuneata*.[36]

3 Synthesis of Amino Acids

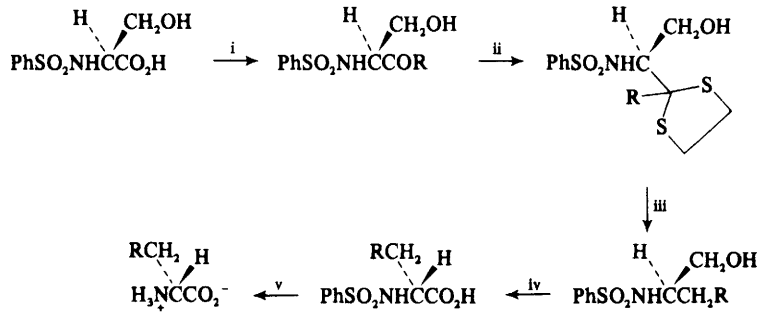
General Methods. – Standard methods employing ammonia in reactions with keto acids to give *N*-acylamino acids as well as amino acids[37] (see also Vol. 15, p. 4) and with 1,1-dichloro-oxiranes to give amino acids[38] and a related use of Me_3N in a synthesis of DL-carnitine by aminolysis of $ClCH_2CH(OH)CH_2CO_2Et$ followed by hydrolysis[39] have been reported. Other standard synthetic procedures used are alkylation of diethyl acetamidomalonate[40,109,130,169] (to give β-pyrazinyl-L-alanine[40]) and of diethyl formamidomalonate,[41] also Strecker[42] and hydantoin[43] alkylation. Alkylation of glycine derivatives is being taken up in more laboratories for the synthesis of α-amino acids.

A later section ('Reactions of Amino Acids') of this chapter also happens to include some general methods of synthesis of amino acids, possibly not the primary objective of the authors concerned, but a growing number of papers is directed at the use of readily available amino acids as starting materials for the synthesis of other amino acids. L-Serine is the starting material in a chirally efficient synthesis of D-α-amino acids $RCH_2CH(\overset{+}{N}H_3)CO_2^-$. *N*-Benzenesulphonyl-L-serine reacts with organo-lithium and -magnesium compounds at −78 °C to give optically pure amino ketones, from which the D-amino acid is obtained through conventional stages (Scheme 1).[44] Alkylation of a ω-iodo or -bromo acid with four equivalents of an organocuprate R_2CuLi gives excellent yields of the substitution products of high optical purity.[45]

A stereoselective synthesis of γ-hydroxyamino acids from α-nitrosoalk-2-enoic amides $MeCH{=}C(NO)CONHBu^t$ involves condensation with an aldehyde.[46]

A new general β-amino acid synthesis is based on the alkylation of *N*-benzyloxyimines with ketene silylacetals [$R^1CH{=}NOCH_2Ph + R^2R^3C{=}C(OR^4)OSiMe_3 \rightarrow PhCH_2ONHCHR^1CR^2R^3CO_2R^4$].[47]

[36] R. L. Sass and M. E. Marsh, *Biochem. Biophys. Res. Commun.*, 1983, **114**, 304.
[37] H. Yamagawa, Y. Makino, K. Sato, M. Nishizawa, and F. Egami, *Adv. Space Res.*, 1983, **3**, 69.
[38] L. Yu and M. Yan, *Gaodeng Xuexiao Huaxue Xuebao*, 1983, **4**, 213 (*Chem. Abstr.*, 1984, **100**, 7086).
[39] Y. Chen, J. Chen, and K. Qian, *Hunan Yixueyuan Xuebao*, 1983, **8**, 82 (*Chem. Abstr.*, 1983, **99**, 122 847).
[40] C. Petermann and J. L. Fauchere, *Helv. Chim. Acta*, 1983, **66**, 1513.
[41] L. Yu and H. Liang, *Beijing Shifan Daxua Xuebao, Ziran Kexueban*, 1983, 94 (*Chem. Abstr.*, 1983, **99**, 105 665).
[42] S. Yu. Sizov, L. V. Semenova, and N. P. Utrobin in ref. 7, p. 38, 42.
[43] D. Mostowicz, W. Abramski, and C. Belzecki, *Pol. J. Chem.*, 1981, **55**, 1387.
[44] P. J. Maurer, H. Takahata, and H. Rapoport, *J. Am. Chem. Soc.*, 1984, **106**, 1095.
[45] A. Bernardini, A. El Hallaoui, R. Jacquier, C. Pigiere, P. Viallefont, and J. Bajgrowicz, *Tetrahedron Lett.*, 1983, **24**, 3717.
[46] B. J. Banks, A. G. M. Barrett, M. A. Russell, and D. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 873.
[47] K. Ikeda, K. Achiwa, and M. Sekiya, *Tetrahedron Lett.*, 1983, **24**, 4707.



Reagents: i, RLi and RMgBr; ii, $HSCH_2CH_2SH$ and $BF_3 \cdot Et_2O$; iii, Raney Ni; iv, O_2–PtO_2; v, 48% aq. HBr

Scheme 1

Asymmetric Synthesis of Amino Acids. – Several reviews have appeared,[48–50] including coverage of non-enzymic transamination[49] and catalysed asymmetric hydrogenation.[50]

Much of the recent literature on this topic reports extensions of studies of groups active over several years. Reductive aminolysis of 4-alkylidene- and -arylidene-oxazolin-5-ones,[51] aminolysis of 4-benzyl- or isopropyl-oxazolin-5-ones,[52] and catalysed asymmetric hydrogenation of *Z*- and *E*-isomers of *N*-benzoyl-α-amino-β-methyl-β-ethylacrylate[53] have raised interest mainly of a mechanistic nature, since enantiomeric discrimination in these reactions is usually low owing to accompanying racemization. The other work based on catalysed asymmetric hydrogenation,[54–57] though from different laboratories, employs rhodium(I)–chiral phosphine catalysis and includes pessimistic assessment[57] of the Ruch and Ugi proposals that enantioselectivity of this process can be predicted. The alternative approach, in which hydrogenation catalysed by achiral means

[48] U. Schöllkopf, *Top. Curr. Chem.*, 1983, **109**, 65.

[49] H. Kuzuhara, *Yuki Gosei Kagaku Kyokaishi*, 1983, **41**, 134.

[50] W. S. Knowles, *Acc. Chem. Res.*, 1983, **16**, 106; B. D. Vineyard, W. S. Knowles, and M. J. Sabacky, *J. Mol. Catal.*, 1983, **19**, 159.

[51] G. V. Chel'tsova, E. I. Karpeiskaya, L. N. Kaigorodova, and E. I. Klabunovskii, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1983, 65; I. E. Khatskevich, I. K. Kalnin, E. I. Karpeiskaya, and E. I. Klabunovskii, *ibid.*, p. 359, 366; E. S. Levitina, L. F. Godunova, E. I. Karpeiskaya, and E. I. Klabunovskii, *ibid.*, p. 1740.

[52] G. V. Chel'tsova, E. I. Karpeiskaya, E. I. Klabunovskii, and E. D. Lubuzh, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1983, 59; L. F. Godunova, E. S. Levitina, E. I. Karpeiskaya, E. I. Klabunovskii, and E. D. Lubuzh, *ibid.*, p. 1733.

[53] N. D. Zubareva, I. E. Khatsevich, T. I. Kuznetsova, Sh. G. Bitiev, I. Kalnins, E. I. Karpeiskaya, A. A. Vedenyapin, and E. I. Klabunovskii, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1983, 477.

[54] D. Sinou, D. Lafont, G. Descotes, and T. Dayrit, *Nouv. J. Chim.*, 1983, **7**, 291.

[55] C. Cativiela, J. A. Mayoral, E. Melendez, R. Uson, L. A. Oro, and M. T. Pinillos, *React. Kinet. Catal. Lett.*, 1982, **21**, 173.

[56] M. Yatagai, M. Zama, T. Yamagishi, and M. Hida, *Chem. Lett.*, 1983, 1203.

[57] H. Brunner, B. Schönhammer, B. Schönhammer, and C. Steinberger, *Chem. Ber.*, 1983, **116**, 3529.

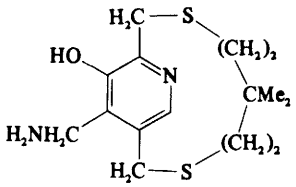
(*e.g.* Pd[58]) is applied to a chiral 3-alkylidene dioxopiperazine, leads to high chiral induction (90.1–98.4%) in most cases.[58] Stereoselective bromination of the same starting material using *N*-bromosuccinimide in methanol[59] gives 3-bromoalkyl-3-methoxypiperazinediones in less substantial enantiomeric excesses.

Several more papers from the Schöllkopf group[60–68] extend the use of bis-lactim ethers derived from dioxopiperazines into synthesis of (*R*)-(−)-isovaline,[65] (*R*)-α-methyl-*S*-benzylcysteine,[66] (*R*)-serine derivatives,[67] and (*R*)-β-fluorovaline and related 2-amino-3-fluoroalkanoic acids.[68] The principle employed in this approach is also exemplified in the asymmetric alkylation of the carbanion derived from a chiral 2-methoxy-3-substituted Δ^1-1,4-tetrahydro-oxazin-3-one.[69]

The remaining papers considered here extend established principles either in the control exerted by a nearby chiral centre over alkylation adjacent to nitrogen[70–73] or in enantioselective decarboxylation of an α-alkyl-α-amino-malonate.[74] In the latter area,[74] employing chiral cobalt(III) complexes (see Vol. 14, p. 8), and in the alkylation of amidines formed by the reaction of (*S*)-*N*-dimethoxymethylprolinol methyl ether with a DL-α-amino acid[73] useful levels of enantiomeric purity are achieved only in the second of these two studies. Schiff bases formed either from chiral carbonyl compounds (D-galacto-dialdehyde[70] or 2-hydroxypinan-3-one[71]) by reaction with a DL-amino acid ester or from a chiral pyridoxamine analogue and a keto acid[72] continue to be used in the asymmetric synthesis of amino acids; alkylation of the DL-amino acid derivatives gives satisfactory enantiomeric purities leading to α-alkyl analogues of the common L-amino acids,[70, 71] while enantiomeric excesses between 60 and 96% have been reported[72] for hydrolysis of the Schiff base formed between an enantiomer of compound (5) and a keto acid in the presence of Zn^{2+} ions.

Models for Prebiotic Synthesis of Amino Acids. – Several reviews of the classic Miller and Urey system for synthesis of amino acids from simple molecules (for example combinations of H_2, CH_4, H_2O, N_2, and NH_3 subjected to a spark

[58] Y. Hashimoto, H. Aoyagi, M. Waki, T. Kato, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1983, **21**, 11.
[59] C. G. Shin, Y. Yonezawa, Y. Sato, T. Nakano, and J. Yoshimura, *Heterocycles*, 1983, **20**, 405.
[60] U. Schöllkopf and H. J. Neubauer, *Synthesis*, 1982, 861.
[61] U. Schöllkopf and Y. C. Chiang, *Synthesis*, 1982, 864.
[62] J. Nozulak and U. Schöllkopf, *Synthesis*, 1982, 866.
[63] U. Schöllkopf, J. Nozulak, and U. Groth, *Synthesis*, 1982, 868.
[64] U. Schöllkopf, *Tetrahedron*, 1983, **39**, 2085.
[65] U. Schöllkopf and R. Lonsky, *Synthesis*, 1983, 675.
[66] U. Groth and U. Schöllkopf, *Synthesis*, 1983, 37.
[67] U. Schöllkopf, U. Groth, M. R. Gull, and J. Nozulak, *Liebigs Ann. Chem.*, 1983, 1133.
[68] U. Groth and U. Schöllkopf, *Synthesis*, 1983, 673.
[69] W. Hartwig and U. Schöllkopf, *Liebigs Ann. Chem.*, 1982, 1956.
[70] I. Hoppe, U. Schöllkopf, and R. Tolle, *Synthesis*, 1983, 789.
[71] J. A. Bajgrowicz, B. Cossec, C. Pigiere, R. Jacquier, and P. Viallefont, *Tetrahedron Lett.*, 1983, **24**, 3721.
[72] Y. Tachibana, M. Ando, and H. Kuzuhara, *Chem. Lett.*, 1982, 1765, 1769.
[73] M. Kolb and J. Barth, *Liebigs Ann. Chem.*, 1983, 1668.
[74] M. J. Jun, N. M. Yoon, and C. F. Liu, *J. Chem. Soc., Dalton Trans.*, 1983, 999; *J. Coord. Chem.*, 1983, **12**, 279.



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discharge) have appeared.[75] The new results seen in the recent literature continue to demonstrate the suitability of an increasing range of simple compounds in this process, experiments supporting the possible roles of newly identified members of the galactic chemistry set. U.v. irradiation of hydrazine, formaldehyde, and water over $CaCO_3$ in an anoxic environment generates amino acids and other compounds of biological interest,[76] while similar treatment of aqueous ammonium acetate creates glycine, alanine, β-alanine, and γ-aminobutyric acid (and possibly also serine, aspartic acid, and valine).[77] The infinite variety of adjustments that can be applied to the Miller–Urey system is not yet quite exhausted; a mixture of steam, N_2, CO_2, and NH_3 (1:1:1:0.1) streamed at elevated temperatures over a fluidized volcanic ash bed subjected to electric discharge gives serine, glycine, alanine, and glutamic acid as major amino acid products.[78]

Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids. – As in previous volumes, only representative citations can be offered to indicate the burgeoning literature covering fermentative production of protein amino acids. Section 16 (Fermentation and Bio-industrial Chemistry) of *Chem. Abstr.* offers easy access to most of the papers and patents in this field. The literature of the biosynthesis of amino acids also can only be covered in a superficial manner here. Many of the papers cited in this section concern the use of one L-amino acid as a substrate for microbial or enzymatic conversion into another. Production of L-lysine has been described,[79] as has the conversion of L-serine into L-tryptophan,[80] phenylalanine into L-Dopa,[81] L-glutamic acid into γ-aminobutyric acid,[82] and L-homocysteine into *S*-adenosyl-L-homocysteine employing beef-liver *S*-adenosyl-L-homocysteine hydrolase.[83]

[75] S. L. Miller and G. Schlesinger, *Adv. Space Res.*, 1983, **3**, 47; G. Schlesinger and S. L. Miller, *J. Mol. Evol.*, 1983, **19**, 376; A. Lazcano, J. Oro, and S. L. Miller, *Precambrian Res.*, 1983, **20**, 259.

[76] C. Folsome, A. Brittain, and M. Zelko, *Origins Life*, 1983, **13**, 49.

[77] M. Akaboshi, K. Kawai, H. Maki, K. Kawamoto, and Y. Honda, *Origins Life*, 1982, **12**, 339.

[78] G. A. Lavrent'ev, T. F. Strigunkova, Z. Yu. Rakilin, L. A. Piskunova, and I. A. Egorov, *Dokl. Akad. Nauk S.S.S.R.*, 1982, **267**, 756.

[79] A. Rutkov, *Acta Microbiol. Bulg.*, 1983, **13**, 33, 40.

[80] L. Nyeste, M. Pecs, B. Sevella, and J. Hollo, *Adv. Biochem. Eng., Biotechnol.*, 1983, **26**, 175; W. G. Bang, S. Lang, H. Sahm, and F. Wagner, *Biotechnol. Bioeng.*, 1983, **25**, 999.

[81] H. J. Wichers, T. M. Malingre, and H. J. Huizing, *Planta*, 1983, **158**, 482.

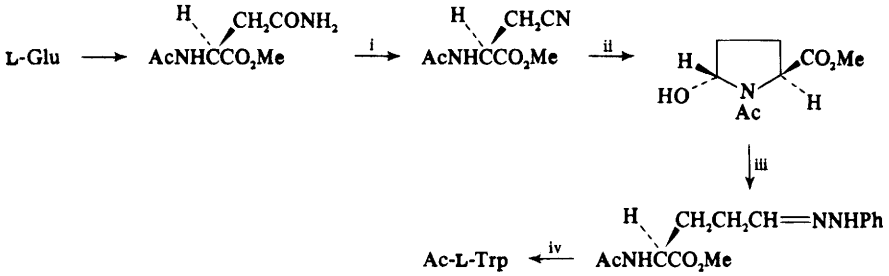
[82] R. Januseviciute, A. Pauliukonis, and D. Kazlauskas, *Khim. Prir. Soedin.*, 1983, 246.

[83] B. Chabannes, A. Gharib, L. Cronenberger, and H. Pacheco, *Prep. Biochem.*, 1983, **12**, 395.

