1
Amino-acids

BY G. C. BARRETT

1 Introduction

The format used in this chapter in previous volumes continues to provide a suitable framework for a review of current literature of amino-acids. There is an additional advantage in retaining a particular format in that readers may more easily trace specific topics back through the recent literature. Biological aspects and patent literature are excluded as in previous volumes of this series, but no other restraints have been imposed so that hopefully all chemistry-based work has been considered for inclusion.

Textbooks and Reviews.—Reference is made to relevant secondary and tertiary literature in the different sections of this chapter.

2 Naturally Occurring Amino-acids

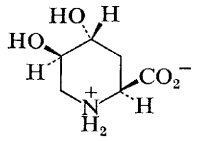
Occurrence of Known Amino-acids.—Reviews of the occurrence of non-protein amino-acids in plants [1–5] place emphasis on imino-acids and on *N*-heterocyclic amino-acids,[3] on D-amino-acids,[5] and on the roles played by free amino-acids,[1, 2, 4] which often exist in extraordinarily high concentrations in seeds [1] (*e.g.* 5-hydroxy-L-tryptophan comprises *ca.* 14% dry weight of seeds of *Griffonia simplicifolia* [6]).

Hydroxylated amino-acids existing in the free form, and their sources, as represented in the recent literature, are: (2*S*,3*R*,4*R*)-4-hydroxyisoleucine in steroidal sapogenin-yielding plants,[7] γ-hydroxyarginine from pea seedlings [8] and from lentil seeds [9] (which also contain γ-hydroxyornithine and homoarginine [9]), (2*S*,4*R*,5*R*)-2-carboxy-4,5-dihydroxypiperidine (1) from seeds of *Julbernardia paniculata*,[10] and its (2*S*,4*R*,5*S*)-isomer from *Derris elliptica*.[11] The (2*S*,4*S*,5*S*)-isomer of (1) is found in seeds of *Isoberlinia*, *Brachystegia*, *Cryptosepalum*,[10] and *Derris elliptica*,[11] but this is the first report [10] of the occurrence of the (2*S*,4*R*,5*R*)-isomer (1). 2,4,5-Trihydroxy-L-phenylalanine (*i.e.* 6-hydroxy-dopa) has been

[1] E. A. Bell, *F.E.B.S. Letters*, 1976, **64**, 29.
[2] A. Kjaer and P. O. Larsen, in 'Biosynthesis', ed. J. D. Bu'Lock (Specialist Periodical Reports), The Chemical Society, London, 1975, Vol. 4, p. 179.
[3] L. Fowden, *Heterocycles*, 1976, **4**, 117.
[4] L. Fowden, in 'Perspectives in Experimental Biology', Proceedings of the 50th Anniversary Meeting of The Society for Experimental Biology, ed. N. Sutherland, Vol. 2, Pergamon Press, Oxford, 1976, p. 263.
[5] T. Robinson, *Life Sci.*, 1976, **19**, 1097.
[6] E. A. Bell, L. E. Fellows, and M. Y. Qureshi, *Phytochemistry*, 1976, **15**, 823.
[7] R. Hardman and I. M. Abu-Al-Futuh, *Phytochemistry*, 1976, **15**, 325.
[8] T. A. Smith and G. R. Best, *Phytochemistry*, 1976, **15**, 1565.
[9] H. Sulser and F. Sager, *Experientia*, 1976, **32**, 422.
[10] P. R. Shewry and L. Fowden, *Phytochemistry*, 1976, **15**, 1981.
[11] M. Marlier, G. Dardenne, and J. Casimir, *Phytochemistry*, 1976, **15**, 183.

isolated from cultures of *Microspira tyrosinatica* grown aerobically in tyrosine-containing media.[12]

γ-Carboxyglutamic acid, a recently-identified addition to the protein amino-acids (Vol. 8, p. 5) has been found in human urine at low concentrations.[13]



(1)

The occurrence of γ-methyl-L-glutamic acid and its γ-ethyl-, -methylene, and -ethylidene analogues in seeds of *Julbernardia*, *Isoberlinia*, *Brachystegia*, and *Cryptosepalum* has been reported.[10]

Reports of studies of basic amino-acids form a large part of a *Proceedings* volume;[14] a report is included[14a] of the presence of basic and *N*-methylated basic amino-acids in frog tissue. Continuing studies of *Reseda odorata* (see Vol. 6, p. 3) reveal the presence of L-saccharopine and of 2*S*-amino-adipic acid.[15] One of the major cell-wall constituents of aerobic coryneform organisms from human skin is L,L-2,6-diaminopimelic acid;[16] however, the claim that this amino-acid exists in the free form in higher plants has been disproved,[17] providing something of a puzzle since the diaminopimelate pathway has been invoked for the biosynthesis of lysine in higher plants.

The first report of the existence of the D-enantiomer of α-amino-n-butyric acid in higher plants (legume seedling extracts) has appeared.[18] Other structurally simple amino-acids located in plants include glycine betaine in wheat,[19] and phenylalanine and tryptophan betaines in *Antiaris africana*.[20]

β-(Isoxazolin-5-on-2-yl)alanine occurs with β-(uracil-3-yl)alanine in root exudates of pea seedlings, and with small amounts of α-amino-γ-(isoxazolin-5-on-3-yl)butyric acid in sweet pea extracts[21] (see also Vol. 7, p. 4). The anthelmintic principle of *Quisqalis indica* is identical with quisqalic acid,[22] isolated from a related source (Vol. 8, p. 3).

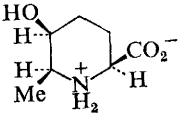
Fermentative production of amino-acids continues to generate a substantial

[12] D. O. Lunt and W. C. Evans, *Biochem. Soc. Trans.*, 1976, **4**, 491.
[13] P. Fernlund, *Clin. Chim. Acta*, 1976, **72**, 147.
[14] (*a*) J. Bolcs, in 'Proceedings of the 15th Hungarian Annual Meeting of Biochemistry', ed. B. Rosdy, Magy. Kem. Egyesulete, Budapest, 1975, p. 119 (*Chem. Abs.*, **85**, 189509); (*b*) E. Tyihak, I. Rusznak, and L. Trezl, *ibid.*, p. 19 (*Chem. Abs.*, **85**, 118182); (*c*) H. Kalasz, G. Kovacs, J. Nagy, J. Knoll, E. Tyihak, and A. Patthy, *ibid.*, p. 73 (*Chem. Abs.*, **85**, 119013); P. Miklos, J. Nagy, H. Kalasz, and J. Knoll, *ibid.*, p. 77 (*Chem. Abs.*, **85**, 119014); (*d*) A. Patthy, E. Tyihak, S. Ferenczi, S. Eckhardt, J. Kralovansky, H. Lapis, and B. Szende, *ibid.*, p. 57 (*Chem. Abs.*, **85**, 139313).
[15] H. Soerensen, *Phytochemistry*, 1976, **15**, 1527.
[16] D. G. Pitcher, *J. Gen. Microbiol.*, 1976, **94**, 225.
[17] P. O. Larsen and F. Norris, *Phytochemistry*, 1976, **15**, 1761.
[18] T. Ogawa, N. Bando, and K. Sasaoka, *Agric. Biol. Chem.*, 1976, **40**, 1661.
[19] R. B. Pearce, R. N. Strange, and H. Smith, *Phytochemistry*, 1976, **15**, 953.
[20] J. I. Okogun, A. I. Spiff, and D. E. U. Ekong, *Phytochemistry*, 1976, **15**, 826.
[21] Y. H. Kuo, F. Lambein, and R. Van Parijs, *Arch. Int. Physiol. Biochim.*, 1976, **84**, 169.
[22] P.-C. Pan, S.-D. Fang, and C.-C. Tsai, *Sci. Sin.*, 1976, **19**, 691.

literature, but can only be given passing mention here; representative papers [23, 24] describe the production of L-isoleucine [23] and the formation of L-norvaline and L-homoisoleucine as by-products in the conversion of threonine into L-isoleucine by *Serratia marcescens*.[24] For a more general review see reference 24*a*.

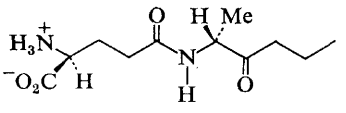
The area in which the non-occurrence of known amino-acids is perhaps a matter of as much importance as their occurrence, *i.e.* in extra-terrestrial samples, is represented by a report of analytical studies on lunar samples brought back on Apollo flights 11—17;[25] no amino-acids are present. The detection of amino-acids in meteorites and rock samples has been reviewed.[79]

New Natural Free Amino-acids.—Amino-acids reported in 1974 as 'unknown' constituents [26] of seeds of *Fagus silvatica* have now been characterized as (2*S*,5*S*,6*S*)-2-carboxy-5-hydroxy-6-methylpiperidine (2) and its 5*R*-epimer.[27]

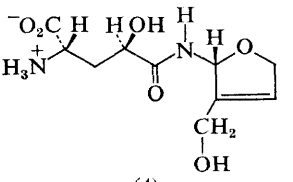


(2)

Further examples of new amino-acids reported in 1976 which also have relatively simple structures are L-2-amino-4-(2′-amino-ethoxy)butanoic acid (which accompanies the *trans*-3-butenoic acid analogue in a culture medium from an unidentified *Streptomycete*; see Vol. 7, p. 3);[28] α-methyl-L-arginine from a related source;[29] *N*-(L-γ-glutamyl)-2-aminohexan-3-one (3) from the mushroom *Russula*



(3)



(4)

ochroleuca;[30] and the L-allo-γ-hydroxyglutamine derivative (4) from *Hemerocallis fulva*.[31] The (*S*)-configuration can be proposed for the chiral centre adjacent to the ketone group in (3) on the basis of the octant rule (negative Cotton effect

[23] S. Ikeda, I. Fujita, and F. Yoshinaga, *Agric. Biol. Chem.*, 1976, **40**, 511; S. Ikeda, I. Fujita, and Y. Hirose, *ibid.*, 1976, **40**, 517; for a review see H. Matsushima, *Hakko Kogaku Zasshi*, 1976, **54**, 340.

