1
Amino-acids

BY G. C. BARRETT

1 Introduction

The coverage given in this chapter draws mainly on the chemical literature, but also on the biochemical and biological literature where material relevant to the chemistry, occurrence, and analysis of amino-acids can be found. However, only brief coverage is given, as in previous years, of the distribution and biological roles of well known amino-acids.

Textbooks and Reviews.—Important new textbooks and symposium proceedings[1–4] cover non-protein amino- and imino-acids,[1] ammonia assimilation and amino-acid metabolism in plants,[2] and recent developments in amino-acid chemistry in the context of peptide and protein synthesis.[3,4] Reviews cover physiological roles for γ-aminobutyric acid (GABA)[5] and its β-hydroxy analogue,[6] crosslinking amino-acid residues in collagen,[7] and the history of the discovery of the existence of asparagine and glutamine residues in proteins.[8] Fowden has reviewed the recent literature for non-protein amino-acids.[9]

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—This section is particularly concerned with the location of well-known amino-acids in unusual situations and of unusual amino-acids in a variety of sources.

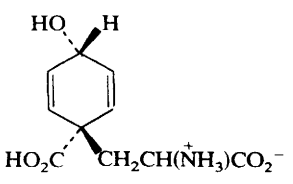
Methods for the isolation of proline and hydroxyproline from fossil bone have been described.[10] L-Canavanine isolated from *Canavalia gladiata* may be

[1] G. A. Rosenthal, 'Plant Nonprotein Amino- and Imino-acids: Biological, Biochemical, and Toxicological Properties', Academic Press, New York, 1982.
[2] B. J. Miflin and P. J. Lea, Encyclopaedia of Plant Physiology (New Series), 1982, Vol. 14A (Nucleic Acids and Proteins in Plants, Part I). p. 5.
[3] 'Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins', ed. B. Weinstein, Dekker, New York, 1982, Vol. 6.
[4] 'Peptides: Synthesis, Structure, Function', Proceedings of the 7th American Peptide Symposium, 1981, ed. D. H. Rich and E. Gross, Pierce Chemical Co., Rockford, Illinois, U.S.A., 1981.
[5] A. Santos-Ruiz, *Rev. Esp. Fisiol.*, 1982, **38,** 1.
[6] A. Mori, *Neurosciences (Kobe, Jpn.)*, 1982, **7,** 236.
[7] D. Fujimoto, *Seikagaku*, 1982, **54,** 314.
[8] C. Chibnall, *Trends Biochem. Sci. (Pers. Ed.)*, 1982, **7,** 191.
[9] L. Fowden in 'Biochemistry of Plants', ed. E. E. Conn, Academic Press, New York, *1981*, Vol. 7, p. 215; see also E. A. Bell, *ibid.*, p. 1.
[10] T. W. Stafford, R. C. Duhamel, C. V. Haynes, and K. Brendel, *Life Sci.*, 1982, **31,** 931.

purified as its flavianic acid salt;[11] the pentacyanoammonioferrate positive spot seen in cellulose t.l.c. of extracts of alfalfa is histidine,[12] not canavanine as claimed earlier.

The simplest non-protein amino-acid, 2-aminobutanoic acid, has been located in mixed rumen ciliate protozoal culture media.[13] Other aliphatic α-amino-acids uncovered recently include L-*threo*-γ-hydroxycitrulline[14] and N^{δ}-benzoyl-γ-hydroxy-L-ornithine[15] from seeds of *Vicia pseudo-orubus*, N^{α}-(γ-glutamyl)-histidine, -ornithine, and -lysine from Shiitake mushroom (*Lentinus edodes*; the first report of the occurrence of these derivatives in mushrooms),[16] L-β-(1,4-cyclohexadienyl)-L-alanine from *Pseudomonas* 1–30,[17] and another 1,4-cyclohexadiene derivative, arogenic acid (1) from *Pseudomonas aureofaciens* as an intermediate in the biosynthesis of phenylalanine and tyrosine.[18]

The methionine adduct of dopa *o*-quinone, which forms during work-up of solutions of these amino-acids and therefore may appear in biological extracts, is proposed to possess structure (2).[19] The natural occurrence of *S*-methyl-L-cysteine and its sulphoxide has been reviewed.[20]



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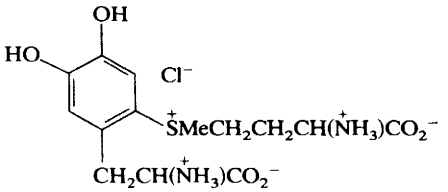


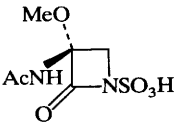
(2)

The possibilities for the existence of amino-acids and other important biochemicals on other planets have been reviewed.[21]

The archetypal β-amino-acid β-alanine has been found in mycelial cell walls of mature *Morchella esculenta*.[22]

New Natural Amino-acids.—*E*-2*S*-Amino-3-methyl-3-pentenoic acid is a new natural amino-acid, found in *Coniogramme intermedia*.[23] The β-lactam (3)[24] and the γ-lactone (4)[25] are cyclized *N*-acetyl-α-amino-acid derivatives isolated from bacterial cultures; (4) has little biological potency.

[11] R. Yang and D. Guo, *Huaxue Shiji*, 1982, 192 (*Chem. Abstr.*, 1982, **97**, 212 638).
[12] G. A. Rosenthal and D. L. Dahlman, *Experientia*, 1982, **38**, 1034.
[13] R. Onodera, K. Miura, and H. Fukuda, *J. Protozool.*, 1982, **29**, 122; R. Onodera and T. Ushijima, *ibid.*, p. 547.
[14] T. Miki and S. Hatanaka, *Phytochemistry*, 1982, **21**, 224.
[15] S. Makisumi, K. Mizusaki, S. Hatanaka, and N. Izumiya, *Phytochemistry*, 1982, **21**, 223.
[16] Y. Aoyagi, T. Sugahara, T. Hasegawa, and T. Suzuki, *Agric. Biol. Chem.*, 1982, **46**, 1939.
[17] N. Onishi, T. Watanabe, K. Izaki, and H. Takahashi, *J. Antibiot.*, 1982, **35**, 90.
[18] B. Keller, E. Keller, O. Salcher, and F. Lingens, *J. Gen. Microbiol.*, 1982, **128**, 1199.
[19] M. N. Gupta and P. J. Vithayathil, *Bioorg. Chem.*, 1982, **11**, 101.
[20] G. A. Maw, *Sulfur Rep.*, 1982, **2**, 1.
[21] B. Nagy, *Naturwissenschaften*, 1982, **69**, 301.
[22] M. E. Jacobs, *Comp. Biochem. Physiol. B*, 1982, **72**, 173.
[23] S. Hatanaka, Y. Murooka, K. Saito, Y. Ishida, and Y. Takeuchi, *Phytochemistry*, 1982, **21**, 453.
[24] W. L. Parker, W. H. Koster, C. M. Cimarusti, D. M. Floyd, W. C. Liu, and M. L. Rathnum, *J. Antibiot.*, 1982, **35**, 189.
[25] W. L. Parker, M. L. Rathnum, and W. C. Liu, *J. Antibiot.*, 1982, **35**, 900.



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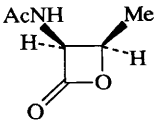
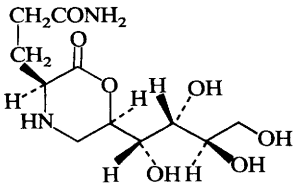


(4)

Re-investigation of agropine from crown-gall tumours (see also Vol. 10, p. 2) shows it to be N^2-(1′-deoxy-D-mannitol-1′-yl)-L-glutamine-1,2′-lactone (5).[26] The amino-glyconic acids as a class would be as well located among amino-acids as among amino-sugars. A compound of this type (6) occurs in *Pseudomonas aeruginosa* 170 005.[27]



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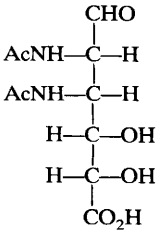


(6)

New Amino-acids from Hydrolysates.—Unusual components of polypeptides and proteins are collected under this heading. The occurrence of *N*-trimethylalanine at the *N*-terminus of histone 2B from *Tetrahymena pyriformis* constitutes the first example of *N*-terminal blocking through methylation in the histone field.[28] Muscle myosin subfragment I has been shown by ^{1}H n.m.r. to carry the same *N*-terminal residue.[29]

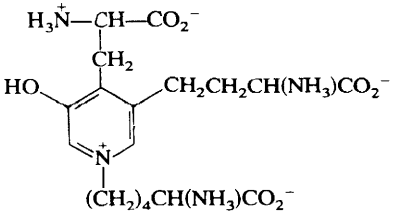
Crosslinking opportunities other than the disulphide grouping of cystine continue to stimulate a considerable amount of research effort, because of the importance of irreversible inter-chain reactions in the ageing process. The structure of pyridinoline (7), a crosslinking diamino-diacid from collagen (see also Vol. 13, p. 2), has been confirmed by f.a.b. mass spectrometry,[30] and the existence of deoxypyridinoline (the analogue of pyridinoline with H in place of the aliphatic hydroxy group) as a new crosslinking residue in collagen has been established by two research groups.[30,31]

'Isodityrosine', an oxidatively coupled dimer of tyrosine involving a diphenyl ether linkage, is a new phenolic crosslinking diamino-diacid found in hydrolysates of cell walls of many higher plants.[32]

[26] M. E. Tate, J. G. Ellis, A. Kerr, J. Tempe, K. E. Murray, and K. J. Shaw, *Carbohydr. Res.*, 1982, **104,** 105.
[27] Yu. A. Knivel, E. V. Vinogradov, A. S. Shashkov, B. A. Omitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 1982, **104,** C4.
[28] M. Nomoto, Y. Kyogoku, and K. Iwai, *J. Biochem.* (*Tokyo*), 1982, **92,** 1675.
[29] G. D. Henry, D. C. Dalgarno, G. Marcus, M. Scott, B. A. Levine, and I. P. Trayer, *FEBS Lett.*, 1982, **144,** 11.
[30] M. Barber, R. S. Bordoli, G. J. Elliott, D. Fujimoto, and J. Escott, *Biochem. Biophys. Res. Commun.*, 1982, **109,** 1041.
[31] T. Ogawa, T. Ono, M. Tsuda, and Y. Kawanishi, *Biochem. Biophys. Res. Commun.*, 1982, **107,** 1252.
[32] S. C. Fry, *Biochem. J.*, 1982, **204,** 449.

3 Synthesis of Amino-acids

General Methods.—Standard methods have been used for the synthesis of β-(3-pyrrolin-*N*-oxylyl)alanine (8) proposed as a paramagnetic amino-acid for use in peptide synthesis.[33] Alkylation of dimethyl acetylaminomalonate, diphenylmethylideneglycine ethyl ester, or diethyl malonate (followed by treatment with diphenylphosphoryl azide and benzyl alcohol to give the *N*-benzyloxycarbonyl amino-acid) was fully studied in this context.[33] Alkylation of acylamidomalonates continues to be widely used for the synthesis of α-amino-acids [homologues of 2-amino-5-(*p*-methoxyphenyl)pentanoic acid[34] and other examples mentioned later in this chapter, refs. 113, 115, 130, 131, 133, and 147]. Improvements have been achieved in the Schiff-base alkylation route,[35,36] where 73—94% yields of monoalkylation products were obtained in most cases using ion-pair extraction or catalytic liquid–liquid or solid–liquid phase-transfer techniques with ethyl *p*-chlorobenzylideneglycinate.[35] Yields of α-methyl-α-amino-acids were equally good in corresponding alkylation reactions of the alanine analogue.[36]



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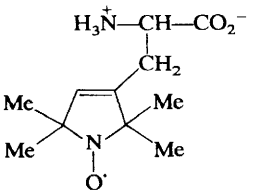


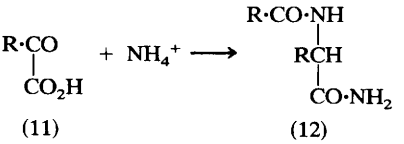
(8)

Alternative methods for the introduction of a nitrogen function adjacent to a carboxy group include amido-alkylation (for β-amino-acid synthesis $R^1{\cdot}CO{\cdot}NH{\cdot}CHR^2{\cdot}SO_2Tol$ has been proposed[37]), a racemization-free synthesis

(9) (10)

of *NN*-dialkylamino-acids (9) → (10),[38] and development of the biogenetically modelled amination of α-keto-acids in aqueous media,[39–41] which has led to

[33] L. Lex, K. Hideg, and H. O. Hankovszky, *Can. J. Chem.*, 1982, **60**, 1448.
[34] N. Kosui, M. Waki, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 918.
[35] L. Ghosez, J.-P. Antoine, E. Deffense, M. Navano, V. Libert, M. J. O'Donnell, and W. A. Brudu, *Tetrahedron Lett.*, 1982, **23**, 4255.
[36] M. J. O'Donnell, B. Le Clef, D. B. Rusterholz, L. Ghosez, J.-P. Antoine, and M. Navano, *Tetrahedron Lett.*, 1982, **23**, 4259.
[37] J. Morton, A. Rahim, and E. R. H. Walker, *Tetrahedron Lett.*, 1982, **23**, 4123.
[38] F. Effenberger, U. Burkard, and J. Willfahrt, *Angew. Chem.*, 1983, **95**, 50.
[39] F. Egami, Y. Makino, K. Sato, and M. Nishizawa, *Proc. Jpn. Acad. Sci., Ser. B*, 1981, **57**, 329.
[40] F. Egami, Y. Makino, M. Nishizawa, and K. Sato, *Nippon Kagaku Kaishi*, 1982, **56**, 537 (*Chem. Abstr.*, 1983, **97**, 198 523).
[41] H. Yanagawa, Y. Makino, K. Sato, M. Nishizawa, and F. Egami, *J. Biochem.* (*Tokyo*), 1982, **91**, 2087.