Biosynthesis of phenylalanine and tyrosine from prephenic acid in *Streptomycetes*[84] and of β-(6-benzylaminopurin-9-yl)alanine from *O*-acetyl-L-serine and 6-benzylaminopurine in higher plants[85] and the unexpected finding that furanomycin arises from two acetate and one propionate unit[86] are typical of the range of biosynthetic work appearing in 1983.

A practical benefit arising from considerable differences in rates of aminoacylase-catalysed reactions is the separation of L-leucine from L-isoleucine by conversion into their *N*-acetyl derivatives and aminoacylase–cobalt(II)-catalysed hydrolysis; conditions have been found that allow L-leucine to crystallize out as the process continues.[87]

Laboratory syntheses of less common L-amino acids from protein amino acids are featured this year, as in most previous volumes in this series; conversion of one protein amino acid into another (synthesis of L-tryptophan from L-glutamic acid[88]) is a relatively rare laboratory operation. L-Glutamic γ-semi-aldehyde, formed from glutamic acid *via* *N*-acetyl-L-asparagine methyl ester (Scheme 2), gives a phenylhydrazone from which *N*-acetyl-L-tryptophan is obtained with negligible racemization.[88] L-Lysine serves as a source of L-pipecolic acid (N^{ϵ}-benzylidene derivative underwent N^{α}-benzylation; treatment with NaOBr then HBr debenzylation gave 19% L-pipecolic acid through treatment with Et_3N),[89] also as a source of L-α-aminoadipic acid (lysine, protected except for the side-chain amino group, underwent successive conversions $-CH_2NH_2 \rightarrow -CH_2NHN{=}NPh$ with $PhN_2^+BF_4^- \rightarrow CH_2OH \rightarrow CO_2H$ in 50% overall yield[90]). An alternative synthesis of DL-α-aminoadipic acid[91] in which hippuric acid is alkylated with



Reagents: i, tosyl chloride, pyridine; ii, H_2/Raney Ni; iii, $PhNHNH_2$; iv, refluxing 0.1M HCl; v, H_3O^+

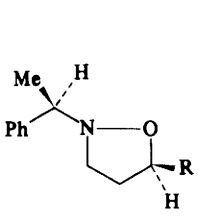
Scheme 2

[84] B. Keller, E. Keller, H. Goerisch, and F. Lingens, *Hoppe-Seyler's Z. Phyiol. Chem.*, 1983, **364**, 455.
[85] I. Murakoshi, C. Koide, F. Ikegami, and K. Nasu, *Chem. Pharm. Bull.*, 1983, **31**, 1777.
[86] R. J. Parry and H. P. Buu, *J. Am. Chem. Soc.*, 1983, **105**, 7446.
[87] J. Martens and H. Weigel, *Liebigs Ann. Chem.*, 1983, 2052.
[88] F. Masumi, H. Takeuchi, S. Kondo, K. Suzuki, and S. Yamada, *Chem. Pharm. Bull.*, 1982, **30**, 3831.
[89] H. Mihara, H. Aoyagi, M. Waki, T. Kato, and N. Izumiya, *Mem. Fac. Sci., Kyushu Univ., Ser. C*, 1983, **14**, 123.
[90] J. E. Baldwin, P. Harrison, and J. A. Murphy, *J. Chem. Soc., Chem. Commun.*, 1982, 818.
[91] N. H. Khan, M. Ali, and H. Panda, *J. Indian Chem. Soc.*, 1982, **59**, 1077.

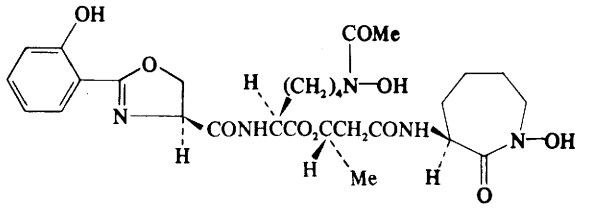
succinic anhydride and the resulting azlactone is cleaved with HI–Red P in refluxing acetic acid has been reported.

Laevulinic acid, converted successively into the 3-bromo derivative, whose oxime was subjected to Beckmann rearrangement, provides a starting material for the synthesis of aspartic acid.[92a] DL-β-Carboxyaspartic acid is accessible through alkylation of the Schiff base Z-N=$CHCO_2Me$ with the anion of di-t-butyl malonate.[92b]

Among a variety of more complex natural amino acids and their derivatives synthesized recently there are to be found examples already mentioned earlier in this chapter. Hypusine has been synthesized[93] through condensation of *N*-benzyloxycarbonyl-L-lysine benzyl ester with the chiral isoxazolidine (6), reduction of the resulting imine with $NaBH_4$, and hydrogenolysis over Pd–C. A synthesis of 9-epihypusine was achieved through the same route using the appropriate isoxazolidine.[93] Another lysine derivative, mycobactin S2 (7),



(6)

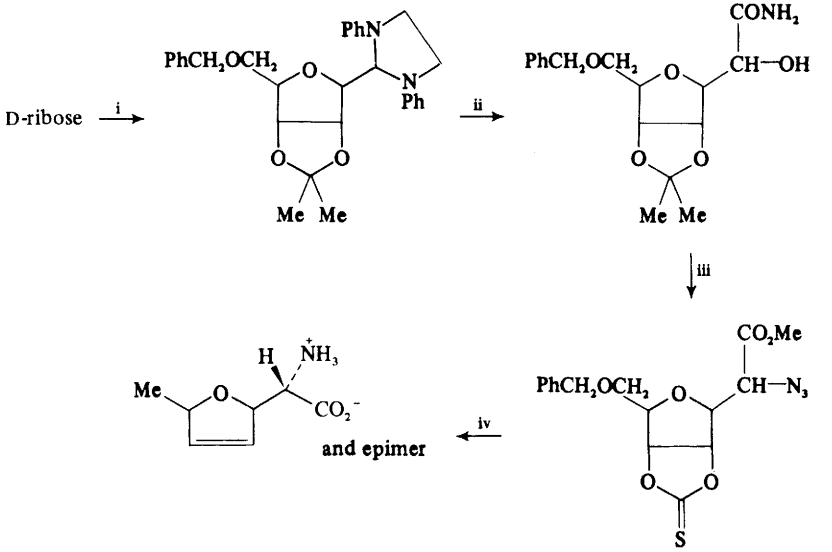


(7)

which differs from mycobactin S in that it carries a methyl group in place of a long hydrocarbon chain, has been synthesized in a route that is unexceptional as far as the amino acid chemistry is concerned.[94] Similarly routine chemistry is involved in the conversion of (*S*)-(−)-phenylalanine into the new derivative (−)-anabellamide [(*S*, *S*)-$PhCONHCH(CH_2Ph)CO_2CH_2CH(NHBz)CH_2Ph$] isolated from *Anaphalis subumbellata*,[95] in the synthesis of all four diastereoisomers of histopine from L-histidine and pyruvic acid,[96] and in the synthesis of the proline analogue of nicotianamine (see p. 24 of this volume, also Vol. 4, p. 4) from L-proline ethyl ester and a protected aspartic semi-aldehyde.[97] More is involved in a synthesis of a diastereoisomer of the antibiotic (+)-furanomycin from D-ribose (Scheme 3), which incidentally confirms the necessity of revision of the stereochemistry assigned to the antibiotic.[98]

Total synthesis of α-allokainic acid has been achieved through a multi-stage procedure (Scheme 4),[99] and reactions of kainic acid itself (modification of the isopropenyl grouping through Pd-catalysed arylation, $CMe=CH_2 \rightarrow CMe=CHAr$,

[92] (*a*) U. R. Joshi and P. A. Limaye, *Indian J. Chem., Sect. B*, 1982, **21**, 1122; (*b*) D. H. Rich and M. K. Dhaon, *Tetrahedron Lett.*, 1983, **24**, 1671.
[93] C. M. Tice and B. Ganem, *J. Org. Chem.*, 1983, **48**, 5048.
[94] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1983, **105**, 240.
[95] S. K. Talapatra, M. K. Pal, A. K. Mallik, and B. Talapatra, *J. Nat. Prod.*, 1983, **46**, 140.
[96] Y. Kitajima, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3870.
[97] J. Faust, A. Priess, K. Schreiber, and H. Ripperger, *Tetrahedron*, 1983, **39**, 1593.
[98] M. J. Robins and J. M. R. Parker, *Can. J. Chem.*, 1983, **61**, 317.
[99] G. A. Kraus and J. O. Nagy, *Tetrahedron Lett.*, 1983, **24**, 3427.



Reagents: i, known sequence; ii, modified Strecker-type synthesis; iii, hydrolysis, $CSCl_2$; iv, Corey–Winter reaction

Scheme 3

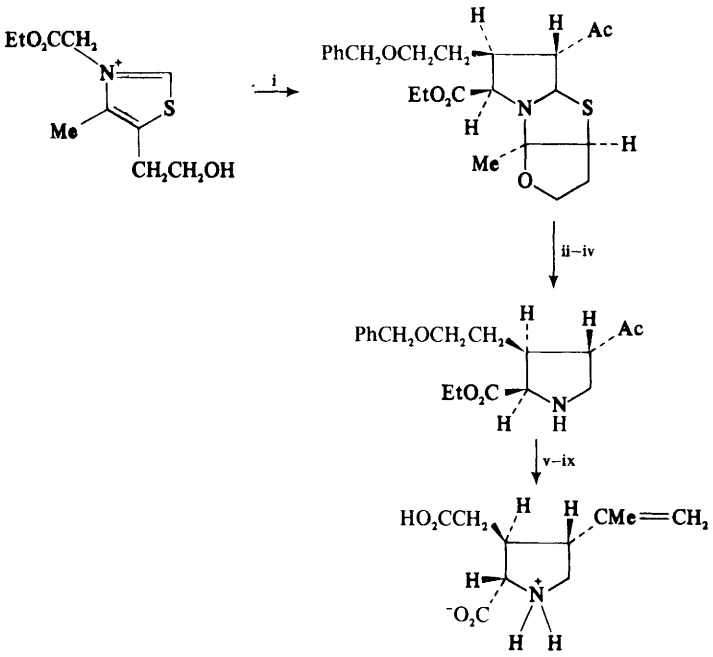
and allylic substitution through alkylation of the π-allylpalladium complex with carbanions from t-butyl acetoacetate or $MeCOCH_2SPh$) have been described.[100]

Synthesis of β- and Higher Homologous Natural Amino Acids. – 4-Amino-alkanoic acids feature in the crop of papers eligible for citation here, including 3-methylstatine analogues $BocNHCHRC(OH)MeCH_2CO_2Et$, prepared from Boc-α-amino acids by treatment with MeLi followed by $MeCO_2Et$ and BuLi,[101] and the use of the corresponding material (Boc-D-alanine) in a related procedure for the synthesis of (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylpentanoic acid, a constituent of bleomycin.[102] A homologue, (*S*)-4-amino-3-hydroxybutyric acid, has been synthesized from D-arabinose *via* conventional stages through a route easily adapted to yield (*S*)-carnitine.[103]

Detoxinine (8) has been synthesized in racemic form through the sequence shown in Scheme 5.[104]

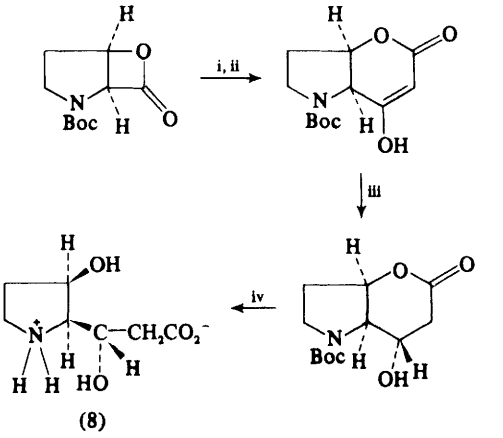
α-Alkyl Analogues of Natural α-Amino Acids. – Preceding sections include descriptions of syntheses of αα-disubstituted α-amino acids through standard methods. Further examples with enhanced interest describe syntheses of α-methyl-

[100] G. A. Conway, J. S. Park, L. Maggiora, M. P. Mertes, N. Galton, and E. K. Michaelis, *J. Med. Chem.*, 1984, **27**, 52.
[101] M. Kawai, A. S. Boparai, M. S. Bernatowicz, and D. H. Rich, *J. Org. Chem.*, 1983, **48**, 1876.
[102] R. M. Di Pardo and M. G. Bock, *Tetrahedron Lett.*, 1983, **24**, 4805.
[103] K. Bock, I. Lundt, and C. Pederson, *Acta Chem. Scand., Ser. B*, 1983, **B37**, 341.
[104] J. Hausler, *Liebigs Ann. Chem.*, 1983, 982.



Reagents: i, $PhCH_2O(CH_2)_2CH{=}CHAc$, Et_3N, MeCN; ii, Bu_3SnH; iii, HCl–EtOH; iv, Boc_2O; v, H_2–Pd/C; vi, Jones oxidation then $Ph_3P{=}CH_2$ on methyl ester; vii, KOH–MeOH; viii, TFA; ix, aq. NaOH, 1 h, 155 °C

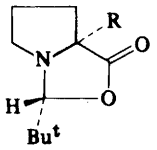
Scheme 4



Reagents: i, Meldrum's acid (2,2-dimethyl-1,3-dioxan-4,6-dione) with 4-(dimethylamino)-pyridine; ii, heat; iii, amine–borane reduction; iv, Boc cleavage and lactone ring-opening

Scheme 5

and -carboxymethyl-ornithine and -arginine,[105] through alkylation of di-lithiated 3-aminopiperidin-2-one Schiff bases followed by cleavage with HCl,[105] and α-alkyl-threonines and -cysteines produced similarly from derived oxazolines.[106] Oxazolidinones (9; R = H), formed by condensing L-proline with pivalic aldehyde, undergo alkylation by electrophiles after lithiation with conservation of the configuration of the proline chiral centre.[107]

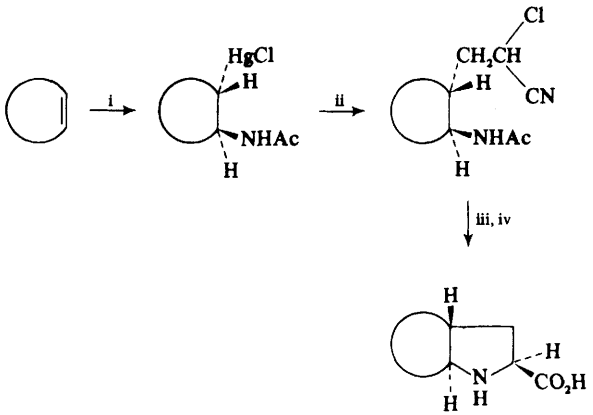


(9)

Synthesis of Other Aliphatic, Alicyclic, and Saturated Heterocyclic Amino Acids. — The Proceedings of the 17th European Peptide Symposium include a number of points of interest of amino acid chemistry, a representative example being a synthesis of 'L-neopentylglycine' (2-amino-4,4-dimethylpentanoic acid) through the sequence $Bu^tCH{=}C(CN)CO_2H \rightarrow Bu^tCH_2CH(CN)CO_2H \rightarrow Bu^tCH_2CH(CONH_2)CO_2H$ followed by Hofmann rearrangement and resolution, using brucine, of the *N*-formyl derivative.[108] Alkylation of ethyl acetamidocyanoacetate with 3-bromomethylcyclohexene gives β-(3-cyclohexenyl)alanine, after alkaline hydrolysis and decarboxylation, which on epoxidation gives a starting material for the synthesis of tetaine analogues.[109] Other straightforward syntheses lead to the 2-aminosuberic acid derivative $BzlOCO(CH_2)_5CH(NHAc)CO_2Et$ through alkylation of benzylideneglycine ethyl ester[110] and to 6-diazo-5-oxo-L-norleucine from methyl *N*-trifluoroacetyl γ-benzyl-L-glutamate through conventional elaboration of the side-chain carboxy group[111] (the foregoing derivative was also elaborated further through its side-chain carboxy group to yield the corresponding oxiranyl ketone[110]).