[24] M. Kisumi, M. Sugiura, J. Kato, and I. Chibata, *J. Biochem.* (*Tokyo*), 1976, **79**, 1021.

[24a] D. R. Daoust, in 'Industrial Microbiology', ed. B. M. Miller and W. Litsky, McGraw-Hill, New York, 1976, p. 106.

[25] C. W. Gehrke, R. W. Zumwalt, K. Kuo, C. Ponnamperuma, and A. Shimoyama, *Origins Life*, 1975, **6**, 541.

[26] I. Kristensen, P. O. Larsen, and H. Soerensen, *Phytochemistry*, 1974, **13**, 2803.

[27] I. Kristensen, P. O. Larsen, and C. E. Olsen, *Tetrahedron*, 1976, **32**, 2799.

[28] J. P. Scannell, D. L. Pruess, H. A. Ax, A. Jacoby, M. Kellett, and A. Stempel, *J. Antibiotics*, 1976, **29**, 38.

[29] H. Maehr, L. Yarmchuk, D. L. Pruess, M. Kellett, N. J. Palleroni, B. La. T. Prosser, and T. C. Demny, *J. Antibiotics*, 1976, **29**, 213.

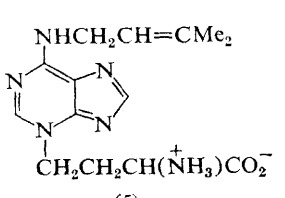
[30] A. Welter, J. Jadot, G. Dardenne, M. Marlier, and J. Casimir, *Phytochemistry*, 1976, **15**, 1984.

[31] G. J. Kruger, L. M. Du Plessis, and N. Grobbelaar, *J. South African Chem. Inst.*, 1976, **29**, 24.

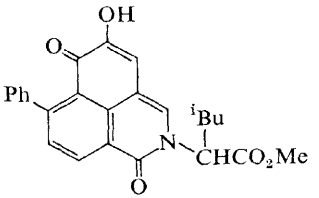
centred at 280 nm), but configurational assignments to acyclic ketones must be regarded as tentative when (as in this case) c.d. data for closely similar compounds of known absolute configuration are not available. The structure (4) was determined by *X*-ray crystal analysis,[31] establishing the configuration at the chiral centre in the dihydrofuran moiety to complete the structure assignment to oxypinnatanine, isolated from *Staphylea pinnata* and *Hemerocallis fulva* in 1973 (Vol. 6, p. 4), with which the amino-acid (4) studied by Kruger and co-workers is identical.[31]

The disulphiram-('antabuse')-like factor of the inky cap mushroom *Coprinus atramentarius* is N^5-(1-hydroxycyclopropyl)-L-glutamine (13), *alias* coprine.[36]

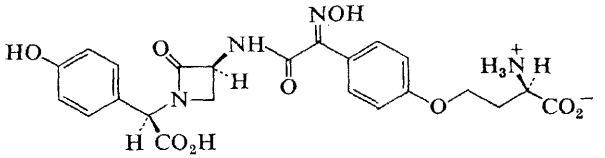
More complex structures are represented in discadenine (5), a spore germination inhibitor from the mould *Dictyostelium discoideum*,[32] the isoleucine derivative (6) (one of two new pigments from flowers of *Lachnanthus tinctoria*),[33] nocardicins



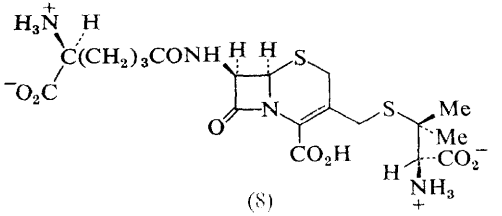
(5)



(6)



(7)



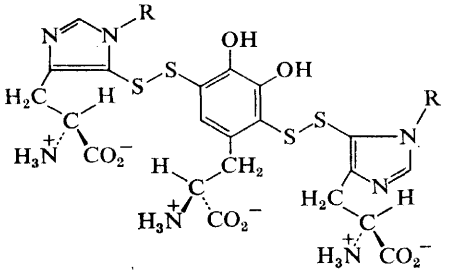
(8)

A and B from *Nocardia uniformis tsuyamaenis* [related to each other as geometrical isomers of the oxime (7)],[34] and the cephalosporin C analogue (8) produced by mutants of *Cephalosporium acremonium*.[35]

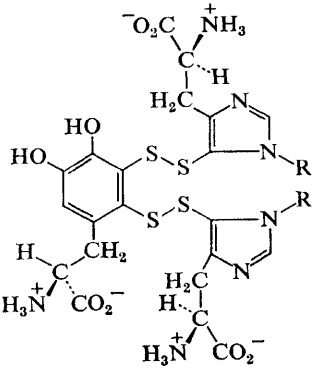
[32] H. Abe, M. Uchiyama, Y. Tanaka, and H. Saito, *Tetrahedron Letters*, 1976, 3807.
[33] A. C. Bazan and J. M. Edwards, *Phytochemistry*, 1976, **15**, 1413.
[34] M. Kurita, K. Jomon, T. Komori, N. Miyairi, H. Aoki, S. Kuge, T. Kamiya, and H. Imanaka, *J. Antibiotics*, 1976, **29**, 1243.
[35] T. Kanzaki, T. Fukita, K. Kitano, K. Katamoto, K. Nara, and Y. Nakao, *Hakko Kogaku Zasshi*, 1976, **54**, 720 (*Chem. Abs.*, **36**, 28440).

New Amino-acids from Hydrolysates.—Novel peptide antibiotics are often composed of an unusual variety of amino-acid residues. The antifungal antibiotic echinocandin B contains (2*S*,3*S*,4*S*)-3,4-dihydroxyhomotyrosine,[37, 38] (2*S*,4*R*,5*R*)-4,5-dihydroxyornithine,[38] and (2*S*,3*S*,4*S*)-4-methyl-3-hydroxyproline [38] among its complement of six amino-acids; the others are L-threonine (twice) and hydroxy-L-proline. A tripeptide from *Streptomyces plumbeus* contains D-2-amino-5-phosphono-3-pentenoic acid,[39] and provides a further example of an α-amino-acid with a phosphorus-containing functional group in the side-chain.

Hydrolysis of adenochrome, the iron(III)-containing pigment from the branchial heart of *Octopus vulgaris*, gives glycine and two novel iron-binding amino-acids, adenochromines A and B [(9) and (10) respectively], in the ratio 2 : 1.[40] Structure assignments were based upon reductive hydrolysis with HI and red phosphorus, which gives a new thiol-containing amino-acid, 5-mercapto-L-histidine, and L-dopa.[40] The adenochromines (9) and (10) are partially methylated (R = Me).[40]



(9; R = H, Me)

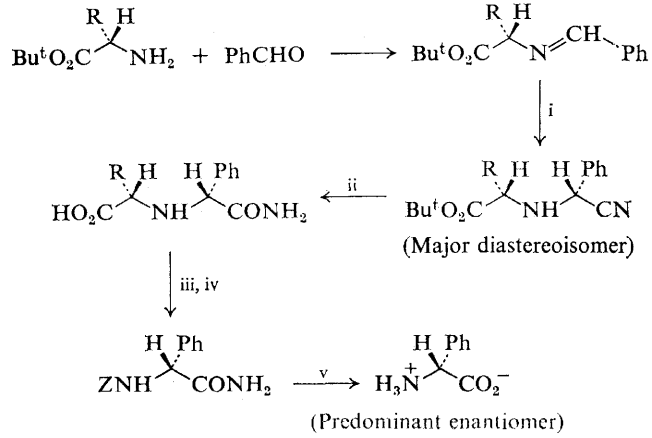


(10)

3 Chemical Synthesis and Resolution of Amino-acids

Asymmetric Synthesis.—Procedures based upon chiral Schiff bases have been developed further, with use of an L-amino-acid t-butyl ester [41] as starting material for the asymmetric synthesis of D-phenylglycine (Scheme 1).[41a] This interesting route reveals clearly the progress which still has to be made towards an economical asymmetric synthesis of α-amino-acids. There is a limitation imposed by the oxidation–decarboxylation cleavage step (the cleavage of the intermediate secondary amine causes destruction of the chiral starting material), and little can be learnt from the relationship between structure and optical yield expected through this route. There is a clue in the fact that of the various L-amino-acid

[36] P. Lindberg, R. Bergman, and B. Wickberg, *J.C.S. Chem. Comm.*, 1975, 946; *J.C.S. Perkin I*, 1977, 604; G. M. Hatfield and J. P. Schaumberg, *Lloydia*, 1975, **38**, 489.
[37] W. Keller-Schierlein and J. Widmer, *Helv. Chim. Acta*, 1976, **59**, 2021.
[38] C. Keller-Juslén, M. Kuhn, H. R. Loosli, T. J. Petcher, H. P. Weber, and A. von Wartburg, *Tetrahedron Letters*, 1976, 4147.
[39] B. K. Park, A. Hirota, and H. Sakai, *Agric. Biol. Chem.*, 1976, **40**, 1905.
[40] S. Ito, G. Nardi, and G. Prota, *J.C.S. Chem. Comm.*, 1976, 1042.
[41] (*a*) S. Yamada and S. Hashimoto, *Chem. Letters*, 1976, 921; (*b*) *Tetrahedron Letters*, 1976, 997.