(11) (12)

the discovery of the novel reaction in which low yields (1—20%) of the corresponding *N*-(2-oxoalkanoyl)amino-acid amide (11) → (12) are formed. The α-amino-acids are easily obtained from these intermediates by acid hydrolysis.

Further exploration of amidocarbonylation routes to α-amino-acids (see Vol. 13, p. 4) has established that allylic alcohols react with acetamide in the presence of $Co_2(CO)_8$ and $HRh(CO)(PPh_3)_3$, reductive carbonylation with a 1:1 mixture of H_2 and CO at 100 atm at 110 °C in dioxane, giving good yields of *N*-acetylamino-acids (the allylic moiety is hydrogenated in this process).[42] Electrochemical reductive carboxylation of *N*-arylideneamines RN=CHAr and RN=CMeAr with CO_2 at a mercury cathode gives predominantly *C*-carboxylation products, and competing reduction of the double bond can be suppressed by increasing the water content of the medium.[43]

Synthesis of amino-acids and peptides exploiting 1,4-opening of β-lactams (see Vol. 13, p. 4) has been reviewed.[44] A forthcoming textbook[45] includes an exhaustive coverage of the synthesis of amino-acids.

A full account has been published of the synthesis of β-amino-acids from *N*-acetyl thioamides and $Ph_3P{=}CHCO_2Me$[46] followed by reduction of the resulting β-acetylaminoacrylate. A total synthesis of iturinic acid, $Me_2CH(CH_2)_8CH(\overset{+}{N}H_3)CH_2CO_2^-$, as its ethyl ester was included as an example of this efficient route.[46] The Reformatzky route to β-amino-acids[47] employing α-bromoalkanoic acids and Schiff bases gives moderate yields.

Most syntheses of amino-acids yield salts from which they may be recovered by passage through columns of crosslinked poly(4-vinylpyridine).[48] Basic amino-acids, however, elute as their mono-acid salts.

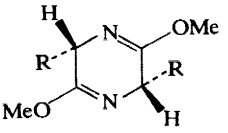
Asymmetric Synthesis.—All the papers encountered in the 1982 literature describe extensions of previously established principles. The general topic has been reviewed.[49]

Representative papers concerned with asymmetric hydrogenation[50,51] continue the use of chiral rhodium–phosphine complexes. Reductive amination of

[42] K. Hirai, Y. Takahashi, and I. Ojima, *Tetrahedron Lett.*, 1982, **23,** 2491.
[43] U. Hess and M. Ziebig, *Pharmazie*, 1982, **37,** 107.
[44] I. Ojima and N. Hatanaka, *Yuki Gosei Kagaku Kyokaishi*, 1982, **40,** 209 (*Chem. Abstr.*, 1982, **97,** 56 205).
[45] G. C. Barrett in 'The Chemistry and Biochemistry of the Amino-acids', ed. G. C. Barrett, Chapman and Hall, London, 1984, Chap. 5; C. N. C. Drey, *ibid.*, Chap. 3.
[46] M. Slopianka and A. Gassauer, *Liebigs Ann. Chem.*, 1981, 2258.
[47] M. Bellassoued, R. Arous-Chtara, and M. Gaudemar, *J. Organomet. Chem.*, 1982, **231,** 185.
[48] D. M. Jewett and R. L. Ehrenkaufer, *Anal. Biochem.*, 1982, **122,** 319.
[49] D. Hoppe, *Nachr. Chem., Tech. Lab.*, 1982, **30,** 782.
[50] N. Izumiya in 'Asymmetric Reactions and Processes in Chemistry', Am. Chem. Soc., Symp. Ser., No. 185, ed. E. L. Eliel and S. Otsuka, American Chemical Society, Washington, D.C., U.S.A., 1982, p. 272.
[51] J.-C. Paulin and H. B. Kagan, *J. Chem. Soc., Chem. Commun.*, 1982, 1261.

4-isopropylidene-2-methyloxazolin-5-ones using (*S*)-phenylethylamine gives *N*-acetyl-L-valine phenylethylamide in 44% enantiomeric excess.[52]

The asymmetric-alkylation approach also offers several alternative methodologies. Schiff bases $Ph_2C{=}NCH_2CO_2R$ (R = Me or Et) give up to 40% enantiomeric excess of the *S*-alanine derivatives after carbanion formation with Pr^i_2NLi and methylation with a 1,2,5,6-di-isopropylidene-D-glucofuranose 3-methanesulphonate.[53] *N*-Benzylidene DL-phenylalanine methyl ester similarly underwent asymmetric methylation with methyl iodide in the presence of chiral lithium (*S*)-2-alkylpyrrolidines.[54] The chiral heterocycles (13) are masked Schiff bases and have been extensively studied[55] (see also Vol. 13, p. 5) in the context of asymmetric synthesis of α-amino-acids and their α-methyl analogues. Better than 95% stereoselectivity can be achieved through anion formation with BuLi, followed by alkylation with an alkyl or benzyl bromide.



(13)

Alkylation of chiral Schiff bases $RCMe{=}NCH_2CO_2^-$, where R is the (*S*)-*o*-*N*-(*N*-benzylpropylamino)phenyl grouping complexed to Cu^{2+}, gives predominantly (95%) *threo*-threonine in at least 97% optical purity when acetaldehyde is the other reactant.[56]

(−)-Menthyl isocyanoacetate $CNCH_2CO_2Men$ gives $H_2N(CH_2)_3CR(\overset{+}{N}H_3)$-$CO_2^-$ through successive alkylation with an alkyl iodide RI and acrylonitrile after anion formation with NaH, followed by acid hydrolysis.[57] Higher homologous amino-acids such as (3*S*,4*S*)- and (3*R*,4*S*)-$Me_2CHCH_2CH(\overset{+}{N}H_3)$-$CH(OH)CO_2^-$ were prepared in several steps from *N*-phthaloyl-L-leucyl chloride through condensation with (−)-menthyl t-butyl malonate and $NaBH_4$ reduction of the resulting β-oxo-ester.[57]

Full details of the enantioselective protonation of phenylglycine Schiff bases with a chiral acid leading to enantiomer excesses up to 70% have been published.[58] The preliminary communication describing this approach was discussed in Vol. 11, p. 16.

Prebiotic Synthesis of Amino-acids.—A number of papers mentioned elsewhere in this chapter have described new possibilities for the synthesis of

[52] G. V. Chel'tsova, E. I. Karpeiskaya, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1981, 2350.

[53] P. Duhamel, J.-Y. Valnot, and J. J. Eddine, *Tetrahedron Lett.*, 1982, **23**, 2863.

[54] T. Yamashita, H. Mitsui, H. Watanabe, and N. Nakamura, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 961.

[55] (*a*) U. Schöllkopf, U. Groth, and W. Hartwig, *Liebigs Ann. Chem.*, 1982, 2407; (*b*) U. Schöllkopf, W. Hartwig, K. H. Posposchil, and H. Kehne, *Synthesis*, 1981, 966; U. Schöllkopf, U. Groth, K. O. Westphalen, and C. Deng, *ibid.*, p. 969; U. Groth, Y. Chiang, and U. Schöllkopf, *Liebigs Ann. Chem.*, 1982, 1756.

[56] Y. N. Belokon', I. E. Zel'tzer, M. G. Ryzhov, M. B. Saporovskaya, V. I. Bakhmutov, and V. M. Belikov, *J. Chem. Soc., Chem. Commun.*, 1982, 180.

[57] M. Kirihata, *Bull. Univ. Osaka Prefect., Ser. B*, 1981, **33**, 135 (*Chem. Abstr.*, 1982, **96**, 143 271).

[58] L. Duhamel and J. C. Plaquevent, *Bull. Soc. Chim. Fr.*, 1982, Part 2, 75.

amino-acids from simple starting materials under ambient conditions. Ammonia and glyoxylic acid yield *N*-oxalylglycine in aqueous solutions,[39–41,59] and u.v. irradiation of these solutions in the presence of an alkene with acetone as sensitizer gives aspartic acid, norvaline, valine, leucine, phenylalanine, and tyrosine.[59]

Simpler reactants such as CO_2[60,61] or methane[61,62] can be caused to react with nitrogen to yield amino-acids, using u.v. light or electric discharges as energy sources,[63] suggesting that the frozen surface of Titan, with its HCN–CH_4–N_2 atmosphere, could indeed have accumulated amino-acids.[61,62]

Glycine is converted into a mixture of seven aliphatic α-amino-acids at 200 °C in contact with N_2 and granite, basalt, or bentonite with or without $MnCO_3$ or Al_2O_3.[64] An entertaining abstract for a paper[65] describing the formation of amino-acids 'in systems not containing any source of N, utilizing compounds with antiseptic properties such as PhOH, resorcinol, *etc.*' hides the fact that the nitrogen molecule is the source of the amino groups in the products. The reaction is light-driven and not a bacterial process; the phenols are oxidized and water is cleaved by photolysis, to provide the energy to drive the (unlikely) reactions.[65]

Aqueous solutions of ammonium salts of dicarboxylic acids irradiated with ultra-short (picosecond) laser u.v. pulses gave the corresponding amino-dicarboxylic acids.[66]

Protein and Other Naturally Occurring Amino-acids.—There is space only for representative papers on production of protein α-amino-acids by fermentation (the formation of L-tryptophan in culture media of azaserine-resistant *Brevibacterium flavum* mutant[67] and of *Escherichia coli* offered L-serine and indole,[68] and L-lysine by *Brevibacterium lactofermentum* mutants[69]). The topic has been reviewed,[70–72] ref. 70 being taken from a volume containing numerous papers on the subject, and ref. 72 being narrower in its scope (L-dopa, L-cysteine, and D-*p*-hydroxyphenylglycine).

Methionine is biosynthesized from 5′-methylthioadenosine *via* 2-oxo-4-methylthiobutyric acid in rat liver.[73]

Coverage of the biosynthesis of the non-protein α-amino-acids is similarly selective. β-Pyrazolyl-L-alanine has 1,3-diaminopropane as precursor for the

[59] M. Nishizawa and F. Egami, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2689.
[60] J. Gribbin, *New Sci.*, 1982, **94**, 413.
[61] M. Ishigami, M. Kinjo, K. Nagano, and Y. Hattori, *Origins Life*, 1982, **12**, 307.
[62] F. Raulin, D. Mourey, and G. Toupance, *Origins Life*, 1982, **12**, 267.
[63] A. R. Bossard, F. Faulin, D. Mourey, and G. Toupance, *J. Mol. Evol.*, 1982, **18**, 173.
[64] Ch. Ivanov and N. Slavcheva, *Dokl. Bolg. Akad. Nauk*, 1981, **34**, 1401.
[65] A. K. Sen, *J. Indian Chem. Soc.*, 1982, **59**, 476 (*Chem. Abstr.*, 1983, **97**, 163 440).
[66] V. S. Letokhov, Yu. A. Matveets, V. A. Semchishen, and E. V. Khoroshilova, *Appl. Phys. B*, 1981, **26**, 243.
[67] I. Shiio, S. Sugimoto, and K. Kawamura, *Agric. Biol. Chem.*, 1982, **46**, 1849.
[68] F. Wagner, S. Lang, W. G. Bang, K. D. Vorlop, and J. Klein, *Enzyme Eng.*, 1982, **6**, 251.
[69] M. E. Schonfeldt, and T. G. Watson, *S. Afr. Food. Rev.*, 1982, **9**, S111 (*Chem. Abstr.*, 1982, **97**, 90 343).
[70] Y. Minota, *Hakko to Kogyo*, 1982, **40**, 292.
[71] H. Enei, H. Shibai, and Y. Hirose, *Ann. Rep. Ferment. Processes*, 1982, **5**, 79.
[72] T. Yamamoto, *Kagaku Gijutsushi MOL*, 1982, **20**, 21 (*Chem. Abstr.*, 1982, **97**, 180 032).
[73] P. S. Backlund, C. P. Chang, and R. A. Smith, *J. Biol. Chem.*, 1982, **257**, 4196.