Proline derivatives and analogues continue to present synthetic challenge, and all the examples representing this year's literature are fully characterized in stereochemical terms. Full details of the synthesis of enantiomers of 5-oxaproline (Vol. 14, p. 11) have been reported.[112] Favorskii ring contraction, a method used before for the synthesis of proline analogues but without specification of stereochemical aspects, is illustrated in the conversion (10) → (11).[113] The same workers

[105] C. G. Unson and B. W. Erickson, *Int. J. Pept. Protein Res.*, 1983, **22**, 50.
[106] D. Seebach and J. D. Aebi, *Tetrahedron Lett.*, 1983, **24**, 3311; D. Seebach and T. Weber, *ibid.*, p. 3315.
[107] D. Seebach, M. Boes, R. Naef, and W. B. Schweizer, *J. Am. Chem. Soc.*, 1983, **105**, 5390.
[108] J. Pospisek and K. Blaha in 'Proceedings of the 17th European Peptide Symposium', ed. K. Blaha and P. Malon, de Gruyter, Berlin, 1983, p. 333.
[109] M. Smulkowski, M. Dzieduszycka, and E. Borowski, *Pol. J. Chem.*, 1982, **56**, 699.
[110] D. H. Rich, J. Singh, and J. H. Gardner, *J. Org. Chem.*, 1983, **48**, 432.
[111] G. R. Pettit and P. S. Nelson, *J. Org. Chem.*, 1983, **48**, 741.
[112] A. Vasella, R. Voeffray, J. Pless, and R. Huguenin, *Helv. Chim. Acta*, 1983, **66**, 1241.
[113] R. Henning and H. Urbach, *Tetrahedron Lett.*, 1983, **24**, 5339.



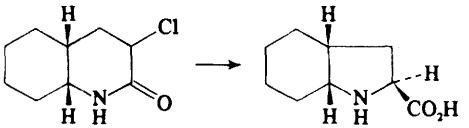
Reagents: i, MeCN, $Hg(NO_3)_2$, NaCl; ii, $CH_2{=}C(CN)Cl$, $NaBH_4$; iii, NaH–DMF; iv, refluxing 5M HCl

Scheme 6

have described a stereospecific synthesis of 4,5-disubstituted proline analogues starting from an alkene (Scheme 6),[114] verifying the stereochemistry through *X*-ray crystal analysis.[115]

Catalytic hydrogenation of 2-ethylpyrrol-1-ine-5-carboxylic acid gave *cis*-5-ethyl-DL-proline, while $NaBH_4$ reduction gave the *cis–trans* mixture from which the *trans* isomer could be isolated *via* *N*-tosyl derivatives.[116] Conventional resolution (employing tartaric acid) completed the task, to provide monomers from which the optically active poly(amino acid)s were prepared.

Higher homologous amino acids are represented in the synthesis of *cis*- and *trans*-2-aminocycloalkaneacetic acids as γ-aminobutyric acid analogues[117] and homo-β-proline analogues.[118]



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[114] R. Henning and H. Urbach, *Tetrahedron Lett.*, 1983, **24**, 5343.
[115] R. Henning, H. Urbach, and E. F. Paulus, *Tetrahedron Lett.*, 1983, **24**, 5347.
[116] W. W. Y. Wang, C. G. Overberger, and C. M. Venkatachalam, *J. Polym. Sci., Polym. Chem. Ed.*, 1983, **21**, 1643.
[117] P. D. Kennewell, S. S. Matharu, J. B. Taylor, R. Westwood, and P. G. Sammes, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2553.
[118] I. M. Labouta, P. Jaconsen, P. Thorbeck, P. Krogsgaard-Larsen, and H. Hjeds, *Acta Chem. Scand., Ser. B*, 1982, **36**, 669.
[119] T. Shono, Y. Matsumura, and K. Inoue, *J. Chem. Soc., Chem. Commun.*, 1983, 1169.

Synthesis of α-Alkoxy-α-amino Acids. – Anodic oxidation of *N,N*-dimethoxycarbonyl-αω-diamino acids in methanol containing tetramethylammonium toluene-*p*-sulphonate gives the ω-methoxy derivative, which can be cyclized *in situ* by adding conc. H_2SO_4 to give saturated heterocyclic amino acids.[119] However, use of NaCl as electrolyte in the same system gives α-methoxy analogues which on cyclization give unsaturated heterocyclic amino acids.[119]

Synthesis of Halogenoalkyl Amino Acids. – All the halogens are represented, in the preparation of 4,4-difluoro-L-proline from the 4-oxoproline and Et_2NSF_3,[120] in the formation of β-fluoro-α-amino acids from cyano- and aminocarbonylaziridines and HF followed by acid hydrolysis,[121] in the formation of *erythro*- or *threo*-4-chloro- or -4-bromo-glutamic acids from the corresponding hydroxyglutamic acids and *N*-methoxycarbonylphthalimide and the phosphorus pentahalide,[122] and in an improved procedure for the synthesis of monoiodohistidine in 57% yield from iodine and the amino acid.[123]

Synthesis of Aliphatic Amino Acids Containing Side-chain Hydroxy Groups. – A convenient synthesis of (*S*)-isoserine from L-malic acid monoamide involves Hofmann rearrangement using $(CF_3CO)_2IPh$ in Ac_2O-pyridine.[124]

Synthesis of Aliphatic Amino Acids Containing Unsaturated Side Chains. – Most space is taken, as usual in this section, by 'dehydroamino acids' (*i.e.* 2-amino-2-alkenoic acids). These can be prepared by dehydration of *N*-Z- or -Boc-2-amino-3-hydroxyalkanoic acids with *N,N'*-dicarbonyldi-imidazole,[125] by dehydration of *N*-acyl-*N*-hydroxy-2-aminoalkanoic acids with $TsCl–Et_3N$,[126] and by $Hg(OAc)_2$ oxidation of vinylogous amino isocyanides followed by treatment with a primary amine.[127]

N-Acetyldehydroalanine may be arylated on the methylene group using an aryl halide with $Pd(OAc)_2$–Ph_3P or $PdCl_2(Ph_3P)_2$, to give homologous *Z*-dehydroamino acids.[128]

βγ-Unsaturated α-alkyl-α-amino acids have been prepared[129] by deconjugative base-catalysed alkylation of methyl α-isocyanoacrylates followed by hydrolysis.

Alkylation of diethyl benzamidomalonate with $Me_3SiC{\equiv}CCH_2Br$ and NaH gives *N*-benzoyl propargylglycine on decarboxylation, which with *N*-iodosuccinimide gives the lactone of *Z*-2-benzamido-4-hydroxy-5-iodopent-4-enoic acid.[130]

[120] J. R. Sufrin, T. M. Balasubramanian, C. M. Vora, and G. R. Marshall, *Int. J. Pept. Protein Res.*, 1982, **20**, 438.
[121] A. I. Ayi and R. Guedj, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2045.
[122] I. M. Kocheva, L. V. Alekseeva, and V. P. Krasnov, *Zh. Org. Khim.*, 1983, **19**, 283.
[123] M. Miyashita, Y. Seyama, K. Kaji, and S. Yamashita, *Chem. Pharm. Bull.*, 1983, **31**, 386.
[124] R. Andruszkiewicz, A. Czerwinski, and J. Grzybowska, *Synthesis*, 1983, 31.
[125] R. Andruszkiewicz and A. Czerwinski, *Synthesis*, 1982, 968.
[126] T. Kolasa, *Synthesis*, 1983, 539.
[127] C. Herdeis and A. Dimmerling, *Arch. Pharm.*, 1984, **317**, 86.
[128] M. Cutolo, V. Fiandanese, F. Naso, and O. Sciacovelli, *Tetrahedron Lett.*, 1983, **24**, 4603.
[129] K. Nunami, M. Suzuki, and N. Yoneda, *Chem. Pharm. Bull.*, 1982, **30**, 4015.
[130] M. J. Sofia, P. K. Chakravarty, and J. A. Katzenellenbogen, *J. Org. Chem.*, 1983, **48**, 3318.

Corresponding phenyl- and benzyl-substituted benzamidomalonate esters give 2-phenyl and 2-benzyl analogues of these unsaturated α-amino acids.[130]

Synthesis of Aromatic and Heteroaromatic Amino Acids. – (3,4-Methylenedioxyphenyl) acetone, NH_3, $CHCl_3$, and a phase-transfer catalyst are said[131] to yield the corresponding Dopa derivative and its amide, from which by conventional resolution and acid hydrolysis L-(−)-α-methylDopa was obtained.[131] Forphenicinol (3-hydroxy-4-hydroxymethylphenyl glycine) is equally easily accessible through Strecker synthesis from the appropriately protected benzaldehyde, resolvable as its *N*-acetyl derivative using aminoacylase.[132] Related amino acids as synthetic targets have included 3′-aryloxytyrosines,[133] 3′-(*N*-4-acetoxy-1,4-benzoxazin-3-onyl)tyrosines,[134] 'cyclopropylphenylalanine' (1-amino-2-arylcyclopropane-1-carboxylic acid),[135] and a variety of 4′-substituted phenylalanines, where the substituent is one of a number of sulphur functional groups formed by elaboration of a $-SO_2Cl$ placed there by reaction of the amino acid with $ClSO_3H$.[136]

The Strecker synthesis has served for the preparation of 3-(1-tetralinyl)- and 3-(5,6,7,8-tetrahydro-5-quinolyl)-alanine,[137] and other standard routes, or modifications of them, have been used for synthesis of 'phosphotryptophan' (from a formylindole and trimethylphosphonoacetate),[138] 2-alkylthio-*N*-hydroxytryptophans [from indoles and $O{=}NC(=CH_2)CO_2Et$],[139] and other tryptophans through Fischer indole synthesis applied to α-methoxy-*N*-benzylpyrrolidines (prepared by electrochemical α-methoxylation – see also ref. 119).[140]

Syntheses of heteroaromatic amino acids using amino acids as starting materials are of the more obvious types (nitration of tryptophan to give the 6-nitro derivative, followed by a variety of modifications to the nitro function,[141] N^{τ}-hydroxyalkylhistidines from protected histidines by reaction with alkyl halides[142]) or of more subtle types (conversion of α-benzyl *N*-benzyloxycarbonyl-D-aspartate to corresponding benzimidazoles using substituted phenylenediamines[143]).

Synthesis of Amino Acids Containing Sulphur. – Many papers dealing with modified cysteines have appeared. β-Chloro-D-alanine has been converted into a range of *S*-substituted D-cysteines through reactions with corresponding thiols

[131] Y. Shi, H. Zhu, Y. Jiang, X. Sun, and H. Hu, *Nanjing Daxue Xuebao, Ziran Kexue*, 1982, 853 (*Chem. Abstr.*, 1983, **98**, 161 140).
[132] H. Morishima, J. Yoshizawa, R. Ushijima, T. Takeuchi, and H. Umezawa, *J. Antibiot.*, 1982, **35**, 1500.
[133] T. Inoue, K. Naitoh, S. Kosemura, I. Umezawa, T. Sonobe, N. Serizawa, N. Mori, and H. Itokawa, *Heterocycles*, 1983, **20**, 397.
[134] T. Ishizaki, Y. Hashimoto, K. Shudo, and T. Okamoto, *Heterocycles*, 1983, **20**, 1481.
[135] H. Kimura and C. H. Stammer, *J. Org. Chem.*, 1983, **48**, 2440; M. Suzuki, S. Kumar, and C. H. Stammer, *ibid.*, p. 4769.
[136] E. Escher, M. Bernier, and P. Parent, *Helv. Chim. Acta*, 1983, **66**, 1355.
[137] E. Reimann and W. Dammertz, *Arch. Pharm.*, 1983, **316**, 297.
[138] S. F. Chen, S. O. Kumar, and M. Tishler, *Tetrahedron Lett.*, 1983, **24**, 5461.
[139] R. Plate, H. C. J. Ottenheijm, and R. J. F. Nivard, *J. Org. Chem.*, 1984, **49**, 540.
[140] T. Shono, Y. Matsumura, and T. Kanazawa, *Tetrahedron Lett.*, 1983, **14**, 1259.
[141] L. L. Melhacb and N. J. Leonard, *J. Org. Chem.*, 1983, **48**, 5130.
[142] J. B. Campbell, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1213.
[143] J. J. Nestor, B. L. Horner, T. L. Ho, G. H. Jones, G. I. McRae, and B. H. Vickery, *J. Med. Chem.*, 1984, **27**, 320.

mediated by an enzyme from *Pseudomonas putida*.[144] Arylation of cysteine by Dopa through oxidation with H_2O_2 in the presence of an iron–EDTA complex gives mixtures of cysteinylDopas,[145] while alkylation using chiral aziridinecarboxylate esters gives lanthionines,[146] *threo*-(—)-*S*-(2-carboxypropyl)-L-cysteine from (*S*)-(—)-$HSCH_2CHMeCO_2Me$,[147] 3-methyl-D-cysteine (a constituent of nisin),[148] and *threo*-3,3′-dimethylcysteine.[149]

Methionine has been converted into *S*-t-butyl homocysteine, exploiting sulphonium salt chemistry; t-butylation followed by equilibration with nucleophiles gave the derivative suitable for use in peptide synthesis as a protected homocysteine.[150] Procedures for the isolation of *S*-adenosyl-L-methionine from baker's yeast have been described,[151] and synthesis of *S*-adenosyl-DL-penicillamine is included in a range of new 5′-substituted *S*-adenosylhomocysteine analogues.[152] Diastereoisomers of L-methionine-*S*,*R*-sulphoximine can be separated after *N*-phthaloylation and cyclization to the corresponding tetrahydro-1,2-thiazin-3-ones by fractional crystallization or chromatography.[153]

2-[(1*S*, 3*S*)-1-Amino-3-carboxy-3-hydroxypropyl]thiazole-4-carboxylic acid, 'fragment D' of the antibiotic nosiheptide, has been synthesized in a multi-step procedure starting with a 4,6-benzylidene-D-2-azido-2,3-dideoxyhexose carrying the necessary chiral centres.[154]

Synthesis of Phosphorus-containing Amino Acids. — (±)-Phosphinothricin has been prepared through alkylation of benzylideneglycine ethyl ester with MeP(O)(OMe)$CH{=}CH_2$ followed by hydrolysis in 6M hydrochloric acid.[155] Synthesis of 'phosphotryptophan' has been mentioned earlier in this chapter.[138]

Amino Acids Synthesized for the First Time. — In addition to many amino acids in this category mentioned elsewhere in this chapter, N^{ϵ}-[(2-chloroethyl)nitrosocarbamoyl]-L-lysine,[156] Dopa analogues in which the phenolic hydroxy groups are replaced by acidic nitrogen functional moieties[157] or linked through $-CH_2-$

[144] T. Nagasawa, H. Hosono, H. Ohkishi, Y. Tani, and H. Yamada, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 809.
[145] S. Ito, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 365.
[146] K. Nakajima, H. Oda, and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 520.
[147] R. J. Parry and M. V. Naidu, *Tetrahedron Lett.*, 1983, **24**, 1133.
[148] T. Wakamiya, K. Fukase, K. Shimbo, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1559; T. Wakamiya, K. Shimbo, T. Shiba, K. Nakajima, M. Neya, and K. Okawa, *ibid.*, 1982, **55**, 3878.
[149] K. Nakajima and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1565.
[150] G. Chassaing, S. Lavielle, and A. Marquet, *J. Org. Chem.*, 1983, **48**, 1757.
[151] S. V. Petrenko, *Khim.-Farm. Zh.*, 1983, **17**, 330.
[152] D. Shire, P. Blanchard, A. Raies, F. Lawrence, M. Robert-Gero, and E. Lederer, *Nucleosides Nucleotides*, 1983, **2**, 21.
[153] Y. Sugiyama and F. C. Wedler, *Tetrahedron Lett.*, 1983, **24**, 1471.
[154] M. Iwakawa, Y. Kobayashi, S. Ikuta, and J. Yoshimura, *Chem. Lett.*, 1982, 1975.
[155] N. Minowa, S. Fukatu, T. Niida, M. Takada, and K. Sato, *Tetrahedron Lett.*, 1983, **24**, 2391.
[156] J. C. Kim and I. S. Cho, *Yakhak Hoechi*, 1983, **27**, 177 (*Chem. Abstr.*, 1983, **99**, 176 244).
[157] N. Zenker, C. N. Talaty, P. S. Callery, J. Wright, L. S. Hubbard, and E. M. Johnson, *J. Heterocycl. Chem.*, 1983, **20**, 435; H. Schmidhammer and K. Hohenlohe-Oehringen, *Sci. Pharm.*, 1983, **51**, 8.

$(CH_2OCH_2)_nCH_2-$,[158] and DL-thyroxine *p*-hydroxyphenyl ether[159] have been described.