Reagents: i, HCN; ii, acid hydrolysis; iii, Bu^{t}OCl; iv, ZCl; v, deprotection

Scheme 1

t-butyl esters tried, the valine derivative gives the highest optical yield (62%) of D-phenylglycine,[41a] suggesting that limited conformational mobility may be linked with high optical yield. L-Alanine is obtained in 65—76% optical yield in a corresponding route starting with methyl pyruvate, and employing H_2–Pd/C for asymmetric reduction to give a secondary amine corresponding to that in Scheme 1.[41b]

A quite different use of chiral Schiff bases has been reported from Yamada's laboratory.[42] Condensation of glycine t-butyl ester with a chiral ketone, (1*S*,2*S*,5*S*)-2-hydroxypinan-3-one, in the presence of BF_3–Et_2O gives the corresponding imine which, on reaction with an alkyl halide after carbanion formation with Pr^i_2NLi, gives a diastereoisomer mixture from which the corresponding D-amino-acid t-butyl ester is released by hydrolysis. Moderate chemical and optical yields (50—79 and 66—83%, respectively) were obtained.[42]

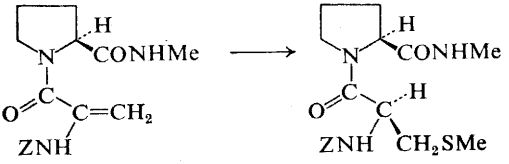
L-Alanine has been obtained in low optical yields by hydrogenolysis of the chiral imines formed by condensation of ethyl pyruvate with chiral amines;[43] in the best case, an optical purity of 46.5% was achieved and this was the case where a bulky amine, S-bornylamine, was used.

Asymmetric catalytic hydrogenation of prochiral acylaminocrotonates $R^1CONHC(=CR^2R^3)COR^4$ is represented in several papers in the recent literature,[44–48] all involving studies of rhodium complexes carrying chiral ligands as homogeneous catalysts. A broad study of the effects of structure of the

[42] S. Yamada, T. Oguri, and T. Shioiri, *J.C.S. Chem. Comm.*, 1976, 136.
[43] S. Kiyooka, K. Takeshima, H. Yamamoto, and K. Suzuki, *Bull. Chem. Soc. Japan*, 1976, **49**, 1897.
[44] V. A. Pavlov, E. I. Klabunovskii, G. S. Barysheva, L. N. Kaigorodova, and Y. S. Airapetov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 2374.
[45] N. Takaishi, H. Imai, C. A. Bertelo, and J. K. Stille, *J. Amer. Chem. Soc.*, 1976, **98**, 5400.
[46] T. Hayashi, T. Mise, S. Mitachi, K. Yamamoto, and M. Kumada, *Tetrahedron Letters*, 1976, 1133.
[47] G. Gelbard, H. B. Kagan, and R. Stern, *Tetrahedron*, 1976, **32**, 233.
[48] K. Achiwa, *J. Amer. Chem. Soc.*, 1976, **98**, 8265.

N-acyl moiety and stereochemistry about the C=C bond on optical yields[47] provides useful source material for the continuing attempts at interpretation of the factors controlling the stereochemical course of this process, using the *O*-isopropylidene-2,3-dihydroxy-bis(diphenylphosphino)butane–RhCl catalyst. A polymer-bound analogue of this particular catalyst has been tested,[45] and in one of two other studies involving phosphorus-containing ligands[46, 48] for the asymmetric synthesis of phenylalanines, optical yields up to 91% have been achieved[48] in the synthesis of dopa and its analogues. Studies of the validity of earlier interpretations of n.m.r. data for the assignment of stereochemistry to 2-acylaminocrotonates have been reported.[49, 50]

Asymmetric induction in favour of the D,L-dipeptide is observed in nucleophilic addition of MeSH to the dehydroalanyl-L-prolinamide (11).[51]



(11)

General Methods of Synthesis of α-Amino-acids.—This section in previous volumes has provided a brief survey of classical methods of amino-acid synthesis, side-by-side with newer methods. The general picture in the 1976 literature is of consolidation of the use of recently-introduced methods, and of continuing use of diethyl acetamidomalonate[52–54] (refs. 128, 131, 134, 246), hydantoins,[55–57] modified Strecker synthesis,[58, 59] β-chloroalanine,[60, 61] and isonitriles ($CNCH_2CO_2Et + RR^1CBrCO_2R^2$ followed by hydrolysis gives $H_3\overset{+}{N}CH(CRR^1CO_2H)CO_2^-$).[62]

A conventional method for the introduction of an α-amino-group into an aliphatic carboxylic acid involves the azido-group as an intermediate, *e.g.* Al–Hg reduction of ethyl 2-azido-2-alkenoates,[63] but it is worth pointing out that the handling of azides can be particularly hazardous under certain circumstances.

[49] A. Srinivasan, K. D. Richards, and R. K. Olsen, *Tetrahedron Letters*, 1976, 891.
[50] R. Glaser and M. Twaik, *Tetrahedron Letters*, 1976, 1219.
[51] U. Schmidt and E. Öhler, *Angew. Chem.*, 1976, **88**, 54; *Angew. Chem. Internat. Ed.*, 1976, **15**, 42.
[52] A. Tun-Kyi and R. Schwyzer, *Helv. Chim. Acta*, 1976, **59**, 2181.
[53] H. Gross and T. Gnauk, *J. prakt. Chem.*, 1976, **318**, 157.
[54] Y. Kataoka, Y. Seto, M. Yamamoto, T. Yamada, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Japan*, 1976, **49**, 1081.
[55] C. Perier, M. C. Ronziere, A. Rattner, and J. Frey, *J. Chromatog.*, 1976, **125**, 526.
[56] T. G. Tsarkova, I. A. Avrutskaya, M. Y. Fioshin, E. P. Krysin, and I. I. Gubenko, *Electrokhimiya*, 1975, **11**, 1803; T. G. Tsarkova, I. A. Avrutskaya, M. Y. Fioshin, and E. P. Krysin, *ibid.*, 1976, **12**, 902.
[57] H. Maehr, L. Yarmchuk, and M. Leach, *J. Antibiotics*, 1976, **29**, 221.
[58] R. F. Eizember and A. S. Ammons, *Org. Prep. Proc. Internat.*, 1976, **8**, 149.
[59] N. Sarda, A. Grouiller, and H. Pacheco, *Tetrahedron Letters*, 1976, 271.
[60] C. De Marco, A. Rinaldi, S. Dernini, and D. Cavallini, *Gazzetta*, 1975, **105**, 1113.
[61] B. Weinstein, K. G. Watrin, J. H. Loie, and J. C. Martin, *J. Org. Chem.*, 1976, **41**, 3634.
[62] M. Bochenska and J. F. Biernat, *Roczniki Chem.*, 1976, **50**, 1195.
[63] C. Shin, Y. Yonezawa, and J. Yoshimura, *Chem. Letters*, 1976, 1095.

Thus,[64] azidoacetic acid, $N_3CH_2CO_2H$, detonates at 90 °C under visible light, and at 25 °C in the presence of Fe or Fe salts.

Improved methods for anion formation from simple glycine derivatives have been developed, so that alkylation studies might then follow to open up general amino-acid syntheses. Preliminary studies of alkylation of the hippuric acid trianion, formed with Pr^i_2NLi and TMEDA, and the correspondingly-formed ethyl hippurate dianion, indicate predominant mono-*C*-alkylation.[65] Mono- or di-alkylation occurs with *N*-benzylideneglycine ethyl ester,[66] while an independent study [42] of Schiff base anion alkylation has taken the general process further in showing that a chiral alkylidene moiety may be mono-alkylated with a small degree of asymmetric induction (see preceding section). Alkylation of the trimethylsilyl *NN*-bis(trimethylsilyl)glycinate anion has been illustrated further (see ref. 123).

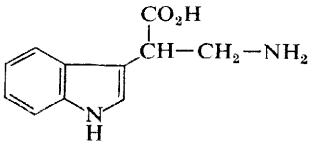
Further results in the application of *N*-acyl α-hydroxyglycines in the synthesis of *C*-aryl- and -heteroaryl-glycines have been reported.[67]

Only 0.08% yield of phenylalanine could be obtained from a reaction mixture including n-octyl phenylthiolacetate $PhCH_2COSC_8H_{17}$, with CO_2, NH_3, $Na_2S_2O_4$, and Schrauzer's iron–sulphur complex $(FeS_4C_4Ph_4)_2$,[68] although higher yields were obtained starting from the keto-acid which is assumed to be the reaction intermediate in this process.

Synthesis of β-Amino- and γ-Amino-acids.—Coverage of amino-acids other than α-amino-acids is justified by the co-occurrence of the various classes of amino-acid in a number of natural products. However, synthetic methods are usually straightforward and difficult to generalize, and only brief mention is possible.

(*S*)-4-Amino-2-hydroxy-n-butyric acid has been synthesized from the corresponding di-amino-acid [69] and from 3-acetoxy-2-methoxy-1-pyrroline.[70] A synthesis of the homologue (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid has been announced.[71]

The tryptophan isomer (12) has been synthesized from indole-3-acetonitrile.[72]



(12)

Prebiotic Synthesis: Model Reactions.—Continuing studies of a familiar type have been described, concerning the formation of nine amino-acids together with

[64] S. J. Borowski and K. Kamholz, *Chem. Eng. News*, 1976, **54**, 5.
[65] A. P. Krapcho and E. A. Dundulis, *Tetrahedron Letters*, 1976, 2205.
[66] G. Stork, A. Y. W. Leong, and A. M. Touzin, *J. Org. Chem.*, 1976, **41**, 3491.
[67] D. Ben-Ishai, I. Sataty, and Z. Bernstein, *Tetrahedron*, 1976, **32**, 1571.
[68] I. Tabushi, Y. Yabushita, and T. Nakajima, *Tetrahedron Letters*, 1976, 4343.
[69] Y. Horiuchi, E. Akita, and T. Ito, *Agric. Biol. Chem.*, 1976, **40**, 1649.
[70] Y. Yamada and H. Okada, *Agric. Biol. Chem.*, 1976, **40**, 1437.
[71] R. Steulmann and H. Klostermeyer, *Annalen*, 1975, 2245.
[72] V. S. Rozhkov, Y. I. Smushkevich, and N. N. Suvorov, *Zhur. org. Khim.*, 1976, **12**, 1076.