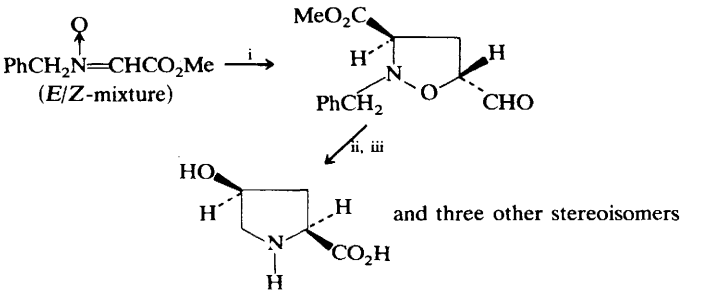
pyrazole moiety in cucumber seeds.[74] Biosynthesis of L-canavanine in jack bean (*Canavalia ensiformis*) has received further detailed study.[75]

Laboratory syntheses of amino-acids that occur in proteins or in other natural sources continue to attract the interest of academic and industrial research groups. Full details of the synthesis of glycine by ammonolysis of trichloroethylene[76] (Vol. 11, p. 9) and of DL-alanine by ammonolysis of 2-chloropropanoic acid in aqueous solution under pressure[77] (Vol. 14, p. 5) have now been published. By-products in the preparation of $MeS\text{-}CH_2CH_2CHO$ from acrolein and methanethiol, for use in the Strecker synthesis of DL-methionine, have been shown to be oligomers $HO[CH(CH_2CH_2SMe)O]_n$ and aldol condensation products of the target aldehyde.[78]

Alternative syntheses have been reported for 4-hydroxy-DL-proline (Scheme 1),[79] L-α-amino-adipic acid from *N*-Boc-L-aspartic acid α-t-butyl ester (Scheme 2),[80] and L-dopa from L-glutamic acid (Scheme 3).[81]

As in two of the three preceding syntheses, cycloaddition offers increasingly attractive possibilities in synthesis; the approach has already been used in syntheses of the anti-tumour compound AT-125 ('acivicin'), and a further synthesis of this amino-acid (14) uses (*S*)-vinylglycine and chlorofulminic acid, ClNCO, from dichloroformaldoxime, $Cl_2C{=}NOH$, and $AgNO_3$.[82]

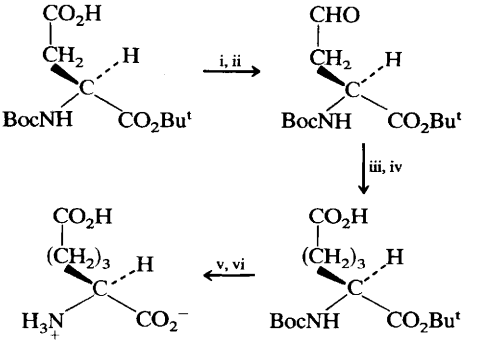
Aliphatic α-amino-acids for which syntheses have been reported recently include β-carboxy-L-aspartic acid. This is prepared by the reaction of $[(NH_3)_5CoO_2CCHO]^{2+}$ with $H_2C(CO_2Et)_2$ in DMSO, then dehydration, giving $[(NH_3)_5CoO_2CCH{=}C(CO_2Et)_2]^{2+}$;[83] the addition of NH_3 [through dissolution



Reagents: i, $CH_2{=}CHCHO$; ii, $H_2/Pd(OH)_2$; iii, hydrolysis

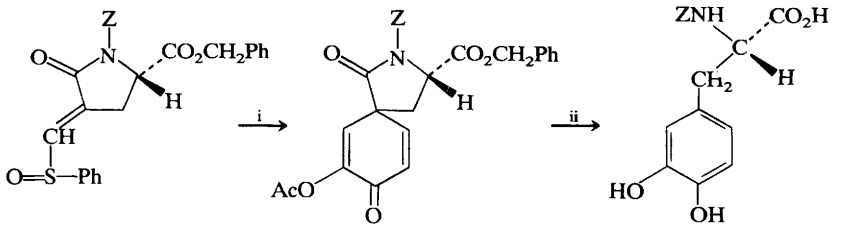
Scheme 1

[74] E. G. Brown, K. A. M. Flayeh, and J. R. Gallan, *Phytochemistry*, 1982, **21**, 863.
[75] G. A. Rosenthal, *Plant Physiol.*, 1982, **69**, 1066.
[76] M. Inoue and S. Enomoto, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 33.
[77] Y. Ogata and M. Inaishi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3605.
[78] V. S. Balakin, B. S. Gorbunov, G. B. Zvegintseva, and L. S. Romanova, *Khim. Promst. (Moscow)*, 1982, 84 (*Chem. Abstr.*, 1982, **97**, 92 707).
[79] J. Hara, Y. Inouye, and H. Kakisawa, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3871.
[80] K. Ramsamy, R. K. Olsen, and T. Emery, *Synthesis*, 1982, 42.
[81] S. Danishefsky and T. A. Craig, *Tetrahedron*, 1981, **37**, 4081.
[82] P. A. Wade, M. K. Pillay, and S. M. Singh, *Tetrahedron Lett.*, 1982, **23**, 4563.
[83] N. E. Dixon and A. M. Sargeson, *J. Am. Chem. Soc.*, 1982, **104**, 6716.



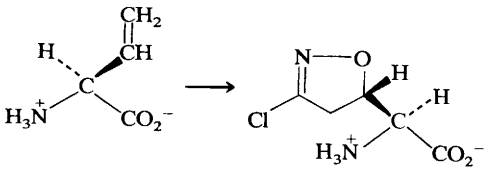
Reagents: i, EtO-COCl; ii, $NaBH_4$, CrO_3-py; iii, $Ph_3P{=}CHCO_2Et$; iv, Pd/C–H_2; v, saponification; vi, 6M HCl

Scheme 2



Reagents: i, $MeOCH{=}C(OAc)C(OSiMe_3){=}CH_2$ in refluxing xylene, 7 h, N_2; ii, hydrolysis

Scheme 3



(14)

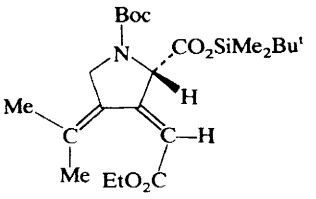
of the cobalt (III) complex in liquid ammonia] gives the malonate from which the target molecule is obtained through hydrolysis and resolution.[83] Stereoselective synthesis of δγ-dihydroxyisoleucine starts with Boc-glycine and $MeC{\equiv}CCH_2OH$, proceeds *via* stereoselective Claisen rearrangement of the *Z*-2-butenyl ester obtained from these reactants, and then *via* elaboration into the appropriate stereoisomer of $CH_2{=}CHCHMeCH(NHBoc)CO_2H$ and iodolactonization to give the lactone of the synthetic objective.[84]

Synthesis of hypusine from N^{α}-benzyloxycarbonyl-L-lysine benzyl ester, through treatment with (*R*)-$ZNHCH_2CH_2CH(OH)CH_2Br$ and deprotection,

[84] P. A. Bartlett, D. J. Tanzella, and J. F. Barstow, *Tetrahedron Lett.*, 1982, **23**, 619.

gave material identical in all respects with the natural compound, thus verifying its absolute configuration.[85] Synthesis of epimers of HO_2CCHMe-(*S*)-Arg-OH from D- or L-alanine and 5-acetylamino-2-bromopentanoic acid followed by conventional conversion of the resulting octopinic acids with $H_2NC(=NH)SMe$ into the octopines confirms the D-configuration of the alanine moiety of the natural (+)-octopine.[86] Similar approaches have verified the L,D-configuration for nopaline (from the crown-gall tumour of *Helianthus annus*) through synthesis from L-arginine and 2-oxoglutaric acid, separation, and assignments of configuration by enzymic methods.[87]

Heterocyclic syntheses in this area include an enantioselective synthesis of (−)-α-kainic acid from γ-ethyl-L-glutamate *via* (15), employing an elegant intramolecular thermal conversion into the protected product (16), proving unambiguously the absolute configuration at C-2.[88] Quisqualamine (17) has been synthesized through a multi-step route starting from ClCONCO and $HONHCH_2CH_2NHAc$.[89] The *N*-terminal amino-acid residue of the nikkomycins I, J, X, and Z, β-hydroxy-β-(5-hydroxy-2-pyridyl)valine, has been synthesized through a lengthy route starting from 6-methyl-3-pyridinol.[90] Of the four stereoisomers produced through this route, one was shown by ^1H n.m.r. and other methods to be identical with the natural amino-acid.[90]



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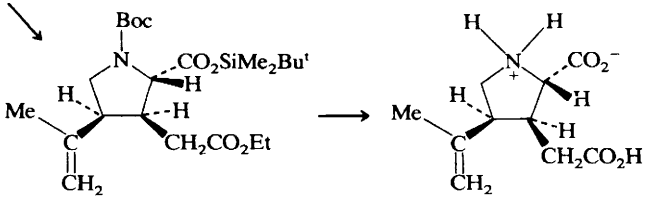

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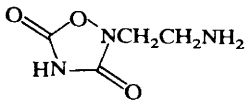
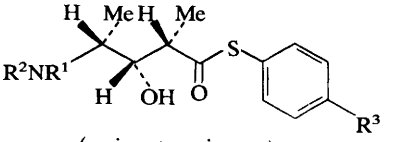


(17)

[85] T. Shiba, H. Akiyama, I. Umeda, S. Okada, and T. Wakamiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 899.
[86] K. Goto, M. Waki, N. Mitsuyasu, Y. Kitajima, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 261.
[87] S. Hatanaka, S. Atsumi, K. Furukawa, and Y. Ishida, *Phytochemistry*, 1982, **21**, 225.
[88] W. Oppolzer and K. Thirring, *J. Am. Chem. Soc.*, 1982, **104**, 1978.
[89] P. Dugenet, J. J. Yaouanc, and G. Sturtz, *Synthesis*, 1982, 781.
[90] W. Hass and W. A. Koenig, *Liebigs Ann. Chem.*, 1982, 1615.

β- and Higher Homologous Amino-acids.—A kinetic study of the reaction of acrylonitrile with aqueous ammonia, through which β-alanine is manufactured, has been reported.[91] The β-amino-acid (3*R*)-amino-(2*S*)-hydroxy-4-phenylbutanoic acid, present in bestatin, has been synthesized starting from Boc-D-phenylalanine 3,5-dimethylpyrazolyl ester, by reduction ($LiAlH_4$/THF) to Boc-D-phenylalaninal and then condensation with HCO_2Et followed by saponification and deprotection.[92] The synthesis of $ZNHCH_2CH_2COCO_2Me$ from $ZNHCH_2CH_2COCH_2S(O)Me$ through bromination (NBS) and methanolysis was followed by bakers' yeast reduction and saponification to give *N*-benzyloxycarbonyl-4-amino-2-hydroxybutanoic acid, a constituent of butirosin.[93] Another γ-amino-acid, this time from bleomycin, has been synthesized by aldol condensation of (*R*)-$R^1NR^2CHMeCHO$ with vinyloxyborane *cis*-$MeCH{=}C(OBR_2)SAr$; the resulting thiolester (18) (R^1 = H, R^2 = Z or Boc, R^3 = phthaloyl) gave the required product after conventional elaboration.[94]



(major stereoisomer)
(18)

α-Alkyl Analogues of Natural Amino-acids.—Candidates for inclusion in this section, whose interest stems largely from the search for enzyme inhibitors, are β-(4-hydroxyphenyl)-α-methylalanine and analogues, prepared from α-nitroalanine ethyl ester and the corresponding benzyl alcohol by condensation in the presence of Bu_4NCl and KF followed by hydrogenation[95] and α-difluoromethylornithine, prepared through the Strecker route as a potent ornithine decarboxylase inhibitor.[96] Other examples of the synthesis of these analogues are included in an earlier section of this chapter[53,54,57] and in later sections.

Other Aliphatic, Alicyclic, and Saturated Heterocyclic α-Amino-acids.—N^{α}-Protected L-2,3-diaminopropanoic acids have been prepared by Curtius rearrangement of corresponding aspartic acid derivatives.[97] L,L-2,5-Diaminoadipic acid has been obtained in the form of its piperidin-2-one by alkylation of $ZNHCH(CO_2H)CO_2Bu^t$ with L-homoserine lactone.[98]

[91] T. Saida and H. Michiki, *Kagaku Gijutsushi MOL*, 1982, **20**, 57 (*Chem. Abstr.*, 1982, **96**, 200 118).
[92] H. Kayahara, J. Kurita, and I. Tomida, *Shinshu Daigaku Nogakubu Kiyo*, 1981, **18**, 103 (*Chem. Abstr.*, 1982, **96**, 85 947).
[93] S. Iriuchijima and M. Ogawa, *Synthesis*, 1982, 41.
[94] M. Narita, M. Otsuka, S. Kabayashi, M. Ohno, Y. Umezawa, H. Morishima, S. Saito, T. Takita, and H. Umezawa, *Tetrahedron Lett.*, 1982, **23**, 525.
[95] B. Renger, *Arch. Pharm. (Weinheim)*, 1982, **315**, 472.
[96] J. E. Seely, H. Poso, and A. E. Pegg, *Biochem. J.*, 1982, **206**, 311.
[97] N. Noguchi, T. Kuroda, M. Hatanaka, and T. Ishimaru, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 633.
[98] D. S. Kemp and E. T. Sun, *Tetrahedron Lett.*, 1982, **23**, 3759.

(*E*)- and (*Z*)-1-amino-2-phenylcyclopropanecarboxylic acids have been prepared by building the cyclopropane ring on to 2-phenyl-4-benzylideneoxazolin-5-one using diazomethane.[99] (*RS*)-2-Cyclopropylglycine and D- and L-proline have been prepared from the 2-cyclopropyl-2-oxoethanoatocobalt(III) complex resulting from the reaction of 5-bromo-2-oxopentanoic acid with the aquapenta-amminecobalt(III) ion.[100] An improvement in the one-step route from L-lysine to L-pipecolic acid (L-piperidine-2-carboxylic acid) involves the use of $Na_2[Fe(CN)_5NO]$ in H_2O at pH 9.5.[101]

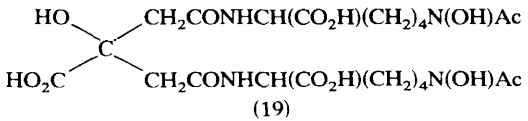
α-Halogenoalkyl Amino-acids.—β-Dibenzylamino-α-fluoroalkanoic acids were obtained from β-hydroxy-α-*NN*-dibenzylamino-acid benzyl esters by treatment with SF_3–NEt_3, *via* the corresponding *NN*-dibenzylaziridinium fluorides.[102]

threo- and *erythro*-β-fluoro-DL-aspartic acid and *threo*-β-fluoro-DL-asparagine, prepared as reported earlier (Vol. 12, p. 11), were assigned their configurations through *X*-ray crystal analysis;[103] tested for cytotoxicity, *threo*-β-fluoro-DL-aspartic acid possessed the greater potency.