Synthesis of Labelled Amino Acids. – Conference proceedings often provide an economical overview of the current status of a narrow field of study, and the increasing interest in amino acids labelled with short-lived isotopes is represented this year.[160] Only sample citations, synthesis of ^{11}C-carboxyl-labelled and ^{13}N-labelled glutamic acid[160] and synthesis of other ^{11}C-carboxyl-labelled amino acids,[161] are given here. Positron emission from ^{11}C-labelled amino acids offers new possibilities in discovering the fate of amino acids metabolized at different sites *in vivo*, providing the main spur to establishing efficient syntheses that can be effected very rapidly; synthesis of L-[1-^{11}C]leucine[162] provides another example of rapid Strecker synthesis and D-amino acid oxidase resolution within 30–40 min from H^{11}CN.

A crop of papers,[163–172] mostly from laboratories whose work has featured in this section in recent volumes, describe syntheses of protein amino acids variously labelled with carbon, hydrogen, and nitrogen isotopes. An extensive account[163] describes syntheses of chiral-methyl valines, whose very high specific radioactivity renders them well suited for biosynthetic studies. The other labelled amino acids described in these papers are (3*RS*)-β-leucine-[2,3-^{3}H],[164] (2*S*,4*S*)- and (2*R*,4*S*)-leucine-[5-^{13}C] from (2*RS*,3*S*)-valine-[4-^{13}C] *via* (3*S*)-isovaleric acid-[4-^{13}C],[165] (2*RS*)-leucine-[1,2-$^{13}C_2$] from $Me_2CHCH_2{}^{13}CHO$,[165] β-hydroxyvaline-[4-^{13}C] and β-hydroxyvaline-[4,4,4-$^2H_3$],[166] L-glutamic acid labelled stereospecifically at C-3 with ^{2}H and non-stereospecifically at C-4 with ^{3}H,[167] L-serine stereospecifically labelled at C-3 with ^{2}H,[168] aspartic and glutamic acids-[1-^{13}C] and glutamic acid-[3-^{13}C],[169] branched-chain amino acids-[1-^{13}C,^{15}N],[170] glutamic acid-[1-^{13}C,^{15}N] and related amino acids,[171] and (*RS*)-glycine-[α-^{2}H].[172]

The other main approach to labelled amino acids employs the amino acids themselves or an amino acid from which the target molecule is accessible. Factors determining the distribution achieved in catalysed ^{3}H–^{1}H exchange with $^3H_2$ have

[158] M. Berthet and E. Sonveaux, *J. Chem. Soc., Chem. Commun.*, 1983, 10.
[159] K. G. Boldt and G. A. Brine, *Org. Prep. Proced. Int.*, 1983, **15**, 137.
[160] M. B. Cohen, L. Spotter, C. C. Chang, and N. S. MacDonald in 'Proceedings of 3rd World Congress, Nuclear Medicine, Biological Advances', ed. C. Raynaud, Pergamon, Oxford, 1983, Vol. 1, p. 632.
[161] Q. Sun, S. Chen, Y. Ye, H. Bao, Y. Li, F. Fan, C. Gu, L. Zhang, and Z. Zhan in ref. 160, p. 650.
[162] J. R. Barrio, R. E. Keen, J. R. Ropchan, N. S. MacDonald, F. J. Baumgartner, H. C. Padgett, and M. E. Phelps, *J. Nucl. Med.*, 1983, **24**, 515.
[163] D. H. G. Crout, M. Lutstorf, and P. J. Morgan, *Tetrahedron*, 1983, **39**, 3469.
[164] D. J. Aberhart and H. J. Lin, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 611.
[165] D. J. Aberhart and B. H. Weiller, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 663.
[166] D. J. Aberhart, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 605.
[167] S. J. Field and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2387.
[168] D. Gani and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2393.
[169] U. Fotader and D. Cowburn, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 1003.
[170] S. S. Yuan, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 173.
[171] O. Bouloussa and P. Dizabo, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 991.
[172] R. H. White, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 787.

been discussed.[173] Alkylation of L-homocysteine with C^3H_3I,[174] formation of L-asparagine-[U-^{13}C] and the corresponding L-aspartic acid,[175] and H_2O_2–Fe^{2+} oxidation of proline-[U-^{14}C] to give the corresponding *trans*-3-hydroxyproline[176] provide routine examples of synthesis of labelled protein amino acids, as do hydrogenation of dehydrophenylalanyl-D-alanine dioxopiperazine with $^2H_2$ followed by hydrolysis,[177] conversion of glycine-[2-^{3}H] into the corresponding creatine,[178] and conversion of L-canaline into L-[guanidino-oxy-^{14}C] canavanine by reaction with *O*-[^{14}C-methyl]isourea.[179]

^{15}N-Labelled L-lysine and L-glutamic acid have been prepared by fermentation (*Brevibacterium flavum*,[180] *Brevibacterium lactofermentum*,[181] respectively) with $^{15}NH_4^+$ salts as nitrogen source, while ^{13}N-labelled leucine and valine have been prepared from the corresponding keto acids, $^{13}NH_3$, and immobilized glutamate dehydrogenase.[182]

Halogen isotopes are discussed in a representative citation from conference proceedings describing ^{18}F-labelled difluoroaminoalkyl analogues of amino acids (*e.g.* conversion of ornithine *via* its lactam into 2-amino-[5-^{18}F]-difluoro-aminopentanoic acid by reaction with $^{18}F_2$ in CF_3CO_2H[183]) and introduction of the same isotope into proline by treatment of *N*-tosyl hydroxy-L-proline methyl ester with $Et_4N^{18}F$.[184] Radio-bromination and -iodination of aromatic amino acids can be effected using the halogen with Chloramine-T or Dichloramine-T.[185]

Resolution of Amino Acids. – The main topic areas (diastereoisomer salt formation, the use of enzymes, chromatographic resolutions, and preferential crystallization) are fully represented in the recent literature. The topic has been reviewed recently.[186]

N-Acylated amino acids have been resolved through diastereoisomeric salt formation (Boc-DL-phenylglycine with phenylethylamine,[187] formyl-DL-neopentylglycine with brucine,[108] *N*-acetyl-3-(3,4-methylenedioxyphenyl)-2-methylalanine with quinine[131]), and *cis*-5-ethyl-DL-proline has been resolved using

[173] B. V. Petrenik, Yu. A. Zolotarev, and N. F. Myasoedov, *Bioorg. Khim.*, 1983, **9**, 1021.
[174] Z. Guo and G. Tang, *He Huaxue Yu Fangshe Huaxue*, 1983, **5**, 255 (*Chem. Abstr.*, 1984, **100**, 34 794).
[175] T. H. Lam, R. Mermet-Bouvier, S. Fermandjian, and P. Fromageot, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 143.
[176] S. G. Ramawamy, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 233.
[177] K. Tanimura, T. Kato, M. Waki, and N. Izumiya, *Tetrahedron Lett.*, 1983, **24**, 3737.
[178] M. M. Daly and I. Lalezari, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 377.
[179] G. A. Rosenthal, K. R. Downum, and J. E. Mattler, *Anal. Biochem.*, 1983, **133**, 277.
[180] C. S. Irving, C. L. Cooney, L. T. Brown, D. Gold, J. Gordon, and P. D. Klein, *Anal. Biochem.*, 1983, **131**, 93.
[181] Z. E. Kahana and A. Lapidot, *Anal. Biochem.*, 1983, **132**, 160.
[182] K. R. Barrio, F. J. Baumgartner, E. Henze, M. R. Stauber, J. E. Egbert, N. S. MacDonald, H. L. Schelbert, M. E. Phelps, and F. T. Liu, *J. Nucl. Med.*, 1983, **24**, 937.
[183] D. Jewett and R. Ehrenkaufer in 'Proceedings of the International Symposium on Synthetic Applications of Isotopically Labelled Compounds', ed. W. P. Duncan and A. B. Susan, Elsevier, Amsterdam, 1983, p. 205.
[184] M. van der Ley, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 453.
[185] G. S. Petzold, *Ber. Kernforschungsanlage Juelich*, 1982 (*Chem. Abstr.*, 1983, **99**, 5984).
[186] T. Kitahara, *Kagaku Kogaku*, 1983, **47**, 277.
[187] A. W. Lipkowski, *Pol. J. Chem.*, 1981, **55**, 1725.

L-tartaric acid.[116] The related diastereoisomeric derivative approach is illustrated in a resolution of DL-cysteine by conversion into diastereoisomeric thiazolidines through reaction with D-(+)-galactose (or any one of a number of aldohexoses or aldopentoses).[188] Related procedures have been used for the resolution of DL-2-amino-4-(aryloxy)butanoic acids[189] and 'cyclopropylphenylalanine' (1-amino-2-phenylcyclopropanecarboxylic acid).[135]

Enzymic methods, such as preferential catalysis of hydrolysis of an *N*-acetyl-L-amino acid in the presence of its enantiomer by aminoacylase, have been used for the resolution of forphenicinol,[132] phenylalanine,[190] and other examples mentioned elsewhere in this chapter. The same principle applied to DL-5-substituted hydantoins using hydantoinase from *Pseudomonas striata*[191] can be particularly satisfactorily conducted on a large scale.

Chromatographic resolution continues along the established paths, but with more discernible attempts to reason out the optimum systems for the purpose. Silica-gel chromatography of *N*-4-nitrobenzoyl-DL-amino acid isopropyl esters with a chiral mobile phase (typically chloroform–n-hexane, *N*-acetyl-L-valine) achieves enantiomeric discrimination through formation of diastereoisomeric chelate-like solvates through intermolecular hydrogen bonds with the D- and L-enantiomers of the amino acid derivative.[192] It is possible that earlier-described silica-gel systems assumed to involve the chiral additive as part of the stationary phase are in fact functioning in the manner now suggested (or *vice versa*). L-Histidine bonded to silica gel[193] and a similar L-valine t-butylamide-bonded stationary phase[194] are described as effective in the resolution of acetylamino acid methyl esters, and further results are reported for L-hydroxyproline bonded to polystyrene and complexed with Cu^{2+} ions for ligand-exchange chromatographic resolutions of DL-amino acids.[195,196]

Preferential crystallization of one enantiomer from supersaturated solutions seeded with one enantiomer is effective for DL-phenylglycine sulphate[197] and for DL-threonine and DL-serine *m*-xylenesulphonates,[198] for salts of *N*-acetyl-DL-phenylalanine with secondary amines,[199] and for ammonium *N*-benzoyl-DL-alaninate.[200] A new approach exploiting the same principle is illustrated in the

[188] J. Martens and K. Drauz, *Liebigs Ann. Chem.*, 1983, 2073.
[189] B. Witczuk, M. Strube, H. Jeschkeit, and G. Kupryszewski, *Pol. J. Chem.*, 1981, **55**, 1511.
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[192] A. Dobashi and S. Hara, *J. Chromatogr.*, 1983, **267**, 11; *Anal. Chem.*, 1983, **55**, 1805; *Tetrahedron Lett.*, 1983, **24**, 1509.
[193] N. Watanabe, *J. Chromatogr.*, 1983, **260**, 75.
[194] X. Xu, Q. Tang, and R. Wang, *Huazue Xuebao*, 1983, **41**, 262.
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[196] V. A. Shirokov, V. A. Tsyryapkin, L. V. Nedospasova, A. A. Kurganov, and V. A. Davankov, *Bioorg. Khim.*, 1983, **9**, 878.
[197] T. Shiraiwa, Y. Ohmichi, K. Iwafuji, K. Fujimoto, and H. Kurokawa, *Nippon Kagaku Kaishi*, 1983, 1070 (*Chem. Abstr.*, 1984, **100**, 7090).
[198] O. Otsuki, *Kemikaru Enjiniyaringu*, 1983, **28**, 148 (*Chem. Abstr.*, 1983, **99**, 122 841).
[199] T. Shiraiwa, S. Taniguchi, A. Ikawa, K. Iwafuji, and H. Kurikawa, *Nippon Kagaku Kaishi*, 1983, 1189 (*Chem. Abstr.*, 1984, **100**, 7097).
[200] T. Shiraiwa, Y. Ohmichi, and H. Kurokawa, *Nippon Kagaku Kaishi*, 1983, 1102 (*Chem. Abstr.*, 1983, **99**, 158 807).

resolution of DL-aspartic acid by seeding a solution with the copper(II) complex of an optically active amino acid, which competitively inhibits the crystallization of one of the enantiomers.[201]

4 Physical and Stereochemical Studies of Amino Acids

Crystal Structures of Amino Acids and Their Derivatives. – Following a listing of amino acids and derivatives other than peptides that have been subjected tc *X*-ray crystal analysis, points of general interest are discussed.

Recent studies have involved *N*,*N*-dimethylglycine hydrochloride,[202] the *N*-chloroacetyl derivative of (–)-isovaline (thus shown to possess the *R*-configuration),[203] L-aspartic acid,[204] DL-glutamic acid monohydrate,[205] L-homoserine,[206] DL-α-methyl-*m*-tyrosine,[207] Boc-L-proline,[208] N^{α}-Boc-N^{π}-benzyloxymethyl-L-histidine (thus confirming other physical evidence that the imidazole substituent is located on the nitrogen atom closest to the chiral centre),[209] L-arginine acetate,[210] L-lysine acetate,[211] L-ornithine L-aspartate hemihydrate,[212] bis(methyl-L-cysteinato) molybdenum dioxygenate,[213] and cyclo(L-asparagyl-L-asparagyl).[214]

Two strong hydrogen bonds per molecule exist in solid *N*,*N*-dimethylglycine hydrochloride,[202] one ($-N-H\cdots Cl^-$) being perpendicular to the plane of the cation. The form of L-aspartic acid studied[204] unusually carries a neutral α-carboxy group and a deprotonated side-chain carboxy group.

In the crystal structures of amino acids generally, a head-to-tail hydrogen-bonded sequence of amino and carboxylate moieties is almost always seen.[215] The structures revealed for the acetates of L-arginine[210] and L-lysine[211] also show the same feature, whereas the parent amino acids and their salts and complexes do not. The weakly acidic acetic acid component therefore interacts with the most basic (side-chain) grouping in these amino acids in the solid state, allowing the head-to-tail arrangement, as preferred by the zwitterionic moiety, to be

[201] K. Harada and N. Fujii, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 653.
[202] B. D. Santarsiero and R. E. Marsh, *J. Crystallogr. Spectrosc. Res.*, 1983, **13**, 245.
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[205] Z. Ciunik and T. Glowiak, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1983, **39**, 1271.
[206] K. K. Chacko, S. Swaminathan, and K. R. Veena, *Cryst. Struct. Commun.*, 1982, **11**, 2057.
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[209] T. Brown, J. H. Jones, and J. D. Wallis, *J. Chem. Soc., Perkin Trans. 1*, 1982, 3045.
[210] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **21**, 223.
[211] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 617.
[212] D. M. Salunke and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 154.
[213] I. Buchanan, M. Minelli, M. T. Ashby, T. J. King, J. H. Enemark, and C. D. Garner, *Inorg. Chem.*, 1984, **23**, 495.
[214] C. Howes. N. W. Alcock, B. T. Golding, and R. W. McCabe, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2287.
[215] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 129.

adopted. The head-to-tail arrangement seen in the crystal structure of L-ornithine L-aspartate is the first example for a mixed amino acid salt.

Nuclear Magnetic Resonance Spectrometry. – ^{1}H and ^{13}C n.m.r. studies are now frequently performed and reported jointly, and this is reflected in the structure of this section. Heavier-element n.m.r. studies of amino acids and their derivatives are reviewed in order of increasing atomic number.

Familiar themes are represented in ^{1}H n.m.r. titration studies of a series of imidazoles (including L-histidine and its isomeric *N*-methyl derivatives) yielding microscopic p*K* values of the different imidazole tautomers[216] and in conformational interpretations [*N*-acetyl-α-aminoisobutyric *N*-methylamide adopts a conformation lacking hydrogen bonds;[217] the flexible thiazolidine ring in 3-thiaproline hydantoin preferentially adopts an envelope conformation (C-4 as the 'flap');[218] the side chains of methionine and norleucine derivatives in MeOH adopt closely similar conformations, while *S*-methylcysteine and norvaline derivatives differ in this respect;[219] five- and seven-membered hydrogen-bonded ring structures are adopted by *N*-acetyl-L-phenylalanine *p*-acetyl- and *p*-valeryl-anilides in non-polar solvents[220]]. ^{13}C n.m.r. studies continue to give crucial information in biosynthetic studies using strategically labelled amino acids, showing for example that, while the *m*,*m'*-dihydroxyphenylglycine and *p*-methyl-*m*,*m'*-dihydroxyphenylglycine moieties of ristocetin can be derived from acetate units, the *p*-hydroxyphenylglycine moiety is derived from tyrosine.[221] ^{1}H and ^{13}C n.m.r. data for tryptophan and derivatives have been collected,[222] mainly to offer help in structure elucidations of indole alkaloids.