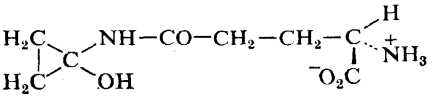
urea in CH_4–NH_3–H_2O mixtures subjected to electric discharge.[73, 74] Amino-acid synthesis resulting from shock waves in HCN–aldehyde–H_2O mixtures proceeds *via* aldimines and α-amino-nitriles as intermediates.[75] An interesting possibility that amino-acids arise, phoenix-like, from volcanic eruptions has been tested in a model system, in which lava at temperatures up to 1050 °C in contact with CH_4 (or CO)–NH_3–H_2O mixtures has been shown to initiate the synthesis of amino-acids and other organic compounds.[76] Other environmental factors which have been considered are transition metal ions in brine (a model for prebiotic sea),[77] and catalysis of condensation reactions by clay.[77, 78] Contrary to previous claims, montmorillonite shows only weak catalytic properties for the polymerization of activated amino-acids.[78]

A late stage in a plausible route for the abiotic synthesis of α-amino-acids, the hydrolysis of amino-malonotrile or acrylonitrile–electrophile adducts, has been shown to proceed under very mild conditions, and to lead to glycine, β-hydroxyaspartic acid, glutamic acid, threonine, and allothreonine.[79]

An extensive review of the prebiotic synthesis of amino-acids, and their detection in meteorites and rock samples, has appeared.[80]

Protein and Other Naturally Occurring Amino-acids.—Several of the papers cited in a preceding section covering general methods of synthesis describe the exploration of new methods for the synthesis of well-known amino-acids. A good deal of effort has been put into developing routes to γ-carboxyglutamic acid, and derivatives suitable for use in peptide synthesis; protected dehydroalanines participate in Michael addition reactions with di-t-butyl malonate,[81] and protected β-chloroalanines give mono-alkylated products with dibenzyl malonate,[61] leading to correspondingly-substituted γ-carboxyglutamic acids. Schwyzer and co-workers have continued their chemical studies of γ-carboxyglutamic acid,[82, 83] with syntheses of γγ′-di-t-butyl ester derivatives and establishment of absolute configuration by chemical correlation.[83]

Syntheses of L-2-oxalylamino-3-aminopropionic acid, an isomer of the neurotoxin from seeds of *Lathyrus sativus*,[84] and of coprine (13), an α-amino-acid from the mushroom *Coprinus atramentarius*,[36] have been announced.

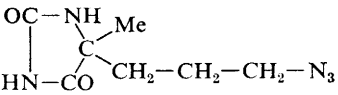


(13)

[73] C. Simionescu, R. Mora, N. Olaru, and E. Ionanid, *Compt. rend.*, 1976, **282**, 679.
[74] A. Constantinescu and C. Liteanu, *Rev. Chim. (Bucharest)*, 1976, **27**, 906.
[75] I. Barak and A. Bar-Nun, *Origins Life*, 1975, **6**, 483.
[76] L. M. Mukhin, V. B. Bondarev, V. I. Kalinichenko, E. N. Safonova, and Y. S. Petrenko, *Doklady Akad. Nauk S.S.S.R.*, 1976, **226**, 1225.
[77] M. Ventilla and F. Egami, *Proc. Japan Acad.*, 1976, **52**, 21 (*Chem. Abs.*, **34**, 117287).
[78] A. Brack, *Clay Miner.*, 1976, **11**, 117 (*Chem. Abs.*, **85**, 187961).
[79] J. W. Thanassi, *J. Mol. Evol.*, 1975, **7**, 65.
[80] A. T. Soldatenkov and I. A. Sytinskii, *Uspekhi Khim.*, 1976, **45**, 329.
[81] S. Bajusz and A. Juhasz, *Acta Chim. Acad. Sci. Hung.*, 1976, **88**, 157.
[82] W. Maerki, M. Oppliger, and R. Schwyzer, *Helv. Chim. Acta*, 1976, **59**, 901.
[83] W. Maerki and R. Schwyzer, *Helv. Chim. Acta*, 1976, **59**, 1591.
[84] G. Wu, S. B. Bowlus, K. S. Kim, and B. E. Haskell, *Phytochemistry*, 1976, **15**, 125.

NN-Disubstituted aspartates may be synthesized by Stevens rearrangement of corresponding quaternary ammonium salts $RR'\overset{+}{N}(CH_2CO_2Me)_2X^-$,[85] while $\beta\beta$-disubstituted analogues have been prepared by alkylation of ethyl isonitriloacetate with α-bromo-esters followed by conventional hydrolysis procedures.[61]

α-Alkyl Analogues of the Protein Amino-acids.—Synthesis of α-methyl-L-arginine *via* the ornithine analogue has been achieved using a hydantoin route frequently adopted previously for similar cases, conventional functional group manipulation procedures being applied to (14).[57] Enzymic conversion into α-methyl-D-arginine and α-methyl-L-ornithine was a late step in the synthesis of a newly-discovered [29] antibiotic from *Streptomyces*.



(14)

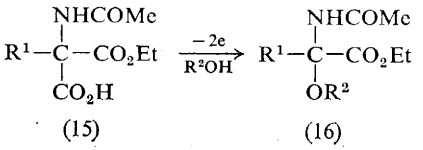
Side-chain Halogenated Analogues of the Protein Amino-acids.—Further details have been published dealing with electrochemical conversion of γγγ-trichloro-L-butyrine derivatives into the dichloro-analogues without racemization (see Vol. 8, p. 10).[86] Also arising from this project are βγ-unsaturated-γγ-dichloro-L-butyrine and γγ-dichloro-L-threonine derivatives.[86]

Photofluorination is demonstrated to be an efficient method for the synthesis of 3-fluoro-D-alanine and its 2-^{2}H analogue from the D-alanines without racemization.[87] Threonine derivatives give 2-amino-3-fluoro-butyric acid analogues on treatment with SF_4 in HF;[88] bromofluorination of 2-amino-pent-4-enoic and -hex-5-enoic acid derivatives followed by conventional aminolysis procedures leads to 4-fluoro-ornithine, -arginine, and -citrulline, and 5-fluoro-lysine, respectively.[89]

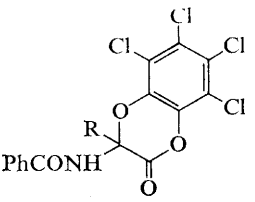
α-Hydroxy-, -Alkoxy-, -Amino-, and -Alkylthio-analogues of the Protein Amino-acids.—α-Amino-acids carrying a hetero-atom at the α-position are of continuing interest not only because of the presence of the $-NH-\underset{|}{C}(X)-CO-$ moiety in gliotoxins and related natural products, but because of their synthetic applications, *e.g.* in the synthesis of dehydro-amino-acids. Mention has been made in a preceding section of continuing studies of the use of α-hydroxyglycine derivatives as starting materials in amino-acid synthesis.[67] *N*-Chlorination of Boc-Val-OMe with Bu^tOCl and treatment with NaOMe gives *N*-t-butoxycarbonyl-α-methoxy-valine methyl ester in 94% yield [90] (see Scheme 2).

A new route to α-alkoxy- and α-acetoxy-α-amino-acids from α-acetamido-malonate half esters, (15) → (16), involves electrolysis of solutions in alcohols or

[85] A. T. Babayan, S. T. Kocharyan, and S. M. Ogandzhanyan, *Armyan. khim. Zhur.*, 1976, **29**, 456.
[86] T. Iwasaki, Y. Urabe, Y. Ozaki, M. Miyoshi, and K. Matsumoto, *J.C.S. Perkin I*, 1976, 1019.
[87] J. Kollonitsch and L. Barash, *J. Amer. Chem. Soc.*, 1976, **98**, 5591.
[88] R. S. Loy and M. Hudlicky, *J. Fluorine Chem.*, 1976, **7**, 421.
[89] V. Tolman and J. Benes, *J. Fluorine Chem.*, 1976, **7**, 397.
[90] H. Poisel and U. Schmidt, *Angew. Chem.*, 1976, **88**, 295; *Angew. Chem. Internat. Edn.*, 1976, **15**, 294.



(15) (16)



(17)

in acetic acid.[91] An unexpected bonus from studies of oxazol-5(4*H*)-ones (already known to be useful intermediates for the activation of the α-C-atom of an α-amino-acid) follows[92] from reaction of *o*-chloranil oxidation products (17) with nucleophiles to give α-alkoxy- (16) and analogous -benzylthio- and -anilino-amino-acids.[92]

Aliphatic α-Amino-acids Carrying Hydroxy-groups and Ether Groups in Side-chains.—5-Hydroxybutylhydantoin serves as starting material for the synthesis of ε-hydroxynorleucine.[55] α-Amino-β-phenyoxypropionic acid has been obtained through a modified Strecker reaction.[59]

Stereoisomers of γ-hydroxyisoleucine isolated as their lactones from photochlorination of D-allo-isoleucine followed by hydrolysis are (2*R*,3*R*,4*R*)-2-amino-3-methyl-4-hydroxyvaleric acid, and its 4*S*-epimer, with 2*S*-epimers of these being obtained by $Ba(OH)_2$ epimerization.[93]

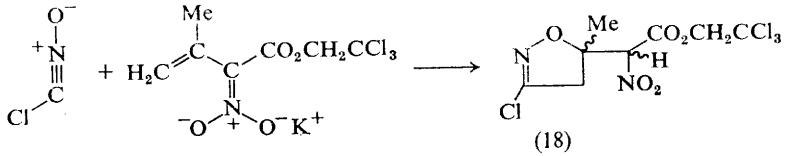
Aromatic and Heterocyclic Amino-acids.—Routine syntheses from aromatic aldehydes leading to *p*-methoxy- and *p*-hydroxyphenylglycine,[58] and to phenylalanine and tryptophan,[56] have been reported, the interest in the latter study laying particularly in the electroreduction of 4-arylidenehydantoins.