α-Difluoromethylornithine is mentioned in the preceding section.[96]

Aliphatic α-Amino-acids Carrying Side-chain Hydroxy and Alkoxy Groups.—N^6-Acetyl-N^6-hydroxylysine, a constituent of aerobactin, has been synthesized from ε-hydroxy-L-norleucine *via* bromination (CBr_4/PPh_3) of its *N*-Boc methyl ester, which was treated with $AcNHOCH_2Ph$, then with anhydromethylenecitryl chloride after removal of the Boc group, giving a blocked form of the synthetic objective (19).[104]

Cleavage of *N*-benzyloxycarbonylaziridine carboxylic esters with alcohols gives substituted serines and threonines.[105]



(19)

Aliphatic Amino-acids with Unsaturated Side Chains.—'Dehydroamino-acids', or α-amino-αβ-alkenoic acids $H_3\overset{+}{N}C(=CR^1R^2)CO_2^-$, can be prepared through aminolysis of 4-alkylidene-oxazolin-5-ones, which are formed from the corresponding saturated α-amino-acids by treatment with dichloroacetic anhydride.[106] A new synthesis of *N*-acetyldehydroamino-acids involves treatment of α-azidoalkanoate esters with acetic anhydride in the presence of

[99] S. W. King, J. M. Riordan, E. M. Holt, and C. H. Stammer, *J. Org. Chem.*, 1982, **47**, 3270.
[100] P. J. Lawson, M. G. McCarthy, and A. M. Sargeson, *J. Am. Chem. Soc.*, 1982, **104**, 6710.
[101] L. Kisfaludy, F. Korenczki, and A. Katho, *Synthesis*, 1982, 163.
[102] L. Somekhkand and A. Shanzer, *J. Am. Chem. Soc.*, 1982, **104**, 5836.
[103] A. M. Stern, B. M. Foxman, A. H. Tashjian, and R. H. Abeles, *J. Med. Chem.*, 1982, **25**, 544.
[104] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1982, **104**, 3096.
[105] K. Nakajima, M. Neya, S. Yamada, and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3049.
[106] D. J. Phelps and F. C. A. Gaeta, *Synthesis*, 1982, 234.

Re_2S_7.[107] Some of the *NN*-diacetyl homologue is also formed in this novel reaction.

$\beta\gamma$-Unsaturated amino-acids have received a good deal of attention, both for the development of new methodology and in extending the usefulness of known processes. Reduction of 5-(α-chloroalkyl)-2-substituted oxazoline-4-carboxylic esters with Zn, followed by hydrolysis, offers a new route to these compounds. The oxazolines are well known as intermediates for the synthesis of β-hydroxy-α-amino-acids[45] and are obtainable from ethyl isocyanoacetate and an α-chloroketone.[108]

Claisen rearrangement of allylic esters of *N*-acyl α-amino-acids using two equivalents of base gives the $\gamma\delta$-unsaturated α-amino-acids in moderate to good yields with a substantial degree of stereoselectivity.[84,109]

Competing routes to L-3,4-didehydroproline supplementing earlier reports involve either Chugaev elimination of 4-hydroxy-L-proline xanthate[110,111] or sulphoxide *syn*-elimination[112] for the selenoxide equivalent[113] from substrates prepared from 4-hydroxy-L-proline. The high regioselectivity in these reactions is notable, less than 10% of the 4,5-dehydro analogue being formed.

Aromatic and Heteroaromatic Amino-acids.—Conventional procedures have been employed in preparations of *p*-chlorophenylalanine (from *p*-chlorobenzyl bromide and diethyl acetamidomalonate),[113] benzoselenophen-3-ylglycine [by amidoalkylation with $ZNHCH(OH)CO_2H$],[114] 6-fluorotryptophan (from the indolylmethyl bromide and diethyl formamido- or acetamido-malonate),[115] and 4, 5-, 6-, and 7-azidotryptophans (from the corresponding indoles and tryptophan synthetase from *Neurospora crassa*).[116]

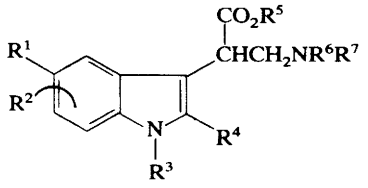
Amino-acids Containing Sulphur.—Electrochemical oxidation of dopa in the presence of cysteine gives mono- and di-cysteinyldopas (attack occurring at the phenolic moiety, giving yields 45, 12, and 8% for 5-, 2-, and 2,5-substitution, respectively).[117]

L-*S*-(2-Amino-2-carboxyethylsulphonyl)-L-cysteine (*alias* cysteine thiolsulphonate) gives *S*-sulpho-L-cysteine and L-alanine-3-sulphinic acid in high yields by treatment with aqueous sodium sulphite, offering convenient preparations of these compounds in view of the accessibility of the thiolsulphonate.[118]

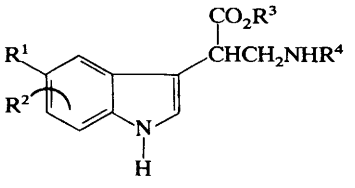
Addition of thiols to 4-methyleneglutamic acid has been used for the

[107] F. Effenberger and T. Beisswenger, *Angew. Chem.*, 1982, **94,** 210.
[108] F. Heinzer and D. Bellus, *Helv. Chim. Acta*, 1981, **64,** 2279.
[109] P. A. Bartlett and J. F. Barstow, *J. Org. Chem.*, 1982, **47,** 3933.
[110] J. R. Dormoy, B. Castro, G. Chappuis, U. S. Fritschi, and P. Grogg in 'Proceedings of the 16th European Peptide Symposium', ed. K. Brunfeldt, Scriptor, Copenhagen, 1981, p. 229.
[111] J. R. Dormoy, *Synthesis*, 1982, 753.
[112] H. Rueger and M. H. Benn, *Can. J. Chem.*, 1982, **60,** 2918.
[113] C. Sun and J. Zhang, *Huaxue Shiji*, 1981, **57,** 28 (*Chem. Abstr.*, 1982, **96,** 123 251).
[114] T. Sadeh, M. A. Davis, R. Gil, and U. Zoller, *J. Heterocycl. Chem.*, 1981, **18,** 1605.
[115] R. Yang and C. Ju, *Shengwu Huaxue Yu Shengwu Wuli Jinzhan*, 1981, **41,** 66 (*Chem. Abstr.*, 1982, **96,** 123 253).
[116] A. Saito and H. C. Rilling, *Prep. Biochem.*, 1981, **11,** 535.
[117] C. Hansson, *Experientia*, 1981, **37,** 1253.
[118] T. Ubuka, M. Kinuta, R. Akogi, S. Kiguchi, and M. Azumi, *Anal. Biochem.*, 1982, **126,** 273.

synthesis of *S*-(4-amino-2,4-dicarboxybutyl)cysteamine and *S*-(4-amino-2,4-dicarboxybutyl)cysteine.[119]



(20)



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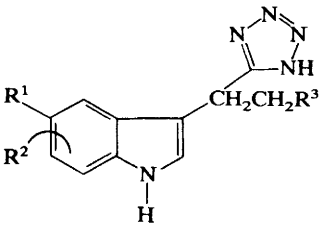


(22)

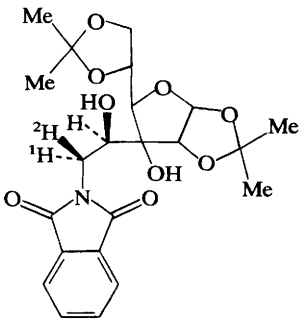
Amino-acids Synthesized for the First Time.—A series of typtophan analogues (20)—(22),[120] aminopiperidinecarboxylic acids related to nipecotic acid (prepared by starting from 5-aminonicotinic acid),[121] and GABA analogues [stereoisomers of *cis*-3-aminocyclohexanecarboxylic acid,[122] (*Z*)- and (*E*)-4-amino-3-(4-chlorophenyl)but-2-enoic acids[123]] include many new amino-acids. Preparations of other new amino-acids have been discussed elsewhere in this chapter.

Labelled Amino-acids.—Continuing the reflection of the high level of interest in the synthesis of isotopically labelled amino-acids by the relatively large amount of space devoted here, the volume of literature and its variety this year have, however, defeated the system used in previous volumes to give some sense of order to the coverage. The synthetic objectives described in the recent literature are all protein amino-acids (with one exception) and are covered in this section in order of increasing molecular complexity.

Chiral glycine enantiomers $H_3\overset{+}{N}C^1H^2HCO_2^-$ have been synthesized from the hexulofuranose derived from D-glucose, as a chiral template, using reactions of its keto group, leading to phthalimidoacetaldehyde by $Pb(OAc)_4$ cleavage of (23).[124]

(3*R*, 4*S*)- and (3*R*, 4*R*)-valine-[4,4-^{2}H, ^{3}H] have been prepared by photolysis of the pyruvyl ester of (*S*)-(−)-$PhCH_2C\dot{H}MeC^2H_2OH$, leading to (*S*)-$PhCH_2CHMeC^2HO$ and thence to (1*R*, 2*R*)- and (1*S*, 2*R*)-alkanes

[119] G. K. Powell, H. C. Winter, and E. E. Dekker, *Biochem. Biophys. Res. Commun.*, 1982, **105**, 1361.
[120] M. E. Safdy, E. Kurchacova, R. N. Schut, H. Vidrio, and E. Hong, *J. Med. Chem.*, 1982, **25**, 723.
[121] P. Jacobsen, K. Schaumburg, J. J. Larsen, and P. Krogsgaard-Larsen, *Acta Chem. Scand., Ser. B*, 1981, **35**, 289.
[122] R. D. Allan, G. A. R. Johnston, and B. Twitchin, *Aust. J. Chem.*, 1981, **34**, 2231.
[123] R. D. Allan and H. Tran, *Aust. J. Chem.*, 1981, **34**, 2641.
[124] K. Kakinuma, N. Imamura, and Y. Saba, *Tetrahedron Lett.*, 1982, **23**, 1697.



(23)

$^3H^2HCHCHMeCH_2Ph$, from which the valine diastereoisomers were prepared through oxidation of the phenyl group to CO_2H, followed by other established stages.[125] Catalytic hydrogenation with H^3H, using Wilkinson's catalyst, showed unusual stereospecificity in leading to a 19:1 mixture of 2*SR*, 3*SR*, 4*RS*-[4-$^3H^2H$]-*N*-acetylvaline and its (3*RS*, 4*SR*)-diastereoisomer, indicating favoured 3-*re*,4-*si* attack on the *S*-component of (2*RS*)-(*E*)-[4-^2H]-2-acetylamino-3-methylbut-3-enoic acid.[126] (2*S*, 3*R*)-Serine-[3-$^2H_1$] and (2*S*, 3*S*)-serine-[2,3-$^2H_2$] have been prepared from the correspondingly labelled aspartic acids by Baeyer–Villiger oxidation of the derived *N*-trifluoroacetyl 2-amino-4-oxopentanoic acids.[127] (2*S*, 3*S*)- and (2*S*, 3*R*)-diastereoisomers, respectively, have been synthesized from (*E*)-$^2HCH{=}C^2HCO_2Me$ and (*Z*)-$^2HCH{=}CHCO_2Et$ *via* bromohydrins, treated with sodium azide followed by Pd-catalysed hydrogenation and hog-kidney acylase I resolution.[128]

L-Tryptophan-[1-^{13}C] has been prepared from DL-serine-[1-^{13}C] and indole using *Escherichia coli* and extended to tryptophan analogues through the use of substituted indoles.[129] [3-^{13}C,3-$^2H_2$]-Tryptophan is available through Mannich condensation of $^2H^{13}C^2HO$ with indole and Me_2NH, followed by condensation with diethyl formamidomalonate and resolution of the derived amino-acid as its *N*-chloroacetyl derivative.[130] Corresponding reactions with substituted indoles and with $^2HC^2HO$ gave other L-tryptophan analogues.[130] ^1H–^2H exchange of tryptophan with ^2H_2O, or hydrolysis of protected tryptophans with NaO^2H–^2H_2O, leads to [2-^2H]-DL-tryptophan.[131] Total synthesis of tryptophan from indole-3-carboxaldehyde by condensation with diethyl acetamidomalonate followed by catalysed addition of $^2H_2$ gave [2,3-$^2H_2$]-DL-tryptophan.[131] A general procedure for the preparation of $\alpha\beta$-deuteriated α-amino-acids giving almost quantitative exchange uses pyridoxal catalysis and reaction times of 2—8 days at 125 °C in ^2H_2O solution.[132]

[125] C. A. Townsend, A. S. Neese, and A. B. Theis, *J. Chem. Soc., Chem. Commun.*, 1982, 116.
[126] D. H. G. Crout, M. Lutstorf, P. J. Morgan, R. M. Adlington, J. E. Baldwin, and M. J. Crimmin, *J. Chem. Soc., Chem. Commun.*, 1981, 1175.
[127] D. Gani and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1982, 867.
[128] L. Slieker and S. J. Benkovic, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 647.
[129] S. S. Yuan and A. M. Ajami, *Tetrahedron*, 1982, **38**, 2051.
[130] W. S. Saari, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 389.
[131] E. Santaniello, M. Ravasi, and F. Astori, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 611.
[132] D. M. Le Master and F. M. Richards, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 639.

Labelled L-methionines described recently are the [methyl-^{13}C] and [3,4-$^{13}C_2$] compound and the [2,3,3-$^{3}H_3$] and [3,3-$^{2}H_2$] analogues of the [methyl-^{13}C] compound.[133] The ^{75}Se analogue of methionine has been synthesized from Me^{75}SeNa and 2-amino-4-bromobutanoic acid.[134]

Several papers describing the synthesis of ^{11}C-labelled amino-acids have appeared in the literature under review, continuing the modest flow of reports on this topic. There is some general interest in, as well as the specific appeal of, this work since the need to achieve good yields in a short time is a consequence of the short half-life of this isotope. L-[^{11}C]Glutamic acid labelled at the carboxy α- or γ-carbon atoms has been prepared by rapid enzyme-catalysed methods;[135] $^{11}CH_3I$ (from $^{11}CO_2$) has been used to prepare L-methionine-[methyl-^{11}C] (within 20 minutes)[136] and thence the *S*-adenosyl derivative by enzyme-catalysed condensation with ATP.[137] ^{11}C-Carboxy-labelled valine, leucine, and aminocyclopentanecarboxylic acid have been prepared from Na^{11}CN by rapid Bucherer–Strecker synthesis.[138]

^{15}N-Labelled amino-acids are readily available from $^{15}NH_4^+$ salts of carboxylic acids using enzymic methods[139] (L-[^{15}N]alanine from pyruvic acid and alanine dehydrogenase[140] and L-[^{15}N]aspartic acid from fumaric acid with immobilized *Escherichia coli B*, this amino-acid being a source of L-[^{15}N]alanine through the agency of immobilized *Pseudomonas dacunhae*[141]).