Because much of the foregoing material is now routine (perhaps an idiosyncratic definition by the Reporter, pressed to select from a broad range of possible citations in certain areas as a result of space limitations imposed on him), more detailed discussion is reserved for more sophisticated instrumental possibilities. Intramolecular separations of hydrogen atoms within the 20 most common amino acids have been determined by n.O.e. studies,[223] while for L-histidine longitudinal and transverse ^{1}H relaxation rates for aqueous solutions give information about molecular motion and conformational changes as a function of time.[224] Other solution studies include ^{1}H, ^{13}C, and INDO studies of L-tryptophan and related compounds[225] and ^{13}C longitudinal relation times (T_1) and

[216] M. Tanokura, *Biochim. Biophys. Acta*, 1983, **742**, 576.
[217] Y. Paterson, E. R. Stimson, D. J. Evans, S. J. Leach, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1982, **20**, 468.
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[219] B. Picur and I. Z. Siemion, *Org. Magn. Reson.*, 1983, **21**, 271.
[220] D. Petkov, P. Ivanov, and I. Stoineva, *Biopolymers*, 1983, **22**, 1489.
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n.O.e. for uniformly ^{13}C-enriched glycine and aspartic acid.[226] Solid-state studies are tending to give information more remote from that which the organic chemist and the analytical chemist need to have to hand, such as ^{2}H n.m.r. spin-lattice relaxation data giving energies for re-orientation of methyl groups in polycrystalline, strategically ^{2}H-labelled amino acids.[227] Similar ^{13}C n.m.r. studies have been described,[228, 229] also ^{13}C chemical-shielding tensors[230] and ^{14}N quadrupole tensors in single crystals of L-threonine and L-serine monohydrate, respectively.[231]

^{15}N n.m.r. can yield information on the barriers to isomerization about the $C-N_2$ bond in guanidinium and guanidino groups in L-arginine; although interconversion is rapid on the n.m.r. time-scale in water–dimethyl sulphoxide through the temperature range −52 to 25 °C, the energy barrier is significantly greater for the guanidinium form.[232] The data can be used to indicate that in this environment approximately one-third of the guanidino form is present as the $RN{=}C(NH_2)_2$ tautomer.[232] The effect of pH on natural-abundance ^{15}N n.m.r. chemical shifts of lysine and ε-(hydroxymethyl)lysine has been interpreted to yield pK_0 values of the nitrogen functional groups.[233]

^{19}F n.m.r. has been used to follow the reaction between *erythro*- or *threo*-β-fluoroaspartic acid and pyridoxal 5′-phosphate, providing stereochemical information for the reaction products through $^{3}J(HF)$ data.[234] Another fundamental application of ^{19}F n.m.r., the pH dependence of chemical shifts for mono-, di-, and tri-fluoro-α-methylalanines and their methyl esters, has been described.[235]

Optical Rotatory Dispersion and Circular Dichroism. — As in many aspects of n.m.r. spectrometry, these techniques are largely free from unexplored fundamental areas deserving publication in the primary literature. Considerable interest remains, however, in applications of these techniques in systems in which an amino acid creates an asymmetric environment for an achiral molecule, for example the positive c.d. induced in catechol crown ethers in the presence of D-glutamic acid hydrochloride.[236] This behaviour can be exploited for the assignment of absolute configuration to an enantiomer of an amino acid. An example of the use of chromophore-substituted amino acids[237] in the manner featured in this

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[235] J. S. Taylor and C. Deutsch, *Biophys. J.*, 1983, **43**, 261.
[236] J. Hu, Z. Guo, and Z. Gao, *Fenzi Kexue Yu Huaxue Yanjiu*, 1983, **3**, 63 (*Chem. Abstr.*, 1983, **99**, 140 331).
[237] N. A. Voskova, V. V. Romanov, G. A. Korshunova, and Yu. P. Shvachkin in 'Proceedings of the 3rd U.S.S.R.–F.R.G. Symposium on the Chemistry of Peptides and Proteins', ed. W. Voelter, E. Wünsch, and Yu. A. Ovchinnikov, de Gruyter, Berlin, 1982, p. 373.

section in every past volume involves the measurement of both c.d. and u.v. spectra near 340 nm of *o*-phthaldialdehyde–mercaptoethanol condensation products formed with mixtures of enantiomers of an amino acid to assess the enantiomeric composition of the sample.

Instrumental advances allowing penetration to shorter wavelengths lead to fuller c.d. spectral information for amino acid derivatives than that obtained in earlier years. Thus, *N*-acetyl-L-tyrosinamide shows positive Cotton effects centred near 230 and 200 nm and negative Cotton effects centred near 215 and 183 nm.[238] Access to vibrational c.d. data is also becoming easier, a recent example establishing a methine C—H stretching chirality rule. L-α-Amino acids in ^2H_2O give vibrational c.d. spectra with a strong positive bias in the CH stretching region.[239] Since the intensity bias seems sensitive to the presence and strength of intramolecular hydrogen bonds, further useful information about fundamental structural features should be expected from this technique.

Mass Spectrometry. – The biological literature is the main source of what is now routine analytical use of this technique, usually in conjunction with g.l.c. Representative papers in this area describe g.l.c.–m.s. estimation of [guanidino-^{15}N]arginine as its tetra-trifluoroacetyl derivative[240] and 1-aminocyclopropane-1-carboxylic acid content of Douglas pine by g.l.c.–m.s. after h.p.l.c. purification of its 2,4-dinitrophenyl derivative.[241] Even the food-analysis literature, however, now contains descriptions of the newer techniques in mass spectrometry, *e.g.* analysis of free amino acids by positive and negative chemical ionization[242] (in contrast to electron-impact m.s., chemical-ionization m.s. yields spectra carrying prominent intact-molecule ion peaks, revealing relatively little fragmentation in the case of amino acid samples). Laser-desorption mass spectrometry of valine similarly yields spectra revealing the minimal fragmentation ion associated with some desorption ionization techniques, valine being characterized by a prominent peak at m/z 118 (*i.e.* M + 1).[243] Secondary-ion emission by bombardment of amino acid films on metal layers results in formation of M + 1 positive ions through proton transfer between adjacent molecules, glycine[244] and phenylalanine[245,246] having been studied in pioneering studies involving irradiation of

[238] E. J. Welsh, S. A. Frangou, E. R. Morris, D. A. Rees, and S. I. Chavin, *Biopolymers*, 1983, **22**, 821.

[239] L. A. Nafie, M. R. Oboodi, and T. B. Freedman, *J. Am. Chem. Soc.*, 1983, **105**, 7449.

[240] I. Nissim, M. Yudkoff, T. Terwilliger, and S. Segal, *Anal. Biochem.*, 1983, **131**, 75.

[241] R. A. Savidge, G. M. C. Mutumba, J. K. Heald, and P. F. Wareing, *Plant Physiol.*, 1983, **71**, 434.

[242] D. Fraisse, F. Maquin, J. C. Tabet, and H. Chaveron in 'Proceedings of the 1st European Conference on Food Chemistry: Recent Developments in Food Analysis', ed. W. Balters, P. Czedik-Eysenburg, and W. Pfannhauser, Verlag Chemie, Weinheim, 1982, p. 190.

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[244] W. Lange, M. Jirikowsky, D. Holtkamp, and A. Benninghoven, *Springer Ser. Chem. Phys.*, 1982, **19** (*Secondary Ion Spectrometry*), 416.

[245] W. Sichtermann, *Springer Ser. Chem. Phys.*, 1983, **25** (*Ion Formation in Organic Solids*), 132.

[246] W. Guthier, O. Becker, S. Della Negra, W. Knippelberg, Y. Le Beyec, U. Weikert, K. Wien, P. Wieser, and R. Wurster, *Int. J. Mass Spectrom. Ion Phys.*, 1983, **53**, 185.

the films by ^{16}N and ^{238}U ions of fixed energies. Formation of layers[247] involves ultra-high-vacuum deposition by a molecular-beam technique, and since the 2000 Å films yield ions whose characteristics depend strongly on the chemical nature of the metal matrix on which they are deposited it is not likely that these ionization techniques will enter rapidly into routine service.

Other Physical Studies. – This section deals with certain other spectroscopic studies, but mainly with a wide range of physical data collected for amino acids and their derivatives.

Raman techniques assist the study of the phase transition that occurs at 80 °C in crystalline L-leucine, supporting i.r. data in identifying a conformational change within the amino acid side chain as being responsible.[248] A conventional i.r.–Raman study of α-glycine and its perdeuteriated derivative at 300 and 85 K has been reported.[249] *N*-Acetylamino acid esters in CCl_4 show additional peaks in the C–O stretching region (1000–1300 cm^{-1}) assigned to Fermi-resonance absorption.[250] Thymine–DL-tryptophan aggregates formed by lyophilization are in the form of stacked pyrimidine–indole moieties.[251]

Helium photoelectron spectra of glycine, sarcosine, and glycine methyl ester and of representative *N*-acetyl amino acids have been interpreted in terms of localized molecular orbitals.[252]

p*K* values 6.97, 9.13, and 9.75 and stability constants for complexes with a range of divalent metal ions have been determined for nicotianamine, a likely phytosiderophoric amino acid (12).[253] The same measurements have been performed for L-Dopa[254] and for *N*-(*m*-substituted phenyl)glycines,[255] and stability constants have been measured for ternary complexes between adenine or cytosine, tryptophan or histidine, and a divalent metal cation.[256] Partial molar volumes at infinite dilution,[257] viscosities,[258] heats of solution,[259] osmotic and activity coefficients,[260] and interfacial properties[261] of amino acids and their

[247] W. Lange, D. Holtkamp, M. Jirikowsky, and A. Benninghoven, *Springer Ser. Chem. Phys.*, 1983, **25** (*Ion Formation in Organic Solids*), 124.
[248] D. Bougeard, *Ber. Bunsenges. Phys. Chem.*, 1983, **87**, 279.
[249] B. Andrews, B. H. Torrie, and B. M. Powell, *Biophys. J.*, 1983, **41**, 293.
[250] V. Slet, *Eesti NSV Tead. Akad. Toim., Keem.*, 1983, **32**, 134 (*Chem. Abstr.*, 1983, **99**, 105 656).
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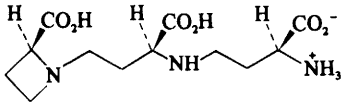
[253] I. Benes, K. Schreiber, H. Ripperger, and A. Kircheiss, *Experientia*, 1983, **39**, 261.
[254] T. Kiss and A. Gergely, *Magy. Kem. Foly.*, 1983, **89**, 81.
[255] R. Chen and H. Lin, *Huaxue Xuebao*, 1983, **41**, 87 (*Chem. Abstr.*, 1983, **98**, 198 683).
[256] M. M. T. Khan and S. Satyanarayana, *Indian J. Chem., Sect. A*, 1983, **22**, 584.
[257] F. Shahidi, *J. Solution Chem.*, 1983, **12**, 295; D. V. Jahagirdar and S. U. Pankanti, *Indian J. Chem., Sect. A*, 1983, **22**, 195; A. K. Mishra, K. P. Prasad, and J. C. Ahluwalia, *Biopolymers*, 1983, **22**, 2397; A. K. Mishra and J. C. Ahluwalia, *J. Phys. Chem.*, 1984, **88**, 86.
[258] S. J. Kim, Y. J. Oh, K. S. Choi, and Y. K. Shin, *Bull. Korean Chem. Soc.*, 1983, **4**, 284; J. S. Sandhu, U. Kashyap, and R. K. Wadi, *J. Indian Chem. Soc.*, 1983, **60**, 192; M. M. Bhattacharyya and M. Sengupta, *Z. Phys. Chem.*, 1982, **133**, 79.
[259] M. Matsumoto and K. Amaya, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2521.
[260] O. D. Bonner, *Physiol. Chem. Phys.*, 1982, **14**, 539.
[261] K. Ogino, H. Yamauchi, and T. Shibayama, *Yukugaku*, 1982, **31**, 1009 (*Chem. Abstr.*, 1983, **98**, 179 843).



(12)

salts have been reported. The last-mentioned study involves the effect of amino acids as solutes on the surface and interfacial tensions between aqueous and non-polar (dodecane or oleic acid) phases;[261] a similar partition study[262] in which distribution coefficients are measured for *N*-acetyl amino acid amides of the twenty protein amino acids and for the extremely lipophilic amino acids carboranylalanine and adamantylalanine yields hydrophobic parameters for the side chains involved. Transport of amino acids across membranes is another related topic of study, moving closer to biological systems; transport of L-cysteine across brush border membranes of rat renal cortex provides a specific example, the movement being inhibited by L-alanine and L-phenylalanine but not by L-glutamic acid or basic L-amino acids.[263] Penetration of L-lysine hydrochloride into lecithin monolayers[264] is another example of 'artificial transport' of glycine or alanine through complexation with the macrocyclic poly(imine) 1,4,7,10-tetra-*N*-benzyl-1,4,7,10-tetra-azadodecane and Ni^{2+} ions, also of phenylalanine or leucine with the same tetra-amine with Cu^{2+} ions;[265] similar results were obtained using a crown-ether analogue.[266] This is an important laboratory model for a plausible *in vivo* mechanism for conversion of zwitterionic amino acids into lipophilic complexes.

Thermochemical studies range from the relatively routine (enthalpies of solution of L-aspartic and glutamic acids in water at low concentrations[267]) to the more adventurous investigation of energetics of protonation of *N*-acetylalanine methyl ester.[268] In comparison with data for *N*,*N*-dimethylacetamide, the amino acid derivative shows higher proton affinity and negative entropy of protonation, indicating the existence of intramolecular hydrogen bonding in the protonated amino acid derivative. Since the internal hydrogen bond decreases the availability of the proton for intermolecular hydrogen bonding, the attachment energy of water to the protonated amino acid derivative is less than that for the protonated dimethylacetamide. Continuation of studies of gas-phase acidity and basicity of glycine sees an extension to various methyl homologues;[269] unlike the crystal-state situation, glycine exists in the non-zwitterionic form in the gas phase.[269]

Easily visible differences in the morphology of centrosymmetric crystals precipitated in the presence of one enantiomer of a chiral amino acid, compared with their appearance when precipitated in the presence of the other enantiomer,

[262] J. L. Fauchere and V. Pliska, *Eur. J. Med. Chem., Chim. Ther.*, 1983, **18**, 369.
[263] B. Stieger, G. Stange, J. Biber, and H. Murer, *J. Membr. Biol.*, 1983, **73**, 25.
[264] M. Nakagaki and E. Okamura, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1607.
[265] H. Tsukube, *J. Chem. Soc., Perkin Trans. 1*, 1983, 29.
[266] H. Tsukube, *J. Membr. Sci.*, 1983, **14**, 155.
[267] X. Ni and R. Hu, *Huaxue Xuebao*, 1983, **41**, 41 (*Chem. Abstr.*, 1983, **98**, 198 682).
[268] M. Meot-Ner, *J. Am. Chem. Soc.*, 1984, **106**, 278.
[269] M. J. Locke and R. T. McIver, *J. Am. Chem. Soc.*, 1983, **105**, 4226.

permit the assignment of absolute configuration to an amino acid[270] (see also Vol. 14, p. 18). The structural requirements of four host molecules (which must be chiral or prochiral) and additives have been outlined; glycine was used in this study as a suitable prochiral host.[270]

Molecular-orbital Calculations. – Major themes over recent years are continued, with papers considering hydrogen bonding and other interactions between amino acids and simple ions or solvent molecules, conformational aspects, and calculations intended to arrive at fundamental data that may be compared with those determined experimentally.