β-Methylphenylalanine has been obtained through the acetamidomalonate route,[54] a point of interest being the stereoselectivity observed in the decarboxylation step (the L-erythro-isomer occurs in bottromycin). ββ-Disubstituted phenylalanines may be obtained from corresponding α-cyano-esters $ArCMe_2CH(CN)CO_2Et$ by conventional elaboration,[94] or from ketones $ArCMe_2COMe$ *via* the keto-acids.[95] The latter route has been applied in the synthesis of ββ-dimethyl-dopa.[95]

Manipulation of the aryl moiety in a phenylalanine has been a well-worked operation over the years, and novel procedures are still being established; *N*-acetyl-3-iodo-L-tyrosinamide gives the thyronine analogue by coupling with di(4-methoxyphenyl)iodonium tetrafluoroborate followed by deprotection with HBr–AcOH, and procedures for further iodination leading to 3,3′-di-iodo- and 3,3′,5′-tri-iodo-L-thyronines have been described.[96]

Isoxazolines are being found in increasing numbers in various classes of natural products, including amino-acids, and a synthesis of the 3-chloroisoxazolin-

[91] H. Horikawa, T. Iwasaki, K. Matsumoto, and M. Miyoshi, *Tetrahedron Letters*, 1976, 191.
[92] J. M. Riordan and C. H. Stammer, *Tetrahedron Letters*, 1976, 1247.
[93] M. Hasan, D. Georgopoulos, and T. Wieland, *Annalen*, 1976, 781.
[94] N. A. Jonsson and L. Mikiver, *Acta Pharm. Suec.*, 1976, **13**, 75.
[95] N. A. Johsson and L. Mikiver, *Acta Pharm. Suec.*, 1976, **13**, 65.
[96] P. Block, *J. Medicin. Chem.*, 1976, **19**, 1067.



(18)

5-ylglycine (18) has been achieved [97] to reward the vigilance of its creators in seeking new applications of cycloaddition reactions for relevant natural products syntheses. The initial exploration of the route involved the methyl α-nitrocrotonate, but difficulty was experienced in hydrolysing the ester at the end of the synthesis while retaining the intact side-chain. The configuration of a crystalline isomer isolated from the diastereoisomeric product mixture was not established.

***N*-Substituted Amino-acids.**—A considerable body of papers has been published concerning particularly the *N*-methyl homologues of the basic amino-acids, as part of a *Proceedings* volume.[14] N^{ε}-Monomethyl-L-lysine is the major reaction product from L-lysine and formaldehyde in dilute neutral solutions, conditions under which no reaction is observed with L-arginine.[14b] A new procedure for obtaining *N*-monomethyl derivatives of simple amino-acids uses conventional reagents (MeI–NaH–THF) after conversion of the amino-acid into the phosphinamide $Ph_2PONHCHRCO_2H$.[98]

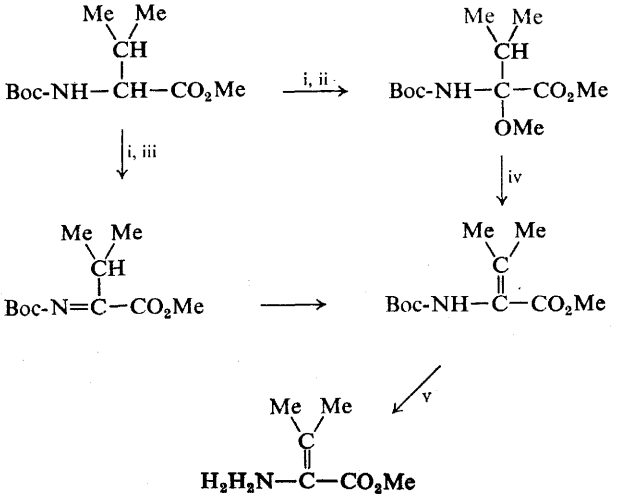
α-Hydrazino-acids may be obtained from amino-acids *via* α-diazo-esters [99] or *via* α-bromo-acids,[100] the latter route being illustrated in the synthesis of 'D-α-hydrazino-ornithine' from L-ornithine.[100, 101]

Amino-acids with Unsaturated Side-chains.—Conventional methods have been used for the synthesis of L-propargylglycine $H_3\overset{+}{N}CH(CH_2C{\equiv}CH)CO_2^-$ (from diethyl acetamidomalonate and propargyl bromide) and its resolution.[52]

Further illustration of the route announced last year (Vol. 8, p. 11) for the conversion of an α-amino-acid into its dehydro-analogue is provided [90] by the alternative reaction sequences in Scheme 2. Methyl dehydrovalinate, perhaps better named methyl 2-amino-3,3-dimethylacrylate, is obtained in 86% yield *via* the α-methoxy-α-amino-acid intermediate. There are alternative routes to these unsaturated α-amino-acid esters, and further examples of their preparation from 2-azido-2-alkenoates by Al–Hg reduction,[63] and of the synthesis of *N*-acyl analogues from *N*-acyl-2-thiono-oxazolidine-4-carboxylates,[102] have been published.

Novel synthetic methods have been described, one [103] exploiting the 'ene' addition pathway in leading to γδ-unsaturated amino-acids from an alkene and an *N*-(arenesulphonyl)imine, p-$MeC_6H_4SO_2N{=}CHCO_2Bu^n$; and the other [104]

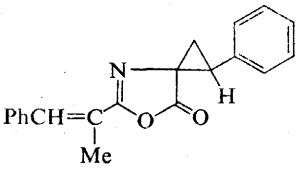
[97] J. E. Baldwin, C. Hoskins, and L. Kruse, *J.C.S. Chem. Comm.*, 1976, 795.
[98] S. Coulton, G. A. Moore, and R. Ramage, *Tetrahedron Letters*, 1976, 4005.
[99] N. Takamura and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 800.
[100] T. Sawayama, H. Kinugasa, and H. Nishimura, *Chem. Pharm. Bull.*, 1976, **24**, 326.
[101] J. G. Johansson, R. M. Parkhurst, and W. A. Skinner, *Indian J. Chem.*, 1976, **14B**, 209.
[102] D. Hoppe and R. Follmann, *Chem. Ber.*, 1976, **109**, 3062.
[103] O. Achmatowicz and M. Pietraszkiewicz, *J.C.S. Chem. Comm.*, 1976, 484.
[104] J. W. Hines, E. G. Breitholle, M. Sato, and C. H. Stammer, *J. Org. Chem.*, 1976, **41**, 1466.



Reagents: i, Bu^{t}OCl; ii, NaOMe; iii, DABCO; iv, HCl; v, NH_3

Scheme 2

unexpectedly yielding DL-styrylglycine rather than a cyclopropylphenylalanine on acid hydrolysis of the oxazolone (19). A reliable synthesis of styrylglycine [104] through the α-amino-nitrile route was developed to provide authentic material for comparison with the hydrolysis product from (19).

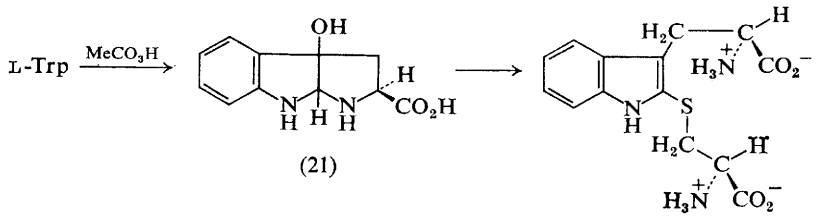


(19)

Amino-acids Containing Sulphur or Selenium.—Addition of thiols to protected amino-acids carrying unsaturated side-chains gives corresponding sulphides.[104, 105] The alternative approach, in which cysteine *S*-alkylation leads to analogous products, is illustrated in a novel synthesis of tryptathionine (20) involving a useful modified tryptophan (21).[106]

Routine studies of the preparation and resolution of sulphoxides of *S*-alkyl- and -propenyl-cysteines [107] and of *S*-(2-methylprop-1-enyl)-L-cysteine [108] are supplemented by interesting studies of cyclization of alkenylcysteine sulphoxides to thiazine-*S*-oxides.[108] Homolanthionine sulphoxides and sulphones are formed from homocystine by treatment with H_2O_2 in acid solution.[109]

[105] J. T. Snow, J. W. Finley, and M. Friedman, *Internat. J. Peptide Protein Res.*, 1976, **8**, 57.
[106] W. E. Savige and A. Fontana, *J.C.S. Chem. Comm.*, 1976, 600.
[107] G. G. Freeman and R. J. Whenham, *Phytochemistry*, 1976, **15**, 521.
[108] J. F. Carson and R. E. Lundin, *J.C.S. Perkin I*, 1976, 1195.
[109] P. Clopath, and K. S. McCully, *Anal. Biochem.*, 1976, **73**, 231.