Alanine labelled with both ^{13}C and ^{15}N has been prepared from Ba$^{13}C_2$ and $^{15}NH_4Cl$ with NaCN through Strecker amination; exchange with $^{2}H_2O$ in the presence of glutamic–pyruvic transaminase and with $^{2}H_2{}^{18}O$ gave 80% and 71.4% of the heavy isotope analogues, respectively.[142]

^{14}C- and ^{3}H-labelled carnitines were prepared by methylation of GABA-$^{14}CO_2H$ with MeI followed by hydroxylation with butyrobetaine hydroxylase from bovine calf liver,[143] demethylation of which (using NaSPh in DMF) followed by methylation with $^{14}CH_3I$ gives the methyl-labelled L-compound.[144]

^{3}H-Labelling methods used for a wide range of amino-acids, catecholamines,

[133] D. C. Billington, B. T. Golding, M. J. Kebbell, I. K. Nassereddin, and I. M. Lockart, *J. Labelled Compd. Radiopharm.*, 1981, **18,** 1773.
[134] H. Yao and C. Dan, *Zhonghua Heyixue Zazhi*, 1982, **2,** 166 (*Chem. Abstr.*, 1982, **97,** 211 697).
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[136] J. Davis, Y. Yano, J. Cahoon, and T. F. Budinger, *Int. J. Appl. Radiat. Isot.*, 1982, **33,** 363.
[137] P. Gueguen, J. L. Morgat, M. Maziere, G. Berger, D. Comar, and M. Maman, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 157.
[138] Y. Ye, R. Hua, Z. Zhou, and Y. Wang, *Nucl. Tech.*, 1981, 44.
[139] Z. E. Kahana and A. Lapidot in 'Stable Isotopes', Anal. Chem., Symp. Ser., Vol. 11, ed. H.-L. Schmidt, H. Förstel, and K. Heinzinger, Elsevier, Amsterdam, 1982, p. 747.
[140] A. Mocanu, G. Niac, A. Ivanhof, V. Gorun, N. Palibroda, E. Vargha, M. Bologa, and O. Barzu, *FEBS Lett.*, 1982, **143,** 153.
[141] Z. E. Kahana and A. Lapidot, *Anal. Biochem.*, 1982, **126,** 389.
[142] S. D. Dimitrijevich, M. D. Scanlon, and M. Anbar, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 573.
[143] D. B. Goodfellow, C. L. Hoppel, and J. S. Turkaly, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 365.
[144] S. T. Ingalls, C. L. Hoppel, and J. S. Turkaly, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 535.

and alkaloids have been described,[145] and ^{13}C-enriched amino-acids have been reviewed.[146]

Resolution of DL-Amino-acids.—The major subdivisions of this topic have been delineated for some time and the recent literature, though increasingly extensive, can be covered efficiently under these headings.

Enzymic methods continue to be used in their classical form [$Ar(CH_2)_nCH(NHAc)CO_2H$ with Taka-acylase,[147,34] α-methyl-tryptophan or -phenylalanine esters with chymotrypsin,[148] *N*-acetyl derivatives of isotopically labelled serines with hog-kidney acylase I,[128] and *N*-phenylacetyl β-(N^1-uracilyl)alanine[149] and related derivatives of the nucleic acid bases[149,150] with penicillin amidase], although the use of immobilized enzymes or intact cells continues to increase. [1-^{14}C]-D-Leucine can be recovered from its DL-form in 83% yield through the action of immobilized L-amino-acid oxidase within 40 minutes, including the time taken for ion-exchange purification.[151] Chymotrypsin in aqueous solution encapsulated in a liquid membrane such as cyclohexane or C_{15} alkanes can effect 70% conversion of DL-phenylalanine methyl ester into L-phenylalanine within 15 minutes, transport through the membrane being mediated by pairing with quaternary ammonium ions.[152] The use of immobilized enzymes or intact cells in the amino-acid area has been reviewed.[153]

Chromatographic methods are being developed successfully into preparative-scale operations, and analytical techniques (covered in Section 6 of this chapter) are also being more widely studied in view of the importance in many areas of determining enantiomer ratios for amino-acid samples. Copper(II) complexation is a feature of several recent papers describing liquid chromatographic methods, using chiral eluents containing the copper(II) complexes of *N*-(toluene-*p*-sulphonyl)-L-phenylalanine and *N*-(toluene-*p*-sulphonyl)-D-phenylglycine over octadecylsilylated silica gel,[154] and similar use of an eluant containing the copper(II) complex of L-aspartyl-L-phenylalanine

[145] J. P. Bloxsidge, J. A. Elvidge, M. Gower, J. R. Jones, E. A. Evans, J. P. Kitcher, and D. C. Warrell, *J. Labelled Compd. Radiopharm.*, 1982, **18,** 1141.

[146] R. E. London in 'NMR Spectroscopy: New Methods and Applications', Am. Chem. Soc., Symp. Ser., No. 191, ed. G. C. Levy, American Chemical Society, Washington, D.C., U.S.A., 1982, p. 119.

[147] N. Kosui, Y. Shimohigashi, M. Waki, T. Kato, and N. Izumiya, *Mem. Fac. Sci., Kyushu Univ., Ser. C*, 1981, **13,** 89 (*Chem. Abstr.*, 1982, **96,** 123 240).

[148] G. M. Anantharamaiah and R. W. Roeske, *Tetrahedron Lett.*, 1982, **23,** 3335.

[149] G. A. Korshunova, Yu. A. Semiletov, O. N. Ryabtseva, and Yu. P. Shvachkin, *Vestn. Mosk. Univ., Khim.*, 1982, **23,** 412 (*Chem. Abstr.*, 1983, **97,** 216 651).

[150] G. A. Korshunova, Yu. A. Semiletov, O. N. Ryabsteva, and Yu. P. Shvachkin, *Vestn. Mosk. Univ., Khim.*, 1982, **23,** 177 (*Chem. Abstr.*, 1983, **97,** 72 728).

[151] G. A. Digenis, R. Goto, J. E. Chaney, and O. Tamemasa, *J. Pharm. Sci.*, 1982, **71,** 818.

[152] T. Scheper, W. Halwachs, and K. Schuegerl, *Chem.-Ing.-Tech.*, 1982, **54,** 696.

[153] I. Chibata in 'Asymmetric Reactions and Processes in Chemistry', Am. Chem. Soc., Symp. Ser., No. 185, ed. E. L. Eliel and S. Otsuka, American Chemical Society, Washington, D.C., U.S.A., 1982, p. 195.

[154] N. Nimura, A. Toyoma, Y. Kasahara, and T. Kinoshita, *J. Chromatogr.*, 1982, **239,** 671.

methyl ester[155] or of *NN*-dimethyl-L-valine or *NN*-di-n-prolyl-L-alanine.[156] Elution with aqueous ammonia effects the resolution of DL-amino-acids over ion-exchange resins carrying imino(methanephosphonic) acid groups complexed to copper(II) through the imino groups.[157] Development of the use of chiral stationary phases continues, silica-bound formyl-L-valinamide being useful for the resolution of *N*-acetyl-DL-amino-acid alkylamides[158] and similar D-phenylglycine modified stationary phases offering convenient gram-scale resolution possibilities.[159] The patent literature[160] contains several recipes for the preparation of chiral supports. The affinity-chromatography approach has been used for the resolution of DL-tryptophan and its analogues over albumin-agarose, the D-enantiomer emerging first from the column.[161] In a newer variation of the chiral support approach, Dnp-amino-acid esters were separated over silica gel coated with the electron donor *P*-(+)-hexahelicene-7,7′-dicarboxylic acid (the L-enantiomer emerges first).[162] Preparative gas-chromatographic separation of volatile amino-acid derivatives over *N*-stearoyl-L-valine t-butylamide has been developed further (see Vol. 14, p. 17),[163] and new knowledge arising from studies of the influence of the structure of the perfluoroacyl group and the ester group on the retention characteristics of enantiomers of derivatized DL-amino-acids[164] and equivalent studies of the effects of the structure of the stearoyl-L-amino-acid t-butylamide on the resolution of *N*-trifluoroacetyl-DL-amino-acid isopropyl esters[165] (see also ref. 323) will assist further development of reliable preparative-scale techniques.

A forty-five page survey of the uses of h.p.l.c. and g.l.c. for resolution of racemates has appeared in a new treatise.[166]

The preferential crystallization route to resolution has been used with DL-amino-acids largely on a trial-and-error basis, but intensive studies of the effects of doping DL-amino-acid solutions with an L- or D-amino-acid of different structure[167—169] have led to greater insight into the process. The

[155] G. Gundlach, E. L. Sattler, and U. Wagenbach, *Fresenius' Z. Anal. Chem.*, 1982, **311,** 684.
[156] S. Weinstein, *Angew. Chem.*, 1982, **94,** 221.
[157] W. Szczepaniak and W. Ciszewska, *Chromatographia*, 1982. **15,** 38 (*Chem. Abstr.*, 1983, **97,** 39 325).
[158] S. Hara, A. Dobashi, and M. Eguchi in 'Asymmetric Reactions and Processes in Chemistry', Am. Chem. Soc., Symp. Ser., No. 185, ed. E. L. Eliel and S. Otsuka, American Chemical Society, Washington, D.C., U.S.A., 1982, p. 266.
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[160] D. W. House, U.S. Pat. 4 324 681 (*Chem. Abstr.*, 1983, **97,** 126 518).
[161] S. Allenmark, B. Bomgren, and H. Boren, *J. Chromatogr.*, 1982, **237,** 473.
[162] Y. H. Kim, A. Balan, A. Tishbee, and E. Gil-Av, *J. Chem. Soc., Chem. Commun.*, 1982, 1336.
[163] M. P. Zabokritskii, B. A. Rudenko, and V. P. Chizhkov, *Dokl. Akad. Nauk SSSR*, 1982, **263,** 1155.
[164] I. Abe, K. Izumi, S. Kuramoto, and S. Musha, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, 1981, **4,** 549.
[165] S. C. Chang, R. Charles, and E. Gil-Av, *J. Chromatogr.*, 1982, **235,** 87.
[166] W. F. Linder in 'Chemical Derivatization in Analytical Chemistry', ed. R. W. Frei and J. F. Lawrence, Plenum Press, New York, 1982, Vol. 2, p. 145.
[167] L. Addadi, Z. Berkovitch-Yellin, N. Domb, E. Gati, M. Lahav, and L. Leiserowitz, *Nature (London)*, 1982, **296,** 21.
[168] L. Addadi, S. Weinstein, E. Gati, I. Weissbuch, and M. Lahav, *J. Am. Chem. Soc.*, 1982, **104,** 4610.
[169] L. Addadi, Z. Berkovitch-Yellin, I. Weissbuch, M. Lahav, L. Leiserowitz, and S. Weinstein, *J. Am. Chem. Soc.*, 1982, **104,** 2075.

dopant binds stereoselectively at the surface of the crystal of the enantiomer of the same configuration and causes gross physical differences in the crystal habit of this enantiomer compared with the appearance of crystals of the other enantiomer and differences in rate of growth of the two crystal types. The implication that absolute configuration can be assigned to enantiomers on this basis was also followed through and shown to be reliable.[168,169] Effects of degree of supersaturation and its control through the addition of acids or bases to the mother-liquor have been studied, leading to crops of crystals of better purity.[170] Details of a particular application of the preferential crystallization phenomenon to the resolution of DL-alanine as its toluene-*p*-sulphonate given in the abstract of a recent paper[171] are sufficiently complete to be followed without the need to resort to the original paper (which occupies seven pages). Either enantiomer may be obtained in 99.9% optical purity after recrystallization.

An example of resolution by crystallization of a diastereoisomeric derivative is included in the synthesis of β-carboxyaspartic acid,[83] the derivative being [(diethyl β-carboxyaspartato)tetramminecobalt(III)] perchlorate.

Further reports from Yamada's group describing the asymmetric transformation of *N*-acyl-DL-amino-acids,[172] combining preferential crystallization of one enantiomer with racemization by traces of acetic anhydride of the other enantiomer, include details of optimized procedures. Optical purities approaching 70% can be expected using an *N*-acyl group that is appropriate for a particular amino-acid.

The remaining topic represented in this section in previous volumes, enantioselective radiolysis of DL-amino-acids, receives a new stimulus with the report that a spin-polarized low-energy positron beam shows some discrimination for degradation of the enantiomers of DL-leucine.[173] It is predicted[173] that enhanced discrimination will be seen in β-irradiative degradation of DL-amino-acids containing heavier elements.

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—The continuing theme of this section involves announcements of new *X*-ray and neutron-diffraction analyses and papers discussing the implications of previously gathered data.

DL-β-Carboxyaspartic acid adopts the dimeric packing mode in the solid state, with unusually strong hydrogen bonding.[174] (−)-Canavanine adopts the zwitterionic form with protonation on the α-amino group rather than on the

[170] S. Asai and S. Ikegami, *Ind. Eng. Chem. Fundam.*, 1982, **21**, 181.
[171] V. Feldnere, A. Vegnere, J. Vitals, and R. Udre, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1982, 310 (*Chem. Abstr.*, 1983, **97**, 110 364).
[172] C. Hongo, S. Yamada, and I. Chibata, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3286, 3291.
[173] D. W. Gidley, A. Rich, J. Van House, and P. W. Zitzewitz, *Nature (London)*, 1982, **297** 639; R. A. Hegstrom, *ibid.*, p. 643.
[174] B. Richey, M. R. Christy, R. C. Haltiwanger, T. H. Koch, and S. J. Gill, *Biochemistry*, 1982, **21**, 4819.

guanidino group;[175] the general behaviour of arginine salts as far as the specific interactions occurring between the guanidino group and the anions are concerned has been surveyed based on published crystal structures.[176]

S-Adenosyl-L-homocysteine[177] and blasticidin S hydrochloride pentahydrate[178] (a cytosine aminonucleoside acylated on the amino group by L-arginine) have received intensive conformational study by *X*-ray[177,178] and other physical and theoretical analytical methods.[177] 3-*N*-Oxalyl-L-2,3-diaminopropanoic acid (an example of a compound exhibiting crystal dimorphism) has been studied by i.r. spectrometry and potentiometric titration as well as by *X*-ray crystal analysis.[179]

Neutron-diffraction data (32 crystal structures) for amino-acids have been studied from the point of view of hydrogen-bond geometries displayed.[180] Of the 168 hydrogen bonds detected, 64 involved zwitterion groups $\overset{+}{N}H_3$ and CO_2^- and 18 were from $\overset{+}{N}H_3$ to sulphate or carbonyl groups, the majority (46) of these —N—H · · · O bonds being three-centred (bifurcated) and 9 being four-centred (trifurcated).