The amide oxygen atom of an *N*-acylated amino acid is calculated[271] to be a stronger proton acceptor than a carboxyl oxygen atom. A summary paper[272] updates the data for geometrical parameters, non-bonded interactions, and hydrogen-bond interactions to take account of experimental information of the last 10 years. Calculations are better able to fit experimental i.r. and Raman spectral parameters and to predict side-chain behaviour of amino acid residues in peptides and proteins more realistically when the revised data are used. Other MO calculations in this general area include hydration geometries of protonated lysine, arginine, and glutamate and aspartate ions,[273] of the 20 common amino acids,[274] of the complex ions $[Cu(Ile)]^+$, $[Cu(Thr)]^+$, and $[Cu(Thr)_2H_{-2}]^{2-}$,[275] of glycine and its zwitterion,[276] and of γ-aminobutyric acid in the presence of Mg^{2+} and Ca^{2+} ions.[277] The relative energies of the three intramolecularly hydrogen-bonded glycine conformers (two with bifurcated hydrogen bonds from $-NH_2$ to carbonyl oxygen and to $-OH$ oxygen, respectively, and one with a hydrogen bond from OH to N) have been computed, indicating the first-mentioned structure to be preferred.[278] Dipole moments, charge density, and ionization potentials have been calculated for neutral and zwitterionic forms of glycine, alanine, serine, cysteine, and selenocysteine.[279]

Factors influencing intermolecular interactions that MO calculations can illuminate include hydrophobicity parameters[280] and amino acid–nucleotide stacking interactions;[281] tryptophan emerges as the residue most able, amongst the aromatic and heteroaromatic amino acids, to form stable interactions of this type. Conformational information arises from calculations concerning *N*-acetyl

[270] I. Weissbuch, L. Addadi, Z. Berkovitch-Yellin, E. Gati, S. Weinstein, M. Lahav, and L. Leiserowitz, *J. Am. Chem. Soc.*, 1983, **105**, 6615.
[271] Z. Berkovitch-Yellin, S. Ariel, and L. Leisorowitz, *J. Am. Chem. Soc.*, 1983, **105**, 765.
[272] G. Nemethy, M. S. Pottle, and H. A. Scheraga, *J. Phys. Chem.*, 1983, **87**, 1883.
[273] G. Ranghino, E. Clementi, and S. Romano, *Biopolymers*, 1983, **22**, 1449.
[274] S. Fraga, *Theochem.*, 1983, **11**, 251.
[275] N. Raos and V. Simeon, *J. Inorg. Biochem.*, 1983, **18**, 133.
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[277] I. O. Blake, A. Les, and S. Rybak, *J. Theor. Biol.*, 1983, **104**, 571.
[278] S. Millefiori and A. Millefiori, *Theochem*, 1983, **8**, 391.
[279] A. E. Smolyar, A. R. Abramov, A. A. Guliev, O. N. Narimanbekov, and T. N. Shakhtakhtinskii, *Azerb. Khim. Zh.*, 1982, 20.
[280] M. Charton and B. I. Charton, *J. Theor. Biol.*, 1982, **99**, 629.
[281] N. V. Kumar and G. Govil in 'Conformation in Biology', ed. R. Srinivasan and R. H. Sarma, Adenine Press, Guilderland, New York, 1983, p. 313.

alanine methylamide,[282] the glycine analogue and near relatives,[283] and the corresponding derivatives of the 20 protein amino acids, together with γ-aminobutyric acid,[284] using up-dated interatomic energies and residue geometries (*cf.* ref. 272). The disulphide linkage in L-cysteine and in simpler disulphides is at its minimum-energy conformation at a dihedral angle close to 90°.[285]

Calculations allowing band assignments in vibrational spectra of cysteine, serine, and β-chloroalanine have been performed, including consideration of seven isotopically substituted analogues.[286]

5 Chemical Studies of Amino Acids

Racemization. – Papers describing mechanistic aspects of amino acid racemization argue in favour[287] of a two-step process with abstraction of the methine proton being rate-determining. There is also evidence[288] that racemization of L-[1-^{2}H]-alanine proceeds with significant internal return of the proton when catalysed by tyrosine phenol lyase and the amino acid racemase from *Pseudomonas striata*, supporting the idea that a single base is involved in the process. Alkaline racemization of *Z*-γ-thiapipecolic acid pentachlorophenyl ester is some seven times faster than that of the γ-thiaproline analogue[289] and some four times faster than that of the pipecolic acid analogue. This confirms the rate-enhancing effect of a nearby sulphur atom[290] and also reveals that finer aspects of the geometrical relationship between the sulphur atom and the chiral centre are important since the rate enhancement associated with the sulphur atom in a five-membered ring is substantially greater than that for an acyclic analogue.[289]

Practical exploitation of amino acid racemization follows familiar lines, with further studies of racemization of α-amino acids in hot AcOH[291] in the presence of 0.05 mol of an aldehyde showing that complete dissolution of the amino acid is not required; the racemate can simply be filtered off after an appropriate time has elapsed. The combination of the same general principle (use of pyridoxal 5′-phosphate with a DL-amino acid ester in neutral aqueous solution) with catalysis of hydrolysis by an esterase allows continuous production of an L-amino acid to be achieved.[292]

[282] J. N. Scarsdale, C. van Alsenoy, V. J. Klimkowski, L. Schaefer, and F. A. Momany, *J. Am. Chem. Soc.*, 1983, **105**, 3438; C. Tosi, *Nuovo Cimento Soc. Ital. Fis. D*, 1983, **2**, 15.

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[286] H. Susi, D. M. Byler, and W. V. Gerasimowicz, *J. Mol. Struct.*, 1983, **102**, 63.

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[291] S. Yamada, C. Hongo, R. Yoshioka, and I. Chibatu, *J. Org. Chem.*, 1983, **48**, 843.

[292] M. Pugniere, A. Commeyras, and A. Previero, *Biotechnol. Lett.*, 1983, **5**, 447.

The less accessible literature carries a recent example of the use of amino acid racemization for dating purposes,[293] ages of shells from Sardinia dated by this method agreeing with relative stratigraphy implications.

General Reactions. – Reactions leading to *N*-substituted derivatives for use in synthesis or for analytical purposes are described in accounts of formylation in refluxing HCO_2H–DMF,[294] t-butoxycarbonylation using the stable reagent t-butyl benzotriazol-1-yl carbonate[295] or using di-t-butyl carbonate,[296] tritylation through initial silylation with $AcN(SiMe_3)_2$ followed by reaction with trityl chloride,[297] phthaloylation using *N*-(ethoxycarbonyl)phthalimide,[298] *N*-benzoylethylation through Mannich reactions with amino acid esters, phenyl alkyl ketones, and paraformaldehyde,[299] and formation of *N*-2,2,2-trichloroethylidene Schiff bases by reaction with chloral followed by dehydration of the resulting aminols with $SOCl_2$.[300] 9-Fluorenylmethyl esters, cleavable under mild conditions using a secondary amine, have been prepared from Boc-amino acids using 9-fluorenylmethanol and dicyclohexylcarbodi-imide.[301] Oxazoles formed by reaction of a *Z*-amino acid with benzoin and treatment of the resulting ester with NH_4OAc are readily cleaved by singlet oxygen to yield the corresponding *Z*-amino acid dibenzoylamide.[302]

Other reactions involving amino and carboxy groups (*i.e.* leading to heterocyclic structures) are illustrated in conversions of amino acids into keto acids[303] and into diketones,[304] by refluxing with trifluoroacetic anhydride in the former case and by successive treatment with trifluoroacetic anhydride, Michael addition to t-butyl acrylate, addition of an organo-magnesium or -lithium compound, and cleavage of the resulting oxazolinols with HCO_2H. 2-Trifluoromethyloxazolin-5-ones are the common intermediate in these two processes. Boroxazolidinones (13) are formed from amino acids with tris-(n-propyl)borane in refluxing xylene.[305]

3-Phenylfuran is one of the products formed in the Maillard reaction between glucose and phenylalanine;[306] this long-established general reaction involving amino acids and carbohydrates has been reviewed.[307] Other reactions of almost equally long acquaintance also continue to be represented in the literature,

[293] P. Wanet, F. Leone, A. Ozer, and A. Ulzegha, *Rend. Semin. Fac. Sci. Univ. Cagliari*, 1982, **52**, 159 (*Chem. Abstr.*, 1983, **99**, 174 860).
[294] J. M. Aizpurua and C. Palomo, *Synth. Commun.*, 1983, **13**, 745.
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[298] C. R. McArthur, P. M. Worster, and A. U. Okon, *Synth. Commun.*, 1983, **13**, 393.
[299] H. Haber and H. G. Henning, *Pharmazie*, 1983, **38**, 509.
[300] G. Giedemann and I. Ugi, *Synthesis*, 1983, 788.
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[302] H. H. Wasserman and T.-J. Lu, *Tetrahedron Lett.*, 1982, **23**, 3831.
[303] C. Klein, G. Schulz, and W. Steglich, *Liebigs Ann. Chem.*, 1983, 1638.
[304] J. Leyendecker, U. Niewöhner, and W. Steglich, *Tetrahedron Lett.*, 1983, **24**, 2375.
[305] G. H. L. Nefkens and B. Zwanenburg, *Tetrahedron*, 1983, **39**, 2995.
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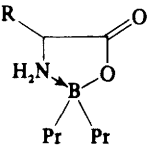

(13)

including deamination by nitrous acid. Alanine methyl ester hydrochloride in acetic acid yields chloro-, acetoxy-, and hydroxy-substitution products and methyl acrylate,[308] and homologues yield products of 1,2-shifts of the β-substituent in yielding β-fluoroalkanoates as well as α-fluoro-substitution products in reaction with $NaNO_2$/HF–pyridine[309] (see also Vol. 14, p. 27). Oxidative degradation of amino acids by Chloramine-T in both acid and alkaline media has been thoroughly re-examined from a mechanistic point of view;[310] in acid media slow *N*-monochlorination of the amino acid is followed by a rapid formation of the *N,N*-dichloro compound, which undergoes elimination of HCl and subsequent hydrolysis. Alternatively, Cl_2 or H_2OCl^+ formed in water by disproportionation of Chloramine-T reacts with the amino acid as just described; in alkaline media the reagents can include HOCl and its anion.[310] The chemistry involved in these pathways, *i.e.* the concerted fragmentation of *N*-chloroamino-acid anions to give imines (which hydrolyse rapidly to amines and carbonyl compounds) and CO_2, proceeds at rates highly dependent upon the amino acid structure (Gly < sarcosine < Thr < Ala < Pro < α-aminoisobutyric acid < 1-aminocyclohexanecarboxylic acid).[311]

Other decarboxylation reactions are featured in the reaction of amino acids with 2,4,6-triphenylpyrylium salts to give corresponding *N*-alkylpyridinium salts[312] and in a moderate-yield pyrolysis (145 °C) of an *N*-acyl dehydroamino acid in the presence of copper and heteroaromatic base.[313]

The classical oxidative degradation of amino acids alluded to in the preceding paragraph has many variations already committed to the literature, and the routine exploitation of these employing metal ions as oxidants continues;[314] use of a diacyl peroxide for the purpose converts an amino acid ester into either an *N*-acyl derivative or an *N*-acyloxy analogue, depending on steric effects

[308] Z. A. Malik, *Pak. J. Sci. Ind. Res.*, 1982, **25**, 5.

[309] S. Hamman and C. G. Beguin, *Tetrahedron Lett.*, 1983, **24**, 57.

[310] B. T. Gowda and D. S. Mahadevappa, *J. Chem. Soc., Perkin Trans. 2*, 1983, 323; D. S. Mahadevappa, M. S. Ahmed, N. M. M. Gowda, and B. T. Gowda, *Int. J. Chem. Kinet.*, 1983, **15**, 775; D. S. Mahadevappa, K. S. Rangappa, N. M. M. Gowda, and B. T. Gowda, *Indian J. Chem., Sect. A*, 1983, **22**, 631.

[311] V. C. Hand, M. P. Snyder, and D. W. Margerum, *J. Am. Chem. Soc.*, 1983, **105**, 4022.

[312] A. R. Katritzky, N. E. Grzeskowiak, N. F. Eweiss, and E. A. Elsherbini, *J. Chem. Soc., Perkin Trans. 1*, 1983, 497.

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imposed by either reactant.[315] A study, non-routine by any standards, involves argon-arc plasma-produced oxidant, probably $HO^{\bullet}$, which effects side-chain oxidation of representative amino acids; for example[316] alanine yields serine and glycine, among other products, in aqueous solutions.

A very considerable emphasis on mechanistic aspects is evident in these studies; aminolysis and hydrolysis studies continue the same theme. Boc-glycine active esters[317] and glycine ethyl ester co-ordinated into mixed copper(II) complexes[318] were the systems on which straightforward structure–reactivity studies were based, while a study of the hydrolysis of *N*-benzyloxycarbonyl-amino acid *p*-nitrophenyl esters in the presence and absence of free amino acids constitutes a more subtle challenge.[319] The high rate seen for the lysine derivative in the absence of an amino acid seems due to intramolecular electrostatic interaction of the positively charged ε-amino group with the negatively charged tetrahedral intermediate, and several other similar conclusions are drawn in this study.[319] General acid catalysis by HCO_3^- of the breakdown of the tetrahedral intermediate that forms during the alkaline hydrolysis of *N*-acetylglycine anilides has been observed.[320]

Enantioselective catalysis is seen in the hydrolysis of amino acid *p*-nitrophenyl esters,[321,322] of *p*-nitrophenyl alkanoates,[323,369] and of histidine methyl ester[324] by poly(acrylamide)s[321] and dodecane-1,10-dioic diamides[323] in which the amide moiety is an L-histidine residue, by micellar *N*-lauroyl- and *N*-palmitoyl-L-histidine and -L-histidyl-L-leucine,[322] and by crosslinked hydrophilic polymer supports bearing L-histidine residues complexed with Ni^{2+} ions.[324] Generally, the greater selectivity is seen with the more hydrophilic carrier molecules (though a brief generalization such as this cannot do justice to the wealth of detail in these studies).

Solvolysis of *N*-[amino(methyl)phosphinyl]-L-phenylalanine esters and amides and their hydroxyphosphinyl and phosphinothioyl analogues involves the heterocyclic intermediate (14).[325]

A variety of functional-group modifications amounting to improvements of known reactions includes conversion of Boc-amino acids into aldehydes *via* *N*-(methoxy)methylamides using $LiAlH_4$,[326] sodium–liquid ammonia reduction

[315] M. Milewska, T. Kolasa, and A. Chimiak, *Pol. J. Chem.*, 1981, **55**, 2215.
[316] K. Harada and M. Takasaki, *Tetrahedron Lett.*, 1983, **24**, 4839.
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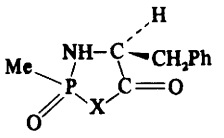
[318] B. Przeczek, H. Langfelderova, and J. Gazo in 'Proceedings of the 9th Conference on Co-ordination Chemistry', p. 349 (*Chem. Abstr.*, 1984, **100**, 7089).
[319] P. Ascenzi, G. Sleiter, and E. Antonini, *Gazz. Chim. Ital.*, 1982, **112**, 307.
[320] D. Petkov and I. Pozharliev, *Izv. Khim.*, 1982, **15**, 247.
[321] I. Cho and J. S. Shin, *Makromol. Chem.*, 1983, **184**, 147.
[322] R. Ueoka, Y. Matsumoto, T. Kikuno, and K. Okada, *J. Mol. Catal.*, 1983, **18**, 267; R. Ueoka and Y. Murakami, *J. Chem. Soc., Perkin Trans. 2*, 1983, 219; see also Y. Ihara, R. Hosako, M. Nango, and N. Kuroki, *J. Chem. Soc., Perkin Trans. 2*, 1983, 5.
[323] M. Kodaka, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2191; see also M. Tanihara and Y. Imanishi, *Polym. J. (Tokyo)*, 1983, **15**, 499.
[324] N. Spassky, M. Reix, M. O. Sepulchre, and J. P. Guette, *Makromol. Chem.*, 1983, **184**, 17.
[325] N. E. Jacobsen and P. A. Bartlett, *J. Am. Chem. Soc.*, 1983, **105**, 1613, 1619.
[326] J. A. Fehrentz and B. Castro, *Synthesis*, 1983, 676.



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of amino acid amides to amino alkanols,[327] and α-methoxylation through anodic oxidation of *N*-acyl amino acid esters in methanol containing an alkali-metal halide or a tetra-alkylammonium halide.[328] Transamination processes in which pyridoxal operates in a membrane is modelled by micellar *N*-lauroyl-pyridoxal Schiff bases, shown to undergo transamination in the absence of metal ions.[329] Corresponding transimination (a deliberately precise term) of pyridoxal 5′-phosphate–ethylamine Schiff base with alanine or aspartic acid has been subjected to kinetic study,[330] indicating a more rapid direct exchange than an alternative 'hydrolysis-followed-by-condensation' pathway. Anchimeric assistance by the 5′-phosphate grouping is also indicated.