(21) (20)

Thioasparagine is formed from β-cyanoalanine derivatives by conventional thioamide synthesis,[110] with benzyl thioaspartates $ArCH_2SCOCH_2CH(\overset{+}{N}H_3)\text{-}CO_2^-$ being formed as a result of *S*-alkylation of the thioamide followed by hydrolysis, in procedures involving deblocking of certain *N*-protected intermediates.[110]

Independent studies leading to the synthesis of L-4-selenalysine, $H_2NCH_2\text{-}CH_2SeCH_2CH(\overset{+}{N}H_3)CO^-$, from β-chloro-L-alanine have been reported,[60, 111] and followed up by a synthesis of 4-selena-homolysine.[112] Selenocysteine, used in the latter synthesis [112] and in an increasing number of studies in plant biochemistry,[113] is easily prepared from selenocystine by $NaBH_4$ reduction.[113]

Phosphorus-containing Amino-acids.—The title of this small section is to be interpreted to refer to amino-carboxylic acids with phosphorus-containing functional groups in side-chains. DL-Phosphinothricin, $HOP(O)MeCH_2CH_2\text{-}CH(\overset{+}{N}H_3)CO_2^-$, has been synthesized by alkylation of diethyl acetamidomalonate with $ClCH_2CH_2OP(O)MeCH_2CH_2Cl$, formed from ethylene oxide and $MePCl_2$ followed by rearrangement.[53]

Further examples of amino-acids of this class are listed in the next section.

A List of Amino-acids which have been Synthesized for the First Time.—In addition to several new amino-acids mentioned elsewhere in the chapter, the following compounds have been synthesized recently for the first time.

Compound	*Ref.*
L-*o*-Carboranylalanine, $B_{10}C_2CH_2CH(\overset{+}{N}H_3)CO_2^-$	114
Selenium analogue of L-tryptophan (Se in place of NH^{indole})	115
Phosphonate analogues of L-aspartic acid	116
Phosphinate analogues of L-aspartic acid	116
5-Aza-analogue of L-arginine	117
5-Aza-analogue of 4,5-dehydro-L-arginine	117
N-Substituted γ-glutamic acid hydrazides	117

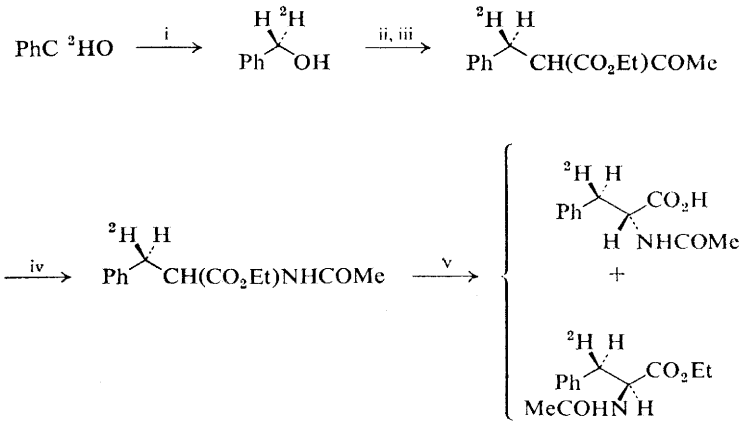
[110] C. Ressler and S. N. Banerjee, *J. Org. Chem.*, 1976, **41**, 1336.
[111] T. Sadeh, M. A. Davis, and R. W. Giese, *J. Pharm. Sci.*, 1976, **65**, 623.
[112] C. DeMarco, S. Dernini, A. Rinaldi, and D. Cavallini, *Gazzetta*, 1976, **106**, 211.
[113] A. Shrift, D. Bechard, C. Harcup, and L. Fowden, *Plant Physiol.*, 1976, **58**, 248.
[114] O. Leukart, M. Caviezel, A. Eberle, E. Escher, A. Tun-Kyi, and R. Schwyzer, *Helv. Chim. Acta*, 1976, **59**, 2184.
[115] L. Liatem and L. Christiaens, *Bull. Soc. chim. France*, 1975, 2294.
[116] M. Soroka and P. Mastalerz, *Roczniki Chem.*, 1976, **50**, 661.
[117] V. Cavrini, A. Chiarini, L. Garuti, G. Giovanninetti, and L. Franchi, *Il Farmaco, Ed. Sci.*, 1976, **31**, 599.

Compound	*Ref.*
p-(Diethyleneimidophosphoramido)phenylalanine	118
β-(1-Chloro-2-naphthyl)-DL-alanine	119
β-(1-Bromo-2-naphthyl)-DL-alanine	119
S-Adenosyl-homocysteine homologues	120, 121
S-Adenosyl-methionine homologues	121
2-Amino-ω-(uracil-1-yl)-n-alkanoic acids	122
2-Amino-ω-(thymin-1-yl)-n-alkanoic acids	122
L-3-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-allofuranos-3-yl)alanine	123
DL-3-(β-D-Ribofuranosyl)alanine	124
(2*R*)-L-Arabino-tetrahydroxybutyl)-(4*R*)-thiazolidinecarboxylic acid, and (2*S*)-epimer	125
	125

Labelled Amino-acids.—Further studies of the selective deuteriation of cobalt(III) complexes $[Co(en)_2(L\text{-amino-acidato})]^{n+}X_n^-$ [126, 127] and $[Co(NH_3)_4(L\text{-amino-acidato})]^+X^-$ (X = Cl, I, or NO_3) have been published (see Vol. 6, p. 20). Separation by ion-exchange chromatography of the diastereoisomeric complexes and α-^{2}H exchange at pH 9.6 led to further diastereoisomeric pairs, which on chromatographic separation and $NaBH_4$ reduction gave optically-pure D- and L-α-^{2}H labelled amino-acids.[126] The stereochemical course of the deuterium exchange reaction is controlled by the amino-acid side-chains since greater stereoselectivity was observed with aspartic and glutamic acid complexes than with the corresponding complexes of the amides of these amino-acids.[127] α-^{2}H-*S*-Benzyl-DL-cysteine has been prepared from benzyl chloromethyl sulphide and diethyl acetamidomalonate with the use of ^2HCl–^2H_2O as reagent for the hydrolysis–decarboxylation step;[128] [ββ-$^2H_2$]- and [αββ-$^2H_3$]-analogues were also prepared *via* the acetamidomalonate route. (β*R*)[β-^{2}H]-L-Homoserine, and (γ*R*)[γ-^{2}H]-DL-homoserine and its (γ*S*)-isomer, have been prepared by routes starting with *cis*-addition of NH_2OH to cinnamic acid, and with enantiomers of $Ph(CH_2)_2CH^2HOH$, respectively.[129] DL-[γ-^{2}H]-Leucine may be prepared by hydroboration of isobutene with $B_2{}^2H_6$, with conventional elaboration and modified acetamidomalonate synthesis.[130] A route providing nearly equal amounts of (2*S*,3*R*)- and (2*R*,3*R*)-[β-^{2}H]-phenylalanines has been developed (Scheme 3).[131] Condensation of 3-(^{2}H-formyl)-indole with hippuric acid and routine elaboration gives (2*S*,3*R*)-[3-^{2}H]-tryptophan.[132] Aromatic

[118] R. Poskiene, K. Karpavicius, A. Puzerauskas, O. V. Kildisheva, and I. L. Knunyants, *Izvest. Akad. Nauk. S.S.S.R., Ser. khim.*, 1976, 407.
[119] T. J. McCord, R. N. Watson, C. E. Du Bose, K. L. Hulme, and A. L. Davis, *J. Medicin. Chem.*, 1976, **19**, 429.
[120] R. T. Borchardt, J. A. Huber, and Y. S. Wu, *J. Org. Chem.*, 1976, **41**, 565.
[121] M. Legraverend and R. Michelot, *Biochimie*, 1976, **58**, 723.
[122] F.-S. Tjoeng, E. Kraas, E. Breitmayer, and G. Jung, *Chem. Ber.*, 1976, **109**, 2615.
[123] A. Rosenthal and A. J. Brink, *Carbohydrate Res.*, 1976, **46**, 289.
[124] A. Rosenthal and A. J. Brink, *Carbohydrate Res.*, 1976, **47**, 332.
[125] R. Bognar, Z. Gyoergydeak, L. Szilagyi, G. Horvath, G. Czira, and L. Radics, *Annalen*, 1976, 450.
[126] W. E. Keyes and J. I. Legg, *J. Amer. Chem. Soc.*, 1976, **98**, 4970.
[127] W. E. Keyes, R. E. Caputo, R. D. Willett, and J. I. Legg, *J. Amer. Chem. Soc.*, 1976, **98**, 6939.
[128] D. A. Upson and V. J. Hruby, *J. Org. Chem.*, 1976, **41**, 1353.
[129] D. Coggiola, C. Fuganti, D. Ghiringhelli, and P. Grasselli, *J.C.S. Chem. Comm.*, 1976, 143.
[130] J. A. Sogn, W. A. Gibbons, and S. Wolff, *Internat. J. Peptide Protein Res.*, 1976, **8**, 459.
[131] U. Nagai and J. Kobayashi, *Tetrahedron Letters*, 1976, 2873.
[132] S. Sawada and M. Kitagawa, *Bull. Koyoto Univ. Educ., Ser. B*, 1975, **47**, 19 (*Chem. Abs.*, **85**, 160484).

amino-acids labelled in the aryl moiety, *viz.* DL-tyrosine-[2′-^{3}H] [133] and 3-(4-azido-3,5-$^3H_2$-phenyl)-L-alanine,[134] have been prepared *via* the benzaldehyde [133] and by catalytic tritiation,[134] respectively. Distribution of the label in generally-



Reagents: i, yeast; ii, TsCl; iii, AcCHNaCO$_2$Et; iv, HN$_3$,H$^+$; v, α-chymotrypsin

Scheme 3

^{3}H-labelled amino-acids may be determined by ^{3}H-n.m.r. spectrometry (see ref. 191).