Statistical analysis of the disposition of side chains seen in *X*-ray structures of amino-acids has been used to derive limiting vicinal coupling constants for the staggered conformations.[181]

Nuclear Magnetic Resonance Spectrometry.—Major themes (conformational analysis,[177] acid–base characteristics) represented over the years in ^1H and ^{13}C n.m.r. studies of amino-acids are being supplemented increasingly by novel variations of the n.m.r. technique. Pioneering and specifically designed studies based on nuclei of higher atomic weight are also applied in the amino-acid area.

High salt concentrations in alkaline solutions of aspartic acid stabilize the two conformations involving *gauche* carboxy groups at the expense of the *anti* conformation.[182] Limiting component vicinal coupling constants for side-chain staggered rotamers of amino-acids have been calculated from statistical analysis of *X*-ray structures.[181]

Double-resonance ^1H n.m.r. has been used to determine solvent exchange rates catalysed by acids or bases of *E*- and *Z*-protons of *N*-acetylasparagine- and -glutamine-*N*-methylamides in water.[183] Relationships between the acidity

[175] A. Boyar and R. E. Marsh, *J. Am. Chem. Soc.*, 1982, **104,** 1995.
[176] D. M. Salunke and M. Vijayan, *Int. J. Pept. Protein Res.*, 1981, **18,** 348.
[177] T. Ishida, A. Tanaka, M. Inoue, T. Fujiwara, and K. Tomita, *J. Am. Chem. Soc.*, 1982, **104,** 7239.
[178] V. Swaminathan, J. L. Smith, M. Sundaralingam, C. Coutsogeorgopoulos, and G. Kartha, *Biochim. Biophys. Acta*, 1981, **655,** 335.
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of *Z*- and *E*-isomers of *N*-nitroso-*N*-alkyl-α-amino-acids and their conformations have been established by measurement of ^{1}H n.m.r. spectra of solutions at varying pH values. *E*-Isomers are in many cases unable to adopt intramolecular hydrogen bonds between nitroso and carboxy groups, and they show stronger acid character.[184]

Proton spin–lattice relaxation for α-aminoisobutyric acid from 120 to 450 K reveals reorientation of amino and methyl groups, for which activation energies were estimated.[185]

A novel heteronuclear spin-echo method allowing assignment of features in a ^{1}H n.m.r. spectrum to both ^{12}C and ^{13}C species opens up interesting possibilities in mechanistic studies, illustrated in following the exchange of ^{13}C between alanine and pyruvic acid catalysed by alanine transferase.[186]

Interactions of the phenyl moiety of L-phenylalanine with 5′-AMP and poly(A) have been investigated by ^{2}H n.m.r., using the $^{2}H_5$-labelled amino-acid.[187]

Reassignment of indole C-5 and C-6 ^{13}C n.m.r. resonances suggested by Gribble and co-workers[188] has been confirmed through consideration of ^{13}C–^{13}C coupling constants observed for tryptophan-[3-$^{13}C_1$].[189] Broad-band proton-decoupled ^{13}C n.m.r. spectra of side-chain derivatives of lysine, serine, histidine, and cysteine were fully assigned to assist n.m.r. studies of crucial regions of enzymes.[190] *cis–trans* isomerism of *N*-acetyl *syn*- and *anti*-5-methylproline methylamide studied by ^{13}C n.m.r. reveals the influence of the steric effect of the 5-methyl group in destabilizing the *trans*-amide isomer but without altering the isomerization barrier.[191]

Many examples of uses of ^{13}C n.m.r. in biosynthetic studies employing ^{13}C-labelled substrates have been described in recent years, and an excellent example of complexities revealed in this way, supported by field-desorption mass spectrometry, is described for $^{13}CO_2$-feeding studies with *Spirulena maxima* and *Synechoccus cedrorum*.[192]

^{14}N nuclear quadrupole resonance data for fourteen *N*-acetylamino-acids show a positive correlation between electron density at N and the Taft inductive parameter σ*.[193] ^{15}N solid-state n.m.r. of histidine permits assignments to be made for isotropic and anisotropic chemical shifts for the imidazole-ring nitrogen atoms in various ionic forms,[194] giving a more precise

[184] B. Liberek, J. Clarkowski, K. Plucinska, and K. Stachowiak, *Org. Magn. Reson.*, 1982, **18,** 143.
[185] S. Idziak, J. Stankowski, and S. Guszczynski, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1980, **28,** 691.
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[191] N. G. Delaney and V. Madison, *Int. J. Pept. Protein Res.*, 1982, **19,** 543.
[192] E. Bengsch, J.-P. Grivet, and H.-R. Schulten in 'Stable Isotopes', Anal. Chem., Symp. Ser., Vol. 11, ed. H.-L. Schmidt, H. Förstel, and K. Heinzinger, Elsevier, Amsterdam, 1982, p. 587.
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[194] M. Munowitz, W. W. Bachovchin, J. Herzfeld, C. M. Dobson, and R. G. Griffin, *J. Am. Chem. Soc.*, 1982, **104,** 1192.

description of tautomeric structure than can be obtained from ^{15}N n.m.r. data on solutions. Spin–lattice relaxation times T_1 and n.O.e. data for alanine, glutamic acid, and arginine in intracellular fluid can be related to the microviscosities of various environments and intermolecular associations between solutes in those environments.[195]

^{17}O n.m.r. of ^{17}O-enriched glycine, glutamic acid, and aspartic acid was particularly studied[196] as part of a study of a range of protein amino-acids.[196,197] High fields are needed to resolve resonances arising from the two carboxy groups of the amino-diacids, but the technique is capable of demonstrating, by concentration-dependence studies, that intramolecular association between the amino group and a side-chain carboxy group does not occur in these compounds.[196]

^{19}F n.m.r. of (+)- or (−)-perfluoro-2-propoxypropionyl amino-acids is a new variation of an established method for determination of enantiomer ratios.[198] Configurational assignments can be made to 2-amino-3-fluoroalkanoate esters by ^{19}F n.m.r., using the effect of complexation of the amino group by 18-crown-6.[199]

Optical Rotatory Dispersion and Circular Dichroism.—Established areas of interest in these techniques are represented in interpretation of c.d. data for Dnp derivatives of α-amino-acids and their β-methyl analogues,[200] where differences in c.d. spectra are associated with effects of the methyl substituent on rotamer populations, and for Dnp derivatives of β- and higher homologous amino-acid arylamides,[201] where characteristic exciton coupling between the aromatic groupings is shown only in those compounds where the amino group is attached to the chiral centre. *N*-Acetylamino-acid methylamides have been studied by c.d. spectrometry from the point of view of interactions between the amide groupings and an aromatic moiety in the side chain.[202] There were indications in the solvent and temperature dependence of c.d. spectra that changes in conformer populations could be discerned by this method.[202]

Novel chromophoric derivatives are represented in complexation of methionine or *S*-ethylcysteine with sodium tetrachloropalladate,[203] whose c.d. spectra are sufficiently responsive to enantiomeric purity to offer a sensitive racemization test for reactions involving these amino-acids. Familiar cobalt(II) and copper(II) complexes of threonine and isoleucine and their epimers have been studied from the point of view of stability in aqueous $NaNO_3$ (*allo* forms are less stable than the natural epimers), and c.d. spectra have been interpreted for the complexes.[204]

[195] K. Kanamoi, T. L. Legerton, R. L. Weiss, and J. D. Roberts, *Biochemistry*, 1982, **21**, 4916.
[196] I. P. Gerothanassis, R. Hunston, and J. Lauterwein, *Helv. Chim. Acta*, 1982, **65**, 1764, 1774.
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[201] M. Kawai and U. Nagai, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 1327.
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[203] T. H. Lam, S. Fermandjian, and P. Fromageot, *J. Chim. Phys. Phys.-Chim. Biol.*, 1982, **79**, 101.
[204] N. Ivivic and V. Simeon, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2581.

Further results from vibrational c.d. studies of amino-acids deal with deuteriated alanines.[205] Insight into the fundamental C—H stretching modes of alanine and the vibrational coupling between the Me—CH stretching modes has been gained by this study. The magnitude and direction of the displacements of the nuclei can be determined by this technique.

Mass Spectrometry.—Field-desorption mass spectra of 19 protected amino-acids have been reported.[206] In nearly all cases the molecular ion is the base peak. The f.d. technique is becoming more widely used and developed in new ways; voltages of around 1 kV applied to the emitter in liquid-ionization mass spectrometry of β-alanine accelerate the desorption of ions but not neutral molecules, and the base peak in this case is due to $M+H^+$ with the protonated dimer $2M+H^+$ particularly abundant.[207]

Secondary-ion mass spectra of phenylalanine have been compared with mass spectra obtained by the three more familiar ionization methods (electron impact, chemical ionization, and field desorption).[208] Bombardment by the primary-ion beam of a solid target (the amino-acid, or its hydrochloride deposited on graphite) produces the secondary ions with intensities determined by both the intensity and nature of the primary-ion beam, xenon being the most suitable.[208]

Laser-microprobe mass spectra of glycine and phenylalanine include quasi-molecular ions $M-1^-$ and $M+1^+$ as well as the decarboxylated molecular ion $M-45^+$.[209]

A more routine study (analytical applications in which the technique is used to support synthesis and structure determination are not included) deals with phenylalanine esters,[210] for which dioxopiperazine formation is an inevitable accompanying process. Trifluoroacetylation prior to mass-spectrometric study suppresses dioxopiperazine formation and increases the possibility of molecular-ion formation.[210]

Other Physical Studies.—The wide range of potentiometric, thermodynamic, and spectrometric studies featured in this section in previous volumes continues to be applied to amino-acids. Some of this seems routine but nevertheless valuable in many ways and occasionally necessary to correct previous erroneous information. For example,[175] pK values for canavanine and canaline have been correctly calculated from reconsideration of titration curves

[205] B. B. Lal, M. Diem, P. L. Polavarapu, M. Oboodi, T. B. Freedman, and L. A. Nafie, *J. Am. Chem. Soc.*, 1982, **104,** 3336; T. B. Freedman, M. Diem, P. D. Polavarapu, and L. A. Nafie, *ibid.*, p. 3343.

[206] D. F. Fraley, M. M. Bursey, D. H. Craig, and B. G. Goldsmith, *Eur. J. Mass Spectrom. Biochem., Med. Environ. Res.*, 1982, **2,** 13.

[207] M. Tsuchiya, T. Nonaka, T. Taira, and S. Tanaka, *Shitsuryo Bunseki*, 1982, **30,** 95 (*Chem. Abstr.*, 1982, **97,** 72 756).

[208] K. D. Klöppel in 'Recent Developments in Mass Spectrometry in Biochemistry, Medicine and Environmental Research', Anal. Chem., Symp. Ser., Vol. 7, ed. A. Frigerio, Elsevier, Amsterdam, 1981, p. 283.

[209] C. Schiller, K.-D. Kupka, and F. Hillenkamp in 'Recent Developments in Mass Spectrometry in Biochemistry, Medicine and Environmental Research', Anal. Chem., Symp. Ser., Vol. 7, ed. A. Frigerio, Elsevier, Amsterdam, 1981, p. 287.

[210] J. Das, *Indian J. Chem., Sect. B*, 1982, **21,** 71.

reported in 1935. Several other pK studies have been reported including DL-tryptophan in water-containing organic solvents[211] and a series of amino-acids in aqueous 2-propanol.[212] β-Carboxy-DL-aspartic acid shows pK_a 0.8, 2.5, 4.7, and 10.9 in water.[174] Revised pK_a values are reported for 3-*N*-oxalyl-L-2,3-diaminopropanoic acid.[179]

Conductimetric titrations of equimolar amounts of L-glutamic acid and L-histidine indicate greater interaction than between L-glutamic acid and D-histidine.[213] No specific interactions were found between L-glutamic acid and the other common basic amino-acids.[213]

Heats of stepwise dissociation of L-aspartic acid were calculated from calorimetric data determined at 15, 25, and 35 °C,[214] and similar studies have been undertaken for L-lysine monohydrochloride[215] and tyrosine derivatives.[216]

Osmotic coefficients of *N*-acetyl derivatives of glycinamide, alaninamide, and leucinamide and calorimetric data for these compounds in equimolal admixture with peptide homologues yield pairwise free-energy and enthalpy parameters.[217] Enthalpy-of-interaction coefficients have been measured calorimetrically for alanine, 2-aminobutanoic acid, norvaline, and norleucine with NaCl in aqueous solutions at 298.15 K.[218] Enthalpies of solution of a series of amino-acid hydrobromides in water–DMF mixtures at 298.15 K, measured calorimetrically, yield better measures of the hydrophobicity of the side chains than those based on transfer properties of amino-acids between immiscible phases.[219] An example of the latter approach has been described[220] for the partition of Dnp-amino-acids in buffered aqueous two-phase polymeric systems (ficoll and dextran). The penetration of leucine and norleucine from aqueous solutions into dimyristoylphosphatidylcholine monolayers has been followed by surface pressure measurements.[221] Ultrasound interferometry provides values of compressibility and solvation numbers of amino-acids in aqueous ethanol.[222] Zwitterion content of glycine in various aqueous media has been measured,[223] and the difference in average volumes of protein amino-acids (181 Å^2) compared with non-protein amino-acids (112 Å^2), in specific volumes (1.28 and 1.1 Å^3 dalton^{-1}, respectively), and in spectral-energy density (15 878 and 13 201 kcal g^{-1}, respectively) has been linked with a hypothesis accounting for the selection of amino-acids for incorporation into proteins.[224]

[211] R. S. Saxena and G. L. Sharma, *J. Indian Chem. Soc.*, 1982, **59,** 413.
[212] B. P. Dey, S. Dutta, and S. C. Lahiri, *Indian J. Chem., Sect. A*, 1982, **21,** 886.
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[222] A. K. Chattopadhyay and S. C. Lahiri, *Electrochim. Acta*, 1982, **27,** 269.
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[224] V. A. Konyshev, *Vapr. Pitan.*, 1982, 24 (*Chem. Abstr.*, 1983, **98,** 1914).