An increasing number of synthetic uses is being found for L-amino acids as chiral agents for biassing the asymmetric synthesis of non-peptide natural products. (*S*)-β-Amino alkanols yield Schiff bases with aldehydes from which (*S*,*S*)-1-aryl-2-phenyl-(2-hydroxymethyl)amines are obtained by reaction with a Grignard reagent.[331] An alternative alkylation procedure in which N-protected L-amino acids are converted into corresponding aminoalkyl ketones by reaction of the lithium carboxylate with an organo-lithium or -magnesium reagent has been described.[332] Asymmetric hydrogenation of *N*-pyruvoyl-(*S*)-amino acid esters[333] and phenylglyoxylic analogues[334] using $NaBH_4$ yields (*R*)-lactoyl derivatives in up to 44% diastereoisomeric purity when the reduction is conducted in alcoholic media and (*S*)-tropoyl analogues in equally modest enantiomeric excess when the reduction is carried out in 1% aqueous THF, respectively. Acid hydrolysis of the products yields the optically active α-hydroxy acids. Schiff bases formed between a ketone and an (*S*)-α-amino acid ester give corresponding secondary amines through hydrogenation over Pd–C, which may be cleaved by *N*-chlorination with t-butyl hypochlorite, dehydrochlorination, and hydrolysis of the resulting imine to give the chiral (*S*)-amine derived from the original ketone,[335] optical yields being dependent on both solvent and structure. Asymmetric conjugate addition of *N*-butyl-lithium to *N*-(cinnamoyl)-*S*-proline in the presence of tertiary amines generates a new chiral centre that is predominantly of *R*-configuration, whereas in the absence of tertiary amines the *S*,*S*-diastereo-

[327] I. Schon, T. Szirtes, T. Uberhardt, and A. Csahi, *J. Org. Chem.*, 1983, **48**, 116.
[328] T. Shono, Y. Matsumura, and K. Inoue, *J. Org. Chem.*, 1983, **48**, 1388.
[329] H. Kondo, J. Kikuchi, and J. Sunamoto, *Tetrahedron Lett.*, 1983, **24**, 2403.
[330] S. H. Weng and D. L. Leussing, *J. Am. Chem. Soc.*, 1983, **105**, 4082.
[331] Y. Suzuki and H. Takahashi, *Chem. Pharm. Bull.*, 1983, **31**, 31.
[332] C. G. Knudsen and H. Rapoport, *J. Org. Chem.*, 1983, **48**, 2260.
[333] T. Munegumi and K. Harada, *Chem. Lett.*, 1983, 1225.
[334] K. Soai, H. Hasegawa, K. Komiya, Y. Shigematsu, and A. Ookawa in 'Proceedings of the 20th American Peptide Symposium', p. 81 (*Chem. Abstr.*, 1983, **99**, 105 651).
[335] N. Ikota, K. Achiwa, and S. Yamada, *Chem. Pharm. Bull.*, 1983, **31**, 387.

isomer predominates. Optical yields are low, and apparently unimportant changes in experimental procedure (the order in which the base and BuLi were added) reverse the diastereoselectivity.[336]

Specific Reactions of Natural Amino Acids and Their Derivatives. – Since this section deals mostly with reactions of side chains of common amino acids, it is incidentally an extension of amino acid synthesis in the sense that the products are often novel amino acids. However, the emphasis is on interesting and useful chemistry.

Regioselective chlorination of *N*-benzoylvaline methyl ester with SO_2Cl_2 and benzoyl peroxide gives β- and γ-chloro derivatives. The proportions ($\beta:\gamma = 2:1$) tend to confirm standard textbook information concerning free-radical attack at saturated branched alkyl groups in favour of substitution of the proton at the tertiary carbon atom of the valine side chain.[337] Other studies of aliphatic hydrocarbon side chains include one on condensation of *cis*-bis(glycinato)copper(II) monohydrate with competition between two aldehydes to yield mixtures of β-hydroxy-α-amino acids (preference for the *threo*-isomer of the bulkier product seems demonstrated – acetaldehyde and benzaldehyde give a reaction mixture containing 66% *threo*-β-phenylserine)[338] and a detailed study of the fragmentation of *cis*-dideuterioaminocyclopropanecarboxylic acid into ethylene (loss of stereochemical integrity is observed under *in vivo* conditions, suggesting a free-radical mechanism) with CN^- being a surprising reaction product.[339]

Pyrolysis studies of amino acids have been featured in this section regularly over the years, because of their relevance to food science on the one hand and the study of the organic content of meteorites on the other. L-Glutamic acid and its salts with metals of Groups I and II undergo successive cyclodehydration, dehydration to 5-carboxy-2-pyrrolidone, and further degradation to pyrrole, as shown by thermal-analysis study through the temperature range to 400 °C.[340]

α-Amino-dicarboxylic acids feature in the enantiodivergent alkylation of L-aspartic acid (Scheme 7).[341] The term 'enantiodivergent': L-aspartic acid is the source of alkylation products of its β-carbon atom, and one of the two pathways shown in Scheme 7 favours the *threo*-alkylation product whereas the other favours the *erythro*-isomer.

An efficient synthesis is announced of *N*-2,2,2-trichloro-ethoxycarbonyl-L-aspartyl and -glutamyl α-ethyl and α-benzyl esters from the corresponding Troc-amino acid anhydrides.[342] One of the recently established protein α-amino-tricarboxylic acids, γ-carboxyglutamic acid, has been shown[343] to react readily with aldehydes in what amounts to an intramolecular Mannich reaction to give

[336] K. Soai, A. Ookawa, and Y. Nohara, *Synth. Commun.*, 1983, **13**, 27.
[337] C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, 1983, 1193.
[338] P. Sharrock, *Polyhedron*, 1983, **2**, 111.
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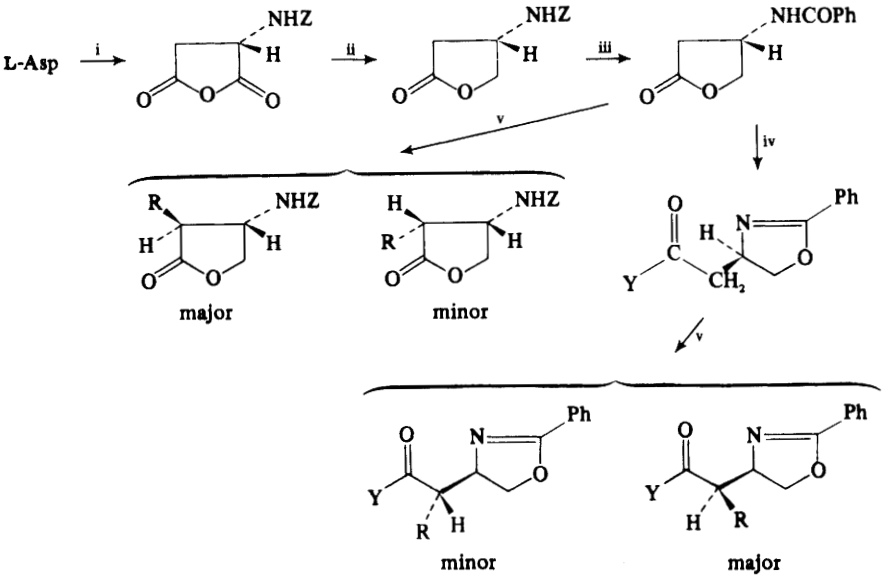
[340] Z. B. Bakasova, V. I. Kegan, D. A. Abdybaliev, and I. G. Druzhinin, *Izv. Akad. Nauk Kirg. SSR*, 1982, 37.
[341] G. J. McGarvey, R. N. Hiner, Y. Matsubara, and T. Oh, *Tetrahedron Lett.*, 1983, **24**, 2733.
[342] J. F. Carson, *Synthesis*, 1983, 669.
[343] R. Capasso, G. Randazzo, and L. Pecci, *Can. J. Chem.*, 1983, **61**, 2657.



Reagents: i, ZCl then Ac_2O; ii, $NaBH_4$; iii, HBr–AcOH then PhCOCl–py; iv, EtO^- or Me_2NH; v, $LiPr_2N$, RX

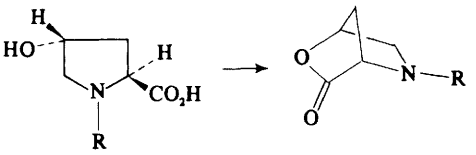
Scheme 7

5-substituted 4,4-dicarboxyprolines. These undergo monodecarboxylation at 100 °C during 30 min.[343]

Side-chain hydroxy groups can participate in elimination, stereospecific conversion of *threo*-β-hydroxy-α-amino acid derivatives into *Z*-dehydroamino acids having been demonstrated using Et_2NSF_3-py.[344] Boc-serine and -threonine can be *O*-acylated under standard conditions by long-chain aliphatic acid chlorides, and elaboration of amino and carboxy groups can then be carried out without disturbing the new side-chain functions.[345] Conversion of *trans*-4-hydroxyprolines (15) into azaoxabicycloheptanes (16) is achievable in one step using $EtO_2CN{=}NCO_2Et$ and Ph_3P;[346] a point of interest is the restriction to rotation imposed on an *N*-acyl group (16; R = MeCO) in its new environment, leading to the existence at ambient temperatures of two rotational isomers.

Several important studies of side-chain thiol, sulphide, and sulphonium groups are represented in the 1983 literature, some of the papers extending earlier studies. Intramolecular transfer of an initially formed *S*-nitroso derivative during the diazotization of L-methionine and *S*-methyl-L-cysteine is suggested by the large rate enhancement seen with these amino acids in comparison with the rate of diazotization of alanine.[347] One enantiomer of *S*-adenosyl-L-methionine

[344] L. Somekh and A. Shanzer, *J. Org. Chem.*, 1983, **48**, 907.
[345] G. V. Marinetti, *Chem. Phys. Lipids*, 1983, **33**, 145.
[346] M. M. Bowers-Nemia and M. M. Joullie, *Heterocycles*, 1983, **20**, 817.
[347] T. A. Meyer and D. L. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 1067.



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(*S*-configuration at sulphur) formed by enzymic synthesis has two pathways open to it in physiological media (pH 7.5, 37 °C), *viz.* inversion at sulphur and nucleophilic cleavage to give homo-L-serine and 5′-deoxy-5′-(methylthio)-adenosine, and factors affecting the balance between these have been studied.[348] Formation of an *S*-centred radical cation through the reaction of methionine with HO• generated from the Ti^{III}–H_2O_2 couple is established as the initiation of oxidative decarboxylation.[349] The α-amino radical formed in this way undergoes reduction considerably more slowly in its protonated form.[350] Copper-catalysed autoxidation of cysteine is accompanied by the formation of H_2O_2,[351] and this is then used for oxidation of further cysteine. The stoicheiometry was established for the ratio of cysteine consumed to H_2O_2 produced as 2:1, and the possibility of a Michaelis–Menten-type mechanism for re-oxidation or oxygenation of copper(I) species produced in the oxidation was also discussed.[351] Straightforward procedures for achieving the reverse process, the reduction of L-cystine, employ electrolysis in aqueous HCl with Pd and graphite electrodes.[352]

Oxidation of selenomethionine by gold(III) in H_2O yields the selenoxide,[353] an observation paralleling the earlier (Vol. 6, p. 32) use of gold(III) for stereospecific oxidation of methionine itself to the sulphoxide. However, selenoxides are considerably more prone to elimination, and the ^1H n.m.r. data on which the assigned chemistry is based may need to be reconsidered to take this into account.

Side-chain aliphatic amino-group chemistry is represented in a clean preparation of N^{α}-benzyloxycarbonyl-N^{ϵ}-Boc-L-lysine from the *Z*-derivative by standard methods but including a recrystallization of the (—)-ephedrine salt,[354] spontaneous N^{ϵ}-methylation and N^{α}-formylation of lysine in formaldehyde solutions and their inhibition by ascorbic acid,[355] N^{ω}-2-(2-trimethylsilylethyl)oxycarbonylation of αω-diamino acid copper(II) complexes,[356] and conversion of ω-guanidino- and -ureido-α-amino acids into α-keto acids without disturbing the side-chain function (*via* 2-trifluoromethyloxazolin-5-ones).[357]

[348] S. E. Wu, W. P. Huskey, R. T. Borchardt, and R. L. Schowen, *Biochemistry*, 1983, **22**, 2828.
[349] M. J. Davies, B. C. Gilbert, and R. O. C. Norman, *J. Chem. Soc., Perkin Trans. 2*, 1983, 731.
[350] K. O. Hiller and K. D. Asmus, *J. Phys. Chem.*, 1983, **87**, 3682.
[351] A. Hanaki and H. Kamide, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2065.
[352] M. Chen, *Huaxue Shiji*, 1983, **5**, 173 (*Chem. Abstr.*, 1983, **99**, 140 338).
[353] A. A. Isab, *Inorg. Chim. Acta*, 1983, **80**, L3.
[354] S. T. Chen and K. T. Wang, *Org. Prep. Proced. Int.*, 1983, **15**, 361.
[355] L. Trezl, I. Rusznak, E. Tyihak, T. Szarvas, and B. Szende, *Biochem. J.*, 1983, **214**, 289.
[356] A. Rosowsky and J. E. Wright, *J. Org. Chem.*, 1983, **48**, 1539.
[357] C. Klein, G. Schulz, and W. Steglich, *Liebigs Ann. Chem.*, 1983, 1623.

α-Amino acids undergo *N*-nitrosation in either crystalline or solution forms when exposed to 1–100 p.p.m. nitrogen oxide-containing atmospheres.[358]

Aromatic side chains undergo modification in non-enzymic oxidation of 5-(*S*-cysteinyl)Dopa into a 1,4-benzothiazine under physiological conditions, mediated by complex formation with various metal ions (however, Cu^{2+}-catalysed oxidation leads to the red-purple pigment trichochrome F),[359] in *O*-alkylation[360] and *O*-phosphorylation of tyrosine under standard conditions,[361] and in electrochemical oxidation of *N*-acetyl tyrosinamide.[362] The tyrosine side chain is, however, the signal for the chymotrypsin-catalysed formation of *N*-acetyl-L-tyrosine ethyl ester from the *N*-acetylamino acid, ethanol, and the immobilized enzyme in a two-phase ($CHCl_3$–H_2O) system.[363]

Tricarbonylchromium complexes form a bridge between aromatic and heteroaromatic examples as far as the organization of this section of the chapter is concerned. Phenylalanine derivatives react with $Cr(CO)_6$ in refluxing water–THF (4:1) to give good yields of the complexes,[364] and tryptophan–$Cr(CO)_3$ complexes suitable for use in peptide synthesis are prepared similarly (but in Bu_2O–THF).[365] Tryptophan derivatives protected at amino and carboxy groups yield cyclic tautomers readily when a methoxycarbonyl *N*-protecting group is involved;[366] in contrast to earlier reports, however, *N*-benzylidene analogues do not readily cyclize to Pictet–Spengler products 1,2,3,4-tetrahydro-β-carbolines (17) in the absence of acids.[367] Free-radical halogenation of protected tryptophans using *N*-bromo- or -chloro-succinimides provides high yields of the 2-halo-substituted analogues.[368] Numerous mechanistic studies involving the histidine imidazole moiety as base catalyst in hydrolysis studies are discussed in the preceding section; the propensity of histidine itself (rather than larger structures containing histidine or imidazole groupings as discussed earlier[321–324]) to catalyse the hydrolysis of *p*-nitrophenyl acetate, though not a new topic for study, has been clarified through comparisons with the behaviour of *N*-methylhistidines.[369] Consideration is given in this study[369] to the protonation state and interactions between the amino and imidazole groups of the histidines, a complex matter

[358] C. Janzowski, R. Klein, R. Preussmann, and G. Eisenbrand, *Food Chem. Toxicol.*, 1982, **20**, 595.
[359] A. Palumbo, G. Nardi, M. D'Ischia, G. Misuraca, and G. Prota, *Gen. Pharmacol.*, 1983, **14**, 253.
[360] W. L. Mendelson, A. M. Tickner, and I. Lantos, *J. Org. Chem.*, 1983, **48**, 4127.
[361] P. F. Alewood, R. B. Johns, R. M. Valerio, and B. E. Kemp, *Synthesis*, 1983, 30.
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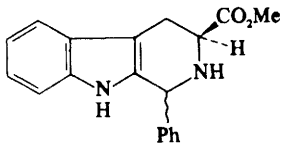
[363] J. L. Vidaluc, M. Baboulene, V. Speziale, A. Lattes, and P. Monsan, *Tetrahedron*, 1983, **39**, 269.
[364] C. Sergheraert, J. C. Brunet, and A. Tartar, *J. Chem. Soc., Chem. Commun.*, 1982, 1417.
[365] C. Sergheraert and A. Tartar, *J. Organomet. Chem.*, 1982, **240**, 163.
[366] M. Taniguchi, A. Gonsho, M. Nakagawa, and T. Hino, *Chem. Pharm. Bull.*, 1983, **31**, 1856.
[367] R. H. Grigg, H. Q. N. Gunaratne, and E. McNaghten, *J. Chem. Soc., Perkin Trans. 1*, 1983, 185.
[368] R. S. Phillips and L. A. Cohen, *Tetrahedron Lett.*, 1983, **24**, 5555.
[369] P. Boschcov, W. Seidel, J. Muradian, M. Tominaga, A. C. M. Paiva, and L. Julio, *Bioorg. Chem.*, 1982, **11**, 383.