A range of familiar chemical operations has been used to create ^{11}C-labelled amino-acids; $^{11}CH_3I$ (from $^{11}CO_2$) has been used to synthesize methyl-labelled L-methionine [135] and DL- or L-ethionine,[136] while carboxyl-^{11}C-labelled DL-phenylglycine and DL-phenylalanine have been prepared by carboxylation of α-lithioisocyanides with $^{11}CO_2$.[137] Some idea of the potential of these compounds in *in vivo* studies is provided by data for methionine-^{11}C, produced by irradiation of methionine in an electron accelerator equipped with a tungsten target; the product is a γ-emitter with half-life 20 min.[138]

S-Ethylthiouronium-^{14}C bromide has been employed in the synthesis of labelled L-arginine from L-ornithine.[139]

Biological synthesis of ^{15}N-labelled amino-acids for n.m.r. studies has been achieved by algae cultured on $^{15}NO_3^{2-}$-containing media.[140] A related approach

[133] P. W. Jeffs, N. Johns, and D. B. Johnson, *J. Labelled Comp. Radiopharm.*, 1976, **12**, 133.
[134] W. Fischli, M. Caviezel, A. Eberle, E. Escher, and R. Schwyzer, *Helv. Chim. Acta*, 1976, **59**, 873.
[135] B. Langstrom and H. Lundqvist, *Internat. Appl. Radiat. Isotopes*, 1976, **27**, 357.
[136] D. Comar, J. C. Cartron, M. Maziere, and C. Marazano, *European J. Nuclear Medicine*, 1976, **1**, 11.
[137] W. Vaalburg, H. D. Beerling Van Der Molen, S. Reiffers, A. Rijskamp, M. G. Woldring, and H. Wynberg, *Internat. J. Appl. Radiat. Isotopes*, 1976, **27**, 153.
[138] G. Gundlach, E. L. Sattler, W. Trampisch, and U. Wagenbach, *Z. Naturforsch., Biosci.*, 1976, **31C**, 377; A. Donnerhack, E. L. Sattler, and W. Trampisch, *Strahlentherapie*, 1976, **151**, 240.
[139] K. V. Viswanathan, V. K. P. Unny, and S. Thyagarajan, *Radiochem. Radioanalyt. Letters*, 1976, **26**, 301.
[140] A. Severge, F. Juttner, E. Breitmayer, and G. Jung, *Biochim. Biophys. Acta*, 1976, **437**, 289.

using a high-methionine-producing mutant of *Saccharomyces cerevisiae* cultured on an $Na_2{}^{75}SeO_3$-containing medium leads to ^{75}Se-selenomethionine.[141]

Resolution of Amino-acids.—The main threads of the various aspects available for study under this heading, which have made up this section of this chapter in recent years, continue to be unravelled further. The chiral macrocyclic polyether project, in which the factors responsible for high chiral recognition of enantiomers of amino-acid perchlorates or amino-acid ester perchlorates [142] are being sought, has been extended to optically-pure 22-crown-6 polyethers bonded to polystyrene;[143] the project has been reviewed.[144] Several other chromatographic resolution procedures have been described, one [145] taking a similar approach to Cram's in bonding dibenzo-18-crown-6 ethers to ion-exchange resins and employing h.p.l.c. techniques, and others using chiral metal complexes, D-$[Co(en)_3]^{3+}$ on a weak acid cation exchange resin [146] and *N*-benzyl-L-leucinatocopper(II) bound to polystyrene,[147] as stationary phases. Copolymers of *N*-acryloyl-L-phenylalanine esters with ethylenediacrylate furnish cross-linked acrylamide-type polymers suitable for the resolution of DL-mandelic acid and related compounds;[148] this method could be tried for the resolution of analogous DL-amino-acid derivatives.

Gas–liquid chromatographic resolution of volatile amino-acid derivatives on an *N*-acyl-L-valinamide [149] on *N*-acyl-L,L-dipeptide ester [150, 151] as stationary phase is represented by further papers from innovators in this field. Higher resolution factors may be achieved when the *N*-acyl moiety of the stationary phase is a bulky alkanoyl grouping.[149] The mechanism of the differential interaction of a chiral stationary phase with enantiomeric amino-acid derivatives has been assumed to be predominantly due to different hydrogen bonding equilibrium constants, but this may need to be re-thought since some resolution can still be achieved with stationary phases incapable of hydrogen bonding.[152]

Stereospecific complex formation forms the basis of a procedure for the resolution of DL-aspartic or glutamic acids, in which combination with (L-argininato)$Cu(ClO_4)_2$ followed by crystallization and destruction of the complex with H_2S gives the amino-acid of moderate optical purity, but in low overall yield.[153]

Stereospecific complex formation of partly-resolved amino-acids with cobalt complexes is reflected in easily measured o.r.d. and c.d. spectra, from which the

[141] M. C. Saiz de Bustamante and C. A. Contreras, *Energ. Nuclear (Madrid)*, 1976, **20**, 305 (*Chem. Abs.*, **85**, 157950).
[142] S. C. Peacock and D. J. Cram, *J.C.S. Chem. Comm.*, 1976, 282.
[143] G. Dotsevi, Y. Sogah, and D. J. Cram, *J. Amer. Chem. Soc.*, 1976, **98**, 3038.
[144] D. J. Cram, R. C. Helgeson, L. R. Sousa, J. M. Timko, M. Newcomb, P. Moreau, F. De Jong, G. W. Gokel, and D. H. Hoffmann, *Pure Appl. Chem.*, 1975, **43**, 327.
[145] E. Blasius, K. P. Janzen, and G. Klautke, *Z. analyt. Chem.*, 1975, **277**, 374.
[146] J. Gaal and J. Inczedy, *Talanta*, 1976, **23**, 78.
[147] E. Tsuchida, H. Nishikawa, and E. Terada, *European Polymer J.*, 1976, **12**, 611.
[148] G. Blaschke and A. D. Schwanghart, *Chem. Ber.*, 1976, **109**, 1967.
[149] U. Beitler and B. Feibush, *J. Chromatog.*, 1976, **123**, 149.
[150] R. Brazell, W. Parr, F. Andrawes, and A. Zlatkis, *Chromatographia*, 1976, **9**, 57.
[151] W. A. Koenig, *Chromatographia*, 1976, **9**, 72.
[152] K. Stoelting and W. A. Koenig, *Chromatographia*, 1976, **9**, 331.
[153] T. Sakurai, O. Yamauchi, and A. Nakahara, *J.C.S. Chem. Comm.*, 1976, 553.

optical purity of the amino-acids may be calculated for samples of about 1 mg [154] (see also Vol. 7, p. 21).

Conventional resolution procedures have been described for 3,4,5-trimethoxyphenylglycine (resolved as its *Z*-derivative using a chiral base),[155] *N*-acylprolines [156] and 3-(3,4-methylenedioxyphenyl)-2-methylalanine hydrochloride [157] (resolved through preferential crystallization), and *N*-benzoyl-amino-acids (use of papain in stereoselective formation of *N*-benzoyl-amino-acid anilides).[158] Novel asymmetric transformation procedures in which more than 50% yield of one enantiomer can be obtained from a racemic amino-acid have been reported for phenylglycine esters, using (+)-tartaric acid and a carbonyl compound,[159] and for the formation of L-lysine from DL-α-amino-ε-caprolactam, based on the preferential crystallization of the L-enantiomer with $NiCl_2$ and EtOH accompanied by the Ni^{II}-catalysed racemization of residual D-enantiomer.[160]

Further studies of preferential adsorption by quartz of one enantiomer from DL-alanine hydrochloride in DMF use an extremely sensitive test system; the 'racemate' is a mixture of ^{3}H-labelled L-alanine and ^{14}C-labelled D-alanine.[161] The conclusion that *l*-quartz preferentially adsorbs L-alanine under these conditions appears to be soundly based, since a discrimination factor larger than would be expected for the different isotopic formulations of the two enantiomers was demonstrated.[161]

Analytical resolution of DL-amino-acids is covered also in section 6 of this chapter.

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and their Derivatives.—A review has appeared [162] of the solid-state hydrogen-bonding patterns for amino-acids established through neutron diffraction studies. A statistical treatment of available data from neutron diffraction studies has been published, which will be useful for computations of preferred conformations within amino-acid residues in peptides.[163]

A substantial amount of the 1976 literature concerns simple compounds, often repeating earlier *X*-ray studies. In this category papers describing L-isoleucine hydrochloride monohydrate (crystal form II),[164] D-allo-isoleucine hydrochloride monohydrate,[165] L-leucine,[166] histidine dihydrochloride,[167] DL-allo-threonine

[154] Y. Fujii, S. Hirasawa, and S. Takahashi, *Chemistry Letters*, 1976, 817.
[155] G. Schmidt and H. Rosenkranz, *Annalen*, 1976, 124.
[156] C. Hongo, M. Shibazaki, S. Yamada, and I. Chibata, *J. Agric. Food Chem.*, 1976, **24**, 903.
[157] S. Yamada, C. Hongo, M. Yamamoto, and I. Chibata, *Agric. Biol. Chem.*, 1976, **40**, 1425.
[158] J. R. Mohrig and S. M. Shapiro, *J. Chem. Educ.*, 1976, **53**, 586.
[159] J. C. Clark, G. H. Phillipps, M. R. Steer, L. Stephenson, and A. R. Cooksey, *J.C.S. Perkin I*, 1976, 471; J. C. Clark, G. H. Phillipps, and M. R. Steer, *ibid.*, 475.
[160] S. Sifniades, W. J. Boyle, and J. F. Van Peppen, *J. Amer. Chem. Soc.*, 1976, **98**, 3738.
[161] W. A. Bonner and P. R. Kavasmaneck, *J. Org. Chem.*, 1976, **41**, 2225.
[162] T. F. Koetzle and M. S. Lehmann, in 'Hydrogen Bond', ed. P. Schuster, G. Zundel, and C. Sandorfy, Vol. 2, North-Holland, Amsterdam, 1976, p. 457.
[163] R. Balasubramanian, *Indian J. Biochem. Biophys.*, 1976, **13**, 7.
[164] K. I. Varughese and R. Srinivasan, *Pramana*, 1976, **6**, 189 (*Chem. Abs.*, **84**, 188004).
[165] K. I. Varughese and R. Srinivasan, *J. Cryst. Mol. Struct.*, 1975, **5**, 317; *Acta Cryst.*, 1976 **B32**, 994.
[166] M. M. Harding and R. M. Howieson, *Acta Cryst.*, 1976, **B32**, 633.
[167] T. J. Kistenmacher and T. Sorrell, *J. Cryst. Mol. Struct.*, 1974, **4**, 419.