Spectrometric techniques not covered in preceding sections include microwave spectrometry (glycine methyl ester carries an internal bifurcated hydrogen bond),[225] near-i.r. differential spectrophotometry (hydration numbers of amino-acids[226]), Raman spectrometry (L-histidine and its derivatives and imidazole analogues show a strong band at 1410 cm^{-1} in ^2H_2O characteristic of the *N*-deuteriated imidazolium ring[227]), and e.n.d.o.r.–e.s.r. spectrometry (*X*-irradiated crystals of L-alanine show evidence of movements of atoms from positions in the undamaged crystals[228] and stable-radical formation as a result of C—N cleavage,[229] while L-cysteine hydrochloride monohydrate undergoes decarboxylation through the same treatment[230]).

Electron diffraction indicates the extended conformation of glycine methyl ester to be the most stable.[231]

Molecular-orbital Calculations.—The hydration geometry of the glycine–water system and intermolecular interactions involved in two different models have been considered.[232] Other molecular-orbital calculations performed on glycine itself include gas-phase protonation by five potential protonating species (H^+, HeH^+, H_3^+, H, and H_2; the first of these is most strongly bound at any of the various points of attack)[233] and ordering of glycine orbitals accompanying conversion from the uncharged to the zwitterionic tautomer in the solid state.[234] A variety of physical parameters has been calculated for the aliphatic protein amino-acids by Boyd's force-field method,[235] and theoretical assessment of relationships between amino-acid structure and propensity towards the formation of supersaturated solutions has been reported.[236] Two possible solutions have been suggested for the mechanism by which one enantiomer is selected from a racemate to account for enantioselective metabolic processes.[237]

Conformational analysis of *N*-acetylglycine *N*-methylamide indicates the intramolecularly hydrogen-bonded form, involving the 7-membered ring structure rather than the 5-membered analogue, to be the most stable form.[238] A similar study for the corresponding derivatives of twenty natural amino-acids confirms both theoretical and experimental results from earlier investigations.[239]

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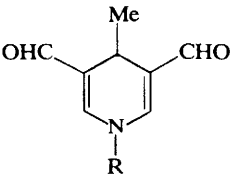
5 Chemical Studies of Amino-acids

Racemization.—As in previous volumes, the topic covers both mechanistic aspects and applications of D:L-ratios in fossil-dating.

Reviews have appeared covering results obtained from determinations of amino-acid D:L-ratios in dating relatively young fossils,[240] including racemization of residues in proteins,[241] in dating Quaternary molluscs, and in controversy associated with the technique.[242] A direct correlation between the ages assigned to samples from a 1800 year old yakusugi tree through measurement of their D:L-aspartic acid ratio and the ages assigned from tree-ring counting indicates close agreement, based on reasonable assumptions of average temperatures experienced by the tree.[243]

Mechanistic studies include OH^--catalysed epimerization of cobalt(III) complexes comprising amino-acids (aspartic acid, asparagine, or glutamic acid) and a chiral 3,7-diazanonane-1,9-diamine as ligands, in which the L-amino-acidato enantiomer is favoured,[244] and racemization rate constants of fifteen amino-acids under the standard protein-hydrolysis regime (110 °C, 6M HCl, in evacuated vacuum-sealed tubes),[245] variations in rate being related to electron-withdrawing character of side chains and to steric hindrance in the neighbourhood of the α-hydrogen atom.[245]

General Reactions.—Reactions at the amino group described in recent papers include important observations concerning reactions of lipid peroxides[246] or linoleic acid hydroperoxides[247] with amino-acids under physiological conditions. In the first of these studies, malondialdehyde, known to be a secondary product of lipid peroxidation, was shown to give fluorescent dihydropyridines (24) with a series of common amino-acids, though with cysteine reacting to



(24)

produce a different type of fluorescent compound.[246] The second study develops the hypothesis that fluorescent compounds formed from the hydroperoxides and glycine, lysine, arginine, histidine, and phenylalanine (but not

[240] S. Matsu'ura and N. Ueta, *Seikagaku*, 1982, **54**, 451.
[241] N. Fujii and K. Harada, *Kagaku No Ryoki*, 1982, **36**, 606.
[242] J. F. Wehmiller, *Quat. Sci. Rev.*, 1982, **1**, 83.
[243] I. Abe, K. Izumi, S. Kuramoto, and S. Musha, *Bunseki Kagaku*, 1982, **31**, 427 (*Chem. Abstr.*, 1982, **97**, 126 438).
[244] M. Yamaguchi, Y. Masui, M. Saburi, and S. Yoshikawa, *Inorg. Chem.*, 1982, **21**, 4138.
[245] R. Liardon and R. Jost, *Int. J. Pept. Protein Res.*, 1981, **18**, 500.
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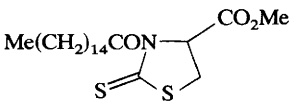
[247] H. Shimasaki, N. Ueta, and O. S. Privett, *Lipids*, 1982, **17**, 878.

from several other common amino-acids) may be an important pointer to irreversible reactions of proteins associated with the ageing process.[247] Familiar reactions which have been conducted with new or modified procedures include trifluoroacetylation of amino-acid esters with *N*-(trifluoroacetyl)nylon 6.6,[248] Schiff-base formation of amino-acids by transamination with benzophenone imine,[249] and preparation of alkanethiosulphenylcarbonylprolines.[250] Further studies of the condensation of amino-acids with formaldehyde[251] and glutaraldehyde[252] as a function of pH have been described (the ε-amino group of lysine is considerably more reactive than the α-amino group),[251] and more routine work is represented in kinetics of *N*-cyanoethylation,[253] *N*-(t-butoxycarbonyl)ation of tyrosines and 5-hydroxytryptophan (accompanied by *O*-t-butoxycarbonylation in all cases except 3-nitrotyrosine),[254] participation in the Mannich reaction,[255] and substitution of the amino-group by F using excess $NaNO_2$ in HF–pyridine.[256] The last-mentioned reaction leads to products of 1,2-shift at the phenyl moiety and corresponding insertion of the fluorine atom at C-3 when phenylalanines are involved (see also Vol. 13, p. 20), and the ratio of direct substitution products to rearrangement products was shown to be controlled by the concentration of HF in the reaction mixture.[256]

N-Acylation of amino-acid esters by the *N*-acylthiazolidinethione (25) yields an excess of the (*R*)-(−) form in the unreacted reagent when the amino-acid has the (*R*)-configuration, offering a novel Horeau-type procedure for the assignment of absolute configuration to chiral amines.[257]



(25)

N-Methylation of αβ-unsaturated α-amino-acids as their esters or dioxopiperazine derivatives can be effected with methyl iodide in the presence of sodium hydride.[258]

Reactions of amino-acids at the carboxy group featured in the recent literature include esterification of *Z*- or Boc derivatives with an alcohol using ethyl dimethylaminocarbodi-imide and 4-(dimethylamino)pyridine[259] and

[248] H. W. Tesch and R. C. Shulz, *Makromol. Chem.*, 1981, **182,** 2981.
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[254] V. P. Pozdnev, *Khim. Prir. Soedin.*, 1982, 129.
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effects of cycloamylose[260] and 2,2′-bipyridylpalladium(II)[261] in accelerating the hydrolysis of α-amino-acid esters. The conversion of amino-acid *p*-nitrophenyl esters into poly(amino-acid)s in aqueous solutions, catalysed by HCO_3^-, proceeds *via* Leuchs anhydrides (oxazolidine-2,5-diones).[262]

Reactions of amino-acids involving both amino and carboxy groups are covered in reviews of α-amino-acids in heterocyclic synthesis[263] and their use in asymmetric synthesis.[264] A mixture of eighteen common protein amino-acids refluxed during six weeks in an aqueous solution containing salts which might have been present in the oceans in prebiotic times is partly converted into mixtures of soluble polypeptides.[265]

Specific Reactions of Natural Amino-acids and Their Derivatives.—This section mostly covers reactions associated with the amino-acid side chains, but also includes work involving specific amino-acids that may be of more general character.

Thermal degradation of DL-glutamic acid gives 3,5,8,10-tetraketoperhydropyrrolo[*a*, *d*]pyrazine *via* pyroglutamic acid.[266] A convenient preparation of *N*-acylpyroglutamic acids[267] using conventional acyl chloride–triethylamine reagent systems and pyroglutamic acid as substrate involves mixed-anhydride formation followed by intramolecular *N*-acylation. γ-Esterification of aspartic and glutamic acids specifically involving the side-chain carboxy group can be accomplished without racemization *via* bis(amino-acidato)copper(II) complexes by benzylic halides.[268] Aspartyl *m*-chlorobenzoyl peroxide rearranges through a radical cage process with migration of the chiral centre, since the hydrogen atoms at the β-carbon atom were shown by labelling studies to undergo racemization.[269] Saponification of Boc-asparagine esters is accompanied by β-amide formation *via* the corresponding succinimide.[270] Decarboxylation kinetics of the new protein amino-acid β-carboxyaspartic acid[271] in 1M HCl and at pH 9.8 in 2M KOH have been described.[272] Carboxymethylenemalonic acid $HO_2CCH{=}C(CO_2H)_2$ is formed in substantial yield under the alkaline hydrolysis conditions, indicating that an alkaline hydrolysis procedure is not necessarily an unambiguous method by which the presence of this amino-acid in proteins can be demonstrated.[272]

Other aliphatic amino-acid chemistry reported recently continues familiar

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[269] S. J. Field and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1982, 591.
[270] I. Schon, *Acta Chim. Acad. Sci. Hung.*, 1982, **109**, 219.
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[272] M. R. Christy and T. H. Koch, *J. Am. Chem. Soc.*, 1982, **104**, 1771.

themes in assigning structures 1-ethyl-3,4-dehydropyrrolidine and 1-ethylpyrrole-2-aldehyde to the major reaction products from threonine and D-xylose heated in water for one hour at 150—160 °C,[273] while glycine and D-xylose give three blue pigments through reaction in slightly alkaline solutions.[274] Further results on oxidative processes undergone by aliphatic amino-acids include analytical possibilities for vanadyl compounds in 5M H_2SO_4 (1 mol proline reduces 4 mol VO_2^+ to give γ-aminobutyric acid and CO_2, but other aliphatic amino-acids do not react)[275] and the useful observation that chromium(III) oxide–pyridine gives reasonable yields of *N*-benzyloxycarbonyl oxamates $ZNH{\cdot}CO{\cdot}CO_2R$ from corresponding serine and threonine esters.[276] Unexpected ^3H loss through Chloramine-T degradation of hydroxyproline produced through metabolism of collagen containing [5-$^3H_1$-^{14}C]proline calls for a revised $^3H/^{14}C$ correction factor (1.68) when quantitative analysis of hydroxyproline is based on specific radioactivity data.[277]

^2H-Labelling studies indicate there to be no exchange of cyclopropane hydrogen atoms during the biogenesis of ethylene from 1-aminocyclopropane carboxylic acid.[278] Stereospecific conversion of 1-amino-2-ethylcyclopropanecarboxylic acid into 1-butene was established by plant-tissue studies using all four stereoisomers of the amino-acid.[279]

A practical separation of L-leucine from L-isoleucine based on the more rapid reaction of leucine with thionyl chloride in ethanol at 60 °C for one hour is accomplished by separating the resulting mixture of leucine ethyl ester hydrochloride from isoleucine hydrochloride.[280]

Weber continues the study of *N*-acetylcysteine (see Vol. 14, p. 24) for easily accomplished reactions that might have some significance in molecular evolution by showing that reaction with pyruvaldehyde at pH 7 in aqueous media containing imidazole gives good yields of *N*-acetyl-*S*-lactoylcysteine.[281] Other reactions involving sulphur-containing side chains include comparison of routes to *N*-trityl-L-homoserine (either from homoserine treated successively with Me_2SiCl_2 or Ph_2SiCl_2 and tritylation or from L-methionine by tritylation followed by methylation and displacement of the sulphonium grouping in OH^-)[282] and base-catalysed exchange behaviour of dehydromethionine.[283]

Aromatic and heteroaromatic side-chain reactions include a remarkably simple preparation of the phenylalanine–$Cr(CO)_3$ complexes through reaction