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requiring assessment of equilibria of various kinds but crucial to underpin the reliability of conclusions drawn in this type of study.

Non-enzymic Models of Biochemical Processes Involving Amino Acids. – Amino acid–nucleotide complexes have been the mainstay of this section over the years and are mentioned elsewhere in this chapter. Reports of other related work describe dissociation by near-u.v. irradiation of DNA complexes with tyrosine, histidine, and tryptophan[370] and extension of stability-constant studies to mixed-ligand complexes $[M^{2+}(ATP)(aa)]^{3-}$ in assessment of metal-ion-promoted hydrophobic interactions between amino acids and nucleotides.[371]

Peptide synthesis has been achieved in aqueous media by reaction of glycine with *N*-acetylglycyl adenylate anhydride[372] and by reaction of tyrosine with the Schiff base of *N*-acetyltyrosinal and hydrogen peroxide.[373]

Effects of Electromagnetic Radiation on Amino Acids. – This section carries summaries of photochemical processes and of processes in which absorption of radiation does not lead to bond-breaking. In the former category, flash photolysis of pyridoxal-derived Schiff bases (yielding decarboxylation products from which pyridoxamine and carbonyl compounds are formed by hydrolysis[374]) and of aqueous tyrosine solutions (yielding degradation products of the initially formed *p*-alanylphenoxyl radical[375]) is accompanied by photo-oxidation studies of histidine using the traditional photosensitizer (Rose Bengal) covalently bonded to a polymer[376] and by photo-oxidation studies of the role of tyrosine and tryptophan as sensitizers in the formation of the superoxide radical anion $O_2^{-\bullet}$ in aqueous solutions.[377]

An unusual type of paper describes a study of radiation damage to crystalline L-valine during electron microscopy at liquid He temperature, which is only some 4–6-fold less than that at room temperature, rather than 70-fold as previously reported.[378] The more familiar studies of irradiation of solid amino acids are illustrated in γ-irradiated L-alanine single crystals which, as studied by e.s.r.

[370] E. M. Mil and V. M. Zhiltsova, *Izvest. Akad. Nauk S.S.S.R., Ser. Biol.*, 1983, 925.
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[372] D. W. Mullins and J. C. Lacey, *J. Mol. Evol.*, 1983, **19**, 173.
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[375] X. Shen, S. Pang, Y. He, and Y. Zhang, *Shengwu Huazue Yu Shengwu Wuli Xuebao*, 1983, **14**, 491 (*Chem. Abstr.*, 1983, **98**, 143 817).
[376] F. I. Llorca, J. L. Iborra, and J. A. Lozano, *Photobiochem. Photobiophys.*, 1983, **5**, 105.
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[378] M. K. Lamrik, D. A. Kopf, and J. D. Robertson, *Nature (London)*, 1983, **301**, 332.

spectroscopy, undergo deamination to form $MeC\dot{H}CO_2H$ radicals,[379] and there are similar studies[380] of γ-irradiated L-leucine. In this work the interest lies, as far as the authors are concerned, in the decay processes of these radicals; in the former case conformational reorientation seems to occur,[379] while a remarkable influence of the atmosphere surrounding the irradiated crystal has been demonstrated (decay in H_2 is much faster than decay in Ar) in the latter study.[380] E.n.d.o.r. evidence indicates the formation of radicals in irradiated *N*-acetylglycine originating at sites of crystal imperfections.[381]

Fluorescence lifetimes of various ionic forms of tryptophan in aqueous solutions are determined by H^+ quenching, and thus an explanation can be found for the longer lifetimes in ^2H_2O.[382] Addition of Et_3N or acid varies the corresponding fluorescence lifetimes observed for tryptophan in MeOH or EtOH.[382] A picosecond time-scale has been established for diffusion-controlled quenching of tryptophan fluorescence, and, since disturbance of the diffusion time by nearby ions and molecules can be thereby measured, parameters such as the interaction radius of the collisional quenching of *N*-acetyltryptophanamide by I^- can be determined.[383] 5-Methoxytryptophan yields a fluorescence spectrum that undergoes a pronounced red-shift as the pH of the solution is raised, owing to deprotonation of the $\dot{N}H_3$ group.[384] This is claimed to be the first direct evidence for the effect of a part of the aliphatic moiety on the energy of an emitting state of the indole moiety in tryptophan.

Tyrosine, and especially its 3,5-dihalo derivatives, is effective in quenching the phosphorescence of the triplet state of acetone.[385]

6 Analytical Methods

Gas-Liquid Chromatography. – A declining number of papers is appearing on this topic, and those that are published amount to extensions or improvements to established methodology. Commonly used perfluoroacyl and -alkyl *N*- and *O*-masking groups for conversion of amino acids into volatile derivatives have been compared,[386] it having been established[387] that *N*-trifluoroacetylamino acid n-propyl esters of 42 amino acids could be separated within about 19 min. Similar assessments of *N*-heptafluorobutyroyl amino acid propyl esters[388] and

[379] V. R. Zaitov, V. A. Onishchuk, and S. Z. Shul'ga in 'Proceedings of the Tihany Symposium on Radiation Chemistry', 1983, Vol. 5, p. 1037 (*Chem. Abstr.*, 1984, **100**, 22978).

[380] M. Mahdavi and M. Dole, *J. Phys. Chem.*, 1983, **87**, 5430; M. Dole and M. Mahdavi in 'Proceedings of the Tihany Symposium on Radiation Chemistry', 1983, Vol. 5, p. 1015 (*Chem. Abstr.*, 1984, **100**, 22977).

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[382] E. Gudgin, R. Lopez-Delgado, and W. R. Ware, *J. Phys. Chem.*, 1983, **87**, 1559.

[383] R. W. Wijnaendts van Resandt, *Chem. Phys. Lett.*, 1983, **95**, 205.

[384] E. F. Gudgin-Templeton and W. R. Ware, *Chem. Phys. Lett.*, 1983, **101**, 345.

[385] E. Rivas-Suarez, L. H. Catalini, E. J. H. Bechara, and G. Cilento, *Photochem. Photobiol.*, 1983, **37**, 93.

[386] G. Gamerith, *J. Chromatogr.*, 1983, **268**, 403.

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[388] S. A. Vitt, E. A. Paskonova, M. B. Saporovskaya, and V. M. Belikov, *Prikl. Biokhim. Microbiol.*, 1983, **19**, 692.

isobutyl esters[389] have been reported, with the analytical objectives of determining muramic acid, diaminopimelic acid, and alanine ratios in bacterial cell walls (employing electron-capture detection),[389] tryptophan at nanogram levels,[390] and 1-methylhistidine in physiological samples.[391]

Estimation of enantiomeric purity of amino acid samples by g.l.c. over chiral stationary phases is illustrated by well established Chirasil-Val g.l.c.,[392] corresponding use of chiral poly(siloxane)s (which have the merit of stability up to 230 °C),[393] and newer instrumental possibilities (two-column g.l.c.).[394]

Ion-exchange Chromatography. – The general area of liquid-chromatographic separation is now overlaid with the h.p.l.c. instrumental facility, and the traditional subdivisions of this section of the chapter are becoming less unambiguous. Readers are therefore directed also to the later h.p.l.c. section for continuation of this coverage of ion-exchange chromatography.

Conventional ninhydrin ion-exchange analysis[395–398] (including assay of pipecolic acid[396] and of tryptophan, 5-methyltryptophan, and glucosamine[397] and identification of EDTA as a source of enhanced ninhydrin colour intensities, probably due to decomposition products of the EDTA contaminant[398]) is being strongly challenged by *o*-phthaldialdehyde–mercaptoethanol reagent systems with fluorimetric quantitation (as illustrated in the estimation of β-hydroxyaspartic acid in protein hydrolysates;[399] see also the later h.p.l.c. section).

Automated amino acid analysis has been reviewed.[400]

Thin-layer Chromatography and Related Techniques. – Screening of physiological samples in a routine manner for specific species has been a major application of t.l.c.[401] and is illustrated[402] in estimation of tyrosine in serum employing fluorescence densitometry. Tyrosine, phenylalanine, and tryptophan derivatives have been subjected to t.l.c. in different solvent systems to determine optimum separation conditions.[403]

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The other papers are concerned with explorations of uncommon or new experimental approaches. Separation of amino acids on stannic tungstate[404] and on ammonium tungstophosphate[405] as ion-exchange materials shows some promise, the latter giving good separation of methyltryptophans. Partition systems ranging from the not unfamiliar polyamide materials (for the separation of *O*-phosphotyrosine, serine, and threonine[406]), reversed-phase materials (C_{18}-bonded silica gel, impregnated silica gel, and acetylated cellulose for the separation of amino acid mixtures, only the first-mentioned offering true reversal of the elution sequence but only when impregnated with dodecylbenzenesulphonic acid[407]), to glass-fibre paper (used in two-dimensional separations of ^{15}N-labelled amino acids[408]) have been tested. Adequate results are given within a short time using relatively small t.l.c. plates in amino acid analysis,[409] a feature emphasized for a pressurized ultra-micro chamber for miniaturized t.l.c., the effect here also being the reduced diffusion of spots.[410]

A mixed hydroxide of iron(III) and bismuth(III) is capable of preferential removal of acidic amino acids from aqueous solutions of pH 4.[411] A chelation principle is probably involved, and desorption can be achieved with Na_3PO_4 buffer at pH 12.2. The technique, used for the separation of tyrosine from aspartic acid, might be suitably adapted for t.l.c. analysis.

High-performance Liquid Chromatography. – Several reviews of this rapidly expanding area have appeared, concentrating solely on amino acid analysis,[412–416] although including some covering narrow aspects (practical procedures,[414] analysis of keto acids formed from amino acids through the use of amino acid oxidase[416]).

The subject appears to have progressed to the point where a few efficient systems have survived from a broad range of possibilities on which the preceding volumes of this Report have drawn. Views differ on whether pre- or post-column derivatization represents the best regime for h.p.l.c. of amino acids, although the preference is usually determined by the stability or otherwise of

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the pigments of fluorophores created by derivatization. Post-column methods are strongly advocated[413] and widely adopted for the dominant *o*-phthaldialdehyde–mercaptoethanol reagent system[417–430] (among these papers there are several[420–423] illustrating pre-column derivatization). There are many points of interest in these studies, but primarily that *o*-phthaldialdehyde fluorimetric amino acid analysis is being taken up widely in preference[425] to ninhydrin methodology. As many as 48 components were identified within 50 min in a standardized use of *o*-phthaldialdehyde for amino acid mixtures,[421] and a parallel study[422] establishes analysis routines for mixtures of 21 common amino acids (but proline, hydroxyproline, and cysteine cannot be detected). Imino acids, however, and other secondary amines, are specifically detectable through hypochlorite cleavage into primary amines in a post-column operation followed by *o*-phthaldialdehyde derivatization and fluorimetric assay.[426,427] Particular amino acids featured in these studies include glutamine,[429] γ-carboxyglutamic acid,[430] cysteinesulphinic acid and cysteic acid,[417] tyrosine and 3-fluorotyrosine,[418] and 1- and 3-methylhistidines.[419] Variations of standard technique include assessment of automated analysis[428] and reversed-phase ion-pair h.p.l.c.,[424] the latter approach also having been used in h.p.l.c. of dansylamino acids[431] (one of a number of papers based on these derivatives[431–434] and related[435] *N*-arenesulphonylamino acids). Post-column derivatization, while used in analysis of *S*-sulphocysteine in urine,[433] is an uncommon variation with these derivatives. Fewer papers have appeared dealing with h.p.l.c. analysis of phenylthiohydantoins;[436–438] they include an example of the unusual pre-column derivatization

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procedure using phenyl isothiocyanate for quantitative analysis of amino acid mixtures.[438] Further studies (Vol. 14, p. 31) of the use of 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole to generate fluorescent derivatives ($\lambda_{emission}$ 524–541 nm, $\lambda_{excitation}$ 467–472 nm) with amino acids (except tryptophan)[439] and of modification of the procedure appropriate for imino acid analysis[440] have been reported.

Surprisingly little interest, relatively speaking, is being shown in estimation of enantiomer purity by h.p.l.c. methods employing mobile phases containing copper(II)-L-amino acids. Separation in this form of resolution applied to dansylamino acids is based on equilibria between ternary complexes.[441,442]

Electrochemical detection[423,443,444] has been favoured for tyrosine[443] and 5-hydroxytryptophan[444] when biological samples were analysed for these amino acids. Related h.p.l.c. studies of aromatic amino acids deal with tryptophan[445–447] and its indoleamine metabolites in brain tissue[446] {sub-picomole amounts of tryptophan are best analysed through the fluorescence of its Pictet–Spengler reaction product, 9-hydroxymethyl-β-carboline, formed with HCHO and $K_3[Fe(CN)_6]$[447]}, Dopa[448] and 5-(*S*-cysteinyl)Dopa,[449] and phenylalanine.[450] Less common amino acids studied include *meso*-alanopine and strombine,[451] members of the mycosporine group,[452] mimosine,[453] and pyroglutamic acid (nanomole level assay as its 4-nitrophenacyl ester[454]).

Fluorescence Methods. – This section offers a safety net for methods not located in the preceding sections. The *o*-phthaldialdehyde–thiol system has been established to react with an amino acid to yield an isoindole. Triphenylmethanethiol has been studied for the first time in this context,[455] confirming the nature of the fluorescent adduct and establishing the effects of amino acid concentration on time to reach maximum fluorescence. The stability of this fluorophore varies with the structure of the amino acid, tryptophan, Dopa, and other amino acids

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that present a hindered environment to the $-NH_2$ group yielding longer-lived fluorescence.[456]

The fluorescent derivative formed between tryptophan and a modified Koshland reagent, 2-carboxy-1-hydroxy-4-naphthylmethyl dimethylsulphonium chloride, shows $\lambda_{emission}$ 416 nm after excitation at 255 nm. A useful aspect is that in acid solutions only tryptophan and cysteine react with this reagent but in neutral solutions histidine, methionine, and amino acids with hydroxy groups in their side chains also react.[457]

Serine levels in blood samples may be estimated by reaction with periodic acid and fluorimetric estimation of the resulting HCHO.[458]

The sole mention of fluoresceamine in this year's chapter, formerly achieving considerable popularity, refers to the estimation of lysine after its enzymic decarboxylation to cadaverine.[459]

Other Methods. – Chemiluminescence generated through reactions of the H_2O_2 formed in the amino acid oxidase-catalysed degradation of an amino acid is directly proportional to the concentration of the amino acid. Concentrations down to 2.5×10^{-7} M[460] or to more than 10-fold lower levels[461] are measurable, and the choice of the enzyme of appropriate specificity permits L-amino acids alone, or D-enantiomers, to be determined.

Potentiometric titration of Boc-amino acids mixed with amino acids gives curves that can be interpreted to reveal relative amounts of the two structural types.[462]

A specific interaction between some amino acids and Trisacryl GF05 gel can be exploited in analytical separations.[463]

Determination of Specific Amino Acids. – The L-amino acids implied can be selectively analysed using lysine decarboxylase immobilized on a CO_2-sensing electrode,[464] lysine oxidase[465] or tyrosinase[466] immobilized on a pO sensor, and asparagine using *Serratia marcescens* immobilized on an NH_3-sensing electrode.[467] Use of an enzyme not linked with an electrochemical assay is represented in L-tyrosine decarboxylase degradation of L-tyrosine and fluorimetry of the tyramine produced in this way.[468] An enzymic deamination method resulting in the conversion of 1-aminocyclopropane-1-carboxylic acid into α-ketoglutaric acid has been described.[469]

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