[273] T. Hara, E. Kubota, and H. Horita, *Chagyo Gijitsu Kenkyu*, 1982, 55 (*Chem. Abstr.*, 1983, **98**, 72 702).
[274] M. Miura and T. Gomyo, *Nippon Nogei Kagaku Kaishi*, 1982, **56**, 417 (*Chem. Abstr.*, 1983, **97**, 182 815).
[275] S. Klein, M. J. Waechter, and M. Hamon, *Analusis*, 1982, **10**, 120.
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with $Cr(CO)_6$ in aqueous THF[284] and an important study of hydroxylation of phenylalanine by H_2O_2 at room temperature in the presence of iron–porphyrin complexes carrying pyridinium groups.[285] High stereoselectivity is observed[286] in the deacylation of *N*-acylphenylalanine *p*-nitrophenyl esters by bilayer vesicular systems containing *Z*-L-leucyl-L-histidine and a quaternary ammonium salt.[287] (−)-Homophenylalanine gives Boc-D-glutamic acid through $RuCl_3$–$NaIO_4$ degradation of its Boc derivative, thus establishing its absolute configuration.[288] Oxidation of tyrosine by ozone gives dopa and its oxidation products as well as *OO'*-dityrosine,[289] the first of these sequences (the conversion of dopa into dopachrome, the first stage of the melanin-forming process) having received detailed kinetic study leading to a revision of the Raper–Mason scheme for this multi-step process.[290] Dimerization through the indole α-position occurs when *N*-acetyltryptophan methyl ester is kept in trifluoroacetic acid solution during three hours at room temperature.[291] With N^{im}-formyltryptophan in liquid HF, cleavage of the protecting group is complete at 0 °C when ethane-1,2-dithiol is present.[292] Another aspect of reactivity of tryptophan derivatives studied in the chemical laboratory is catalytic transfer hydrogenation using HCO_2H–Pd (2,3-dihydrotryptophans are found as side-products),[293] while plant biochemical studies are represented in a study of L-tryptophan catabolism *via* kynurenic acid to 5-(2-carboxyethyl)-4,6-dihydroxypicolinic acid in papaverine-degrading *Nocardia* species.[294]

Further studies (see Vol. 14, p. 25) of π-benzyloxymethylhistidines as protected derivatives for racemization-free coupling to the carboxy group of this amino-acid demonstrate the security in this approach.[295] Exchange of the histidine imidazole C-2 hydrogen atom, as far as the role of metal catalysis is concerned, has been clarified and compared with the comparable process in nucleic acid bases.[296] Alternative routes to Boc-(N^{im}-trityl)histidine have been compared.[297]

Straightforward reactions applied to a variety of amino-acids, and differences accounted for, represent a useful area of study in support of peptide synthesis. A much faster reaction between proline and 1-fluoro-2,4-dinitrobenzene than seen with glycine in 30% aqueous DMSO is accounted for by NaOH catalysis applying only to the proline reaction in the complex-forming pre-equilibrium stage common to both reactions.[298]

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Non-enzymic Models of Biochemical Processes Involving Amino-acids.—This section, occasionally included in this chapter in previous volumes, usually covers observations of complexation phenomena of potential relevance to metabolic and other biochemical processes. Examples this year are the interaction seen between D-cycloserine and DNA from *E. coli* mutants,[299] pyridoxal phosphate–copper(II)-catalysed elimination of tryptophans [and its acceleration by poly(4-vinyl-*N*-dodecylpyridinium salts)],[300] and complexation by inclusion through the side chain of L-phenylalanine (but not L-tyrosine) into cyclohexaamylose.[301]

Effects of Electromagnetic Radiation on Amino-acids.—Photo-oxidation studies of tryptophan and its derivatives continue unabated, irradiation by u.v. or visible light leading to kynurenines[302–304] involving superoxide intermediates.[302] A comparison of dye-sensitized[303,304] with chemically generated singlet oxygen[304] reactions is included in this group of papers. Further results from the study of γ-irradiation of aqueous solutions of tyrosine and the correlation of the characteristic blue fluorescence with the formation of dityrosine have been reported.[305] Cleavage of the pyridinium ring of pyridinoline (7) as a result of u.v. photolysis, giving hydroxylysine, has been studied for its pH dependence.[306] A variation on experimental conditions generally used in this type of study has been applied to u.v. irradiation of lysine, producing glycine, alanine, threonine, and seven other ninhydrin-positive products, with degradation under nitrogen proceeding faster than under hydrogen.[307] Irradiation of tyrosine at 240—370 nm gives intensely absorbing initial products ($\lambda\lambda_{max}$ 260, 270 nm).[308] Effects of $HClO_4$ and HCO_2H on the radiolytic oxidation of cysteine solutions have been evaluated.[309]

Radical formation in photoionization of phenylalanine, tyrosine, or tryptophan in aqueous solution,[310] in photolysis at 340—380 nm or 280—320 nm of tryptophan and thymine (producing a thymine free radical through dissociation of the excited state of the tryptophan–thymine charge-transfer complex, or of products of reaction of solvated electrons with thymine, respectively),[311] and in radiolysis of *N*-acetylamino-acids in the solid state (yielding CO_2 and products of N–C^{α} cleavage)[312] has been studied by e.s.r.

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Tryptophan fluorescence[313–315] and phosphorescence[316] have been studied by the use of a new instrument capable of sub-nanosecond resolution[313] or by conventional methods particularly aimed at evaluating restricted molecular motion in cell membranes.[314,315]

Continuing studies of chiral discrimination in irradiation of D- or L-amino-acids generally reproduce the ambiguous conclusions arising from 0—11 MeV longitudinally polarized proton irradiation of solid L-leucine (1.1—1.7% racemization of D- or L-leucine accompanies 39—55% degradation)[317] and ^{90}Y β-irradiation of D- or L-alanine (greater relative radical formation in the D-enantiomer)[318] or β-irradiation of leucine (slight discrimination) and cysteine and tryptophan (no discrimination).[173]

6 Analytical Methods

Gas–Liquid Chromatography.—Clear advantages inherent in the technique of g.l.c. compared with some other separation methods outweigh the apparent drawbacks in the need to convert the amino-acid mixture into volatile derivatives. Procedures are well established for the preparation of *N*-trifluoroacetylamino-acid n-butyl esters,[319,320] s-butyl esters,[321] isobutyl esters,[322] isopropyl esters,[323] butylamides,[323] *N*-heptafluorobutyrylamino-acid isobutyl esters,[324] *N*-ethoxycarbonylamino-acid methyl esters,[325] oxazolidinones,[326] and (+)-3-methyl-2-butyl esters of *N*-methylamino-acids and their *N*-trimethylsilyl derivatives.[327] Objectives of these studies, often in combination with mass-spectrometric detection,[319–321] include the estimation of ^{15}N-abundance data,[319] determination of side-chain alkylated tyrosines and lysines,[320] separation of all isomers of five-carbon β-, γ-, and δ-aminoalkanoic acids,[321] determination of the imino-acids strombine and alanopine at ≥ 0.05 μg levels,[322] estimation of asparagine and glutamine,[325] and resolution of α-methyl-α-amino-acids[323] and *N*-methylamino-acids[327] either over chiral stationary phases[323] (see also refs. 163—166) or as diastereoisomeric derivative mixtures over coated capillaries.[327]

Determination of Kovat's retention indices for *N*-trimethylsilylamino-acids

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on fused-silica capillary columns coated with Carbowax or silicone oil has been reported.[328]

Ion-exchange Chromatography.—Leaving routine work aside, papers representative of continuing exploratory studies involve analysis at 10—100 picomole levels using *o*-phthaldialdehyde–mercaptoethanol fluorimetric estimation[329] and the virtues of D-glucosaminic acid as an early-eluting internal standard.[330]

Thin-layer Chromatography.—Considerable development of this technique still seems possible in the amino-acid field. Prior derivatization of an amino-acid mixture using 7-chloro-4-nitrobenzene-2-oxa-1,3-diazole followed by fluorimetric densitometry allows estimation of 3- and 4-hydroxyprolines[331] and of histidine and its 1- and 3-methyl derivatives[332] at 10—30 picomole levels. Even greater sensitivity accompanies the conversion of amino-acids into dimethylaminoazobenzenesulphonyl derivatives before t.l.c. (see also Vol. 14, p. 30).[333] Homocystine analysis using the silver nitroprusside spray reagent[334] is the subject of one of several papers concentrating on problems of t.l.c. analysis of sulphur-containing amino-acids (methionine and cystine,[335] cysteine and cystine[336]). Representative papers from the more routine areas of application deal with tryptophan and its metabolites[337] and two-dimensional t.l.c. of lysine and hydroxylysine in hydrolysed blood-serum proteins.[338]

Amino-acid derivatives receiving attention are *N*-acetylamino-acids (paper chromatography)[339] and Dnp-amino-acids (over-pressured t.l.c. using $CHCl_3$: CCl_4 : butanone : 1-propanol : methanol : acetic acid = 30:30:20:30:15:2).[340]

Assessments of improvements in techniques included in some of the preceding papers are supplemented by reports describing the separation of 35 amino-acids on Avicel F layers[341] and comparisons of silica gel, cellulose, and ion-exchange layers.[342]

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High-performance Liquid Chromatography.—This continues to be the growth area in amino-acid analysis. The general objectives, the establishment of acceptable separation parameters at maximum sensitivity, continue to provide challenges in a wide range of applications. Prior derivatization of amino-acid mixtures by dansylation[343–348] (down to 1—2 picomole levels[344]), 4-dimethylaminoazobenzene-4′-sulphonylation (down to 5—10 picomole levels,[349] but the limit is 458 picomole with hydroxyproline[350]), and reaction with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole,[351] with fluorescamine,[347] and particularly with *o*-phthaldialdehyde and mercaptoethanol (a range of values down to less than 1 picomole[352])[352—360] has featured prominently in recent studies. Many of these studies have been conducted with representative amino-acid mixtures, but some have concentrated on specific amino-acids (lysine,[347] neurotransmitter amino-acids,[358] and proline and hydroxyproline[360]). Other studies concentrating on particular amino-acids include those on tryptophan and its metabolites,[361] branched-chain amino-acids,[362] iodinated tyrosines and analogues,[363] and specific derivative-formation methods (betaines after benzyl ester formation,[364] *S*-adenosylmethionine based on its reaction with dopamine to give 3-methoxytyramine,[365] and phenylalanine or tyrosine after conversion into *trans*-cinnamic acid and *p*-coumaric acid, respectively, by the action of phenylalanine–ammonia lyase[366]). The *S*-adenosylmethionine assay[365] is notable in permitting its estimation at 1 picomole levels using less than 1 mg adrenal tissue.

The general topic of picomole-level amino-acid analysis has been reviewed,[367] and the construction of an amino-acid analyser based on standard

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h.p.l.c. equipment using either ninhydrin or *o*-phthaldialdehyde down to 10 picomole levels, requiring less than 45 min for the analysis of protein hydrolysates, has been described.[368] Analogues of tetraiodothyroxine are useful as internal standards in quantitative amino-acid analysis by h.p.l.c.[369]

While phenylthiohydantoins remain the most widely studied amino-acid derivative by h.p.l.c.,[370—373] owing to the potential of this technique in support of peptide sequencing, other derivatives have also received attention. *N*-Acetylamino-acid *N*-methylamides have been used to determine capacity factors in reversed-phase h.p.l.c., and the relationship of these factors to side-chain hydrophobicity has been discussed.[374]

Electrochemical detection in amino-acid h.p.l.c. is being taken up in more laboratories, recent studies concentrating on 5-hydroxytryptophan (100 picogramme levels can be handled),[375] tryptophan itself,[376] and phenylalanine, tyrosine, and *m*-tyrosine.[377] The latter two papers include comparisons of fluorimetric and voltammetric estimations, showing the superiority of electrochemical detection.[376]

Preparative liquid-chromatographic separation of amino-acids and peptides on Amberlite XAD-4 (a polystyrene–divinylbenzene copolymeric reversed-phase adsorbent) allows the use of mixed solvents and acidic or basic solvents that cannot be used with silica and alkylated silicas.[378] Copper(II) ions may be added to the aqueous mobile phase in reversed-phase liquid chromatography to modify retention times of amino-acids through complexation.[379] The complexes show strong u.v. absorption, and this permits samples containing as little as 10 ng per 10 μl to be detected.

Fluorescence Methods.—Fluorimetry based on *o*-phthaldialdehyde reagent systems requires care in eliminating interference from impurity artefacts when the greatest sensitivity is sought.[380] Three- to five-fold higher values for histidine in urine are obtained using the fluorescamine procedure in place of the *o*-phthaldialdehyde method.[381] The fluorescent adduct of histidine with *o*-phthaldialdehyde, but not that with 3-methylhistidine, is destroyed by reaction with formaldehyde, permitting the estimation of the latter in the presence of the former.[382]

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Amperometric determination of amino-acids with a passivated copper electrode[383] and emission spectrometric assay of ^{15}N-labelled amino-acids[384] represent two areas where less-routine studies are being extended.

Determination of Specific Amino-acids.—This section covers quantitative analysis of specific amino-acids by modifications of general analytical methods. The high proportion of electrochemical procedures is notable.

Potentiometric determination of L-alanine, L-serine, tyrosine, and histidine at milligram levels using a copper(II)-sensitive electrode and copper(II) sulphate-containing electrolyte gives accurate results (error range 0.2—2.7%).[385] Immobilized L-tyrosine decarboxylase[386] or bacteria exercising the same function[387] have been employed in potentiometric assay for L-tyrosine. Amperometric titration using potassium iodate is advocated for estimations of cysteine and cystine.[388] Cyclic oxidative voltammetry of methionine[389] provides data in support of electrochemical studies.

Spectrophotometric assay of hydroxyproline in serum based on its oxidation to a red pyrrole dye,[390] Sakaguchi and Millon colorimetric procedures for arginine and tyrosine, respectively,[391] and assay of *N*-carbamoyl-β-alanine through spectrophotometry at 466 nm after reaction with antipyrine and diacetylmonoxime (Prescott–Jones method)[392] represent a much larger body of more routine work.

Enzymatic methods have been applied for the estimation of L-ornithine in serum, L-ornithine carbamoyltransferase effecting its conversion into citrulline, which is assayed colorimetrically after reaction with diacetyl semithiocarbazide.[393] Levels of L-canavanine in plants are determined by arginase-catalysed hydrolysis to canaline, whose amino-oxy functional group reacts quantitatively with pyridoxal 5′-phosphate (a process conveniently followed spectrophotometrically).[394]

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