hydrobromide,[168] α-methyl-DL-tyrosine,[169] and allo-4-hydroxy-L-proline,[170] have appeared. 2′,3′-Dimethyl-3,5-di-iodo-DL-thyronine[171] and 3′-isopropyl-3,5-di-iodo-L-thyronine[172] have been subjected to *X*-ray crystal analysis and other physical studies; the latter compound is particularly interesting since it is the most potent known thyromimetic agent.[172]

The absolute configuration (2*S*,3*R*) has been assigned to 3-amino-2-hydroxy-4-phenyl-butanoic acid, present in bestatin, through *X*-ray crystal analysis of its methyl ester hydrobromide.[173] *X*-Ray studies similarly leading to stereochemical information for uncommon amino-acids have been carried out with ibotenic acid,[174] quisqalic acid,[175] oxypinnatanine,[31] and DL-1-aza-3-thiacyclohexane-6-carboxylic acid monohydrate,[176] while derivatives of common amino-acids which have been studied include *N*-carboxy-anhydrides of glycine[177] and alanine,[178] *N*-acetyl-L-glutamine,[179] N^{α}-toluene-*p*-sulphonyl-L-arginine methyl ester hydrochloride,[180] and the cysteine derivatives (2*R*,4*R*)-*S*-carboxymethyl-cysteine sulphoxide,[181] the corresponding sulphone,[182] and α-(*S*-cysteinyl)-thymine.[183]

N.M.R. Spectrometry.—General trends in n.m.r. studies appearing in the literature of 1976 are reflected in papers on amino-acids, where more confident exploration of nuclei other than ^{1}H and ^{13}C is being undertaken. Continuing consolidation of well-established areas of study is also represented in the 1976 literature.

Conformational studies of protein amino-acids in aqueous solutions are reaching something like a point of culmination, with a mass of data now available relating to the torsion angles about α-CH—β-CH_2 bonds. The three minimum energy staggered conformations for this bond are represented in different proportions for the various β-substituted alanines, and component vicinal coupling constants for the rotamers have been determined.[184] A similar objective is represented in studies of the three-carbon carboxyl-C to β-H coupling constants for aspartic acid and for derivatives of histidine and of cysteine;[185]

[168] P. Swaminathan and R. Srinivasan, *J. Cryst. Mol. Struct.*, 1975, **5**, 203.
[169] O. Gaudestad, A. Mostad, and C. Roemming, *Acta Chem. Scand.*, 1976, **B30**, 507.
[170] N. Shamala, T. N. G. Row, and K. Venkatesan, *Acta Cryst.*, 1976, **B32**, 3267.
[171] J. K. Fawcett, N. Camerman, and A. Camerman, *Canad. J. Chem.*, 1976, **54**, 1317.
[172] J. K. Fawcett, N. Camerman, and A. Camerman, *J. Amer. Chem. Soc.*, 1976, **98**, 587.
[173] H. Nakamara, H. Suda, T. Takita, T. Aoyagi, H. Umezawa, and Y. Iitaka, *J. Antibiotics*, 1976, **29**, 102.
[174] P. W. Borthwick and E. G. Steward, *J. Mol. Struct.*, 1976, **33**, 141.
[175] J. L. Flippen and R. D. Gilardi, *Acta Cryst.*, 1976, **B32**, 951.
[176] J. M. Medard, Y. Manguen, and N. Rodier, *Cryst. Struct. Comm.*, 1976, **5**, 843.
[177] H. Kanazawa, Y. Matsuura, N. Tanaka, M. Kakudo, T. Komoto, and T. Kawai, *Bull. Chem. Soc. Japan*, 1976, **49**, 954.
[178] H. Kanazawa, Y. Matsuura, N. Tanaka, M. Kakudo, T. Komoto, and T. Kawai, *Acta Cryst.*, 1976, **B32**, 3314.
[179] M. R. Narasimhamurthy, K. Venkatesan, and F. Winkler, *J.C.S. Perkin II*, 1976, 768.
[180] Y. Barrans and M. Cotrait, *Acta Cryst.*, 1976, **B32**, 2346.
[181] J. A. Staffa, C. Zervos, A. D. Mighell, and C. R. Hubbard, *Acta Cryst.*, 1976, **B32**, 3132.
[182] C. R. Hubbard, A. D. Mighell, J. A. Staffa, C. Zervos, and J. P. Konopelski, *Acta Cryst.*, 1976, **B32**, 2723.
[183] H. M. Beman, D. E. Zacharias, H. L. Carrell, and A. J. Verghase, *Biochemistry*, 1976, **15**, 463.
[184] J. Feeney, *J. Magnetic Res.*, 1976, **21**, 473.
[185] W. G. Espersen and R. B. Martin, *J. Phys. Chem.*, 1976, **80**, 741.

values 1.3 ± 0.3 Hz for the gauche conformation and 9.8 ± 0.3 Hz for the anti conformation lead to a conclusion that the carboxylate anion–β-substituent relationship is predominantly anti in aqueous solutions of these amino-acids.[185]

Useful generalizations have been reported for ^{13}C-chemical shift data for amino-acids.[186] The pH-dependence of ^{14}N-linewidths has been determined for glycine, alanine, lysine, and proline,[187] and temperature-dependence [188] and pH-dependence [140, 188] of ^{13}C—^{15}N coupling constants for ^{15}N-amino-acids have been assessed.

A routine use of ^{1}H-n.m.r. for the precise analysis of mixtures of phenylglycine with its dihydro-, tetrahydro-, and hexahydro-analogues has been described,[189] using tetramethylammonium bromide as internal standard. ^{3}H-N.m.r. spectra of ^{3}H-phenylalanine obtained through the Pt-catalysed $^{3}H_2O$ exchange procedure show an average 26% exchange in the side-chain, mostly at the β-position, with 74% exchange of the phenyl protons, fairly equally distributed.[190] This spectrometric assay has also been applied to other generally-3H-labelled amino-acids.[190]

Fundamental studies of polycrystalline amino-acids at 130—500 K point to origins of spin–lattice relaxation in reorientation of protonated amino-groups and this results in the relaxation of other protons by spin-exchange.[191] Perturbation of spin-lattice relaxation times of lanthanide(III) ion binding can be exploited in conformational assignments to L-hydroxyproline in $^{2}H_2O$.[192] Further studies of ^{2}H and ^{14}N pure quadrupole resonance in amino-acids have been reported.[193]

Amino-acid derivatives studied by n.m.r. include *N*-acetyl-L-proline deuteromethyl ester (^{1}H and ^{13}C-n.m.r. data interpreted in terms of rotational barriers in support of previous conclusions),[194] N^{α}-acetyl-L-prolinamide (^{1}H n.m.r. conformational studies),[195] and Boc-amino-acid esters of glycine, alanine, and phenylalanine (continuing ^{1}H and ^{13}C-n.m.r. studies of conformational equilibria involving the amide moiety).[196]

Interaction between aromatic amino-acids and nucleic acid models has already been extensively studied and is now supplemented by studies of solutions containing adenosine-5′-phosphate and phenylalanine,[197] tryptophan,[197] or *O*-methyl-tyrosine,[198] and the solution behaviour of adenin-(5′-*N*)-yl derivatives

[186] J. C. MacDonald, G. C. Bishop, and M. Mazurek, *Canad. J. Chem.*, 1976, **54**, 1226.
[187] E. A. Cohen, A. M. Shiller, S. I. Chan, and S. L. Manatt, *Org. Magnetic Res.*, 1975, **7**, 605.
[188] F. Blomberg, W. Maurer, and H. Rueterjans, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 1409.
[189] R. J. Warren, J. E. Zarembo, D. B. Staiger, and A. Post, *J. Pharm. Sci.*, 1976, **65**, 138.
[190] J. M. A. Al-Rawi, J. A. Elvidge, J. R. Jones, V. M. A. Chambers, and E. A. Evans, *J. Labelled Comp. Radiopharm.*, 1976, **12**, 265.
[191] E. R. Andrew, W. S. Hinshaw, M. G. Hutchins, and R. O. I. Sjoeblum, *Mol. Phys.*, 1976, **31**, 1479.
[192] F. Inagaki, M. Tasumi, and T. Miyazawa, *J.C.S. Perkin II*, 1976, 167.
[193] M. J. Hunt and A. L. Mackay, *J. Magnetic Res.*, 1976, **22**, 295.
[194] B. P. Roques, S. Combrisson, and R. Wasylishen, *Tetrahedron*, 1976, **32**, 1517.
[195] L. Pogliani, M. Ellenberger, J. Valat, and A. M. Bellocq, *Internat. J. Peptide Protein Res.*, 1975, **7**, 345.
[196] H. Kessler and M. Molter, *J. Amer. Chem. Soc.*, 1976, **98**, 5969.
[197] S. V. Zenin, *Mol. Biol. (Moscow)*, 1976, **10**, 981.
[198] S. V. Zenin, *Studies Biophys.*, 1976, **55**, 175.

of phenylalanine, tyrosine, and tryptophan methyl esters.[199] Related studies [200] show that lysine and arginine form complexes with DNA, polynucleotides, nucleosides, and nucleic acid bases.

Optical Rotatory Dispersion and Circular Dichroism Spectra.—A positive Cotton effect at 215 nm in the c.d. spectrum of *N*-(γ-glutamyl)-2-amino-3-hexanone, isolated from the mushroom *Russula ochroleuca*, allows the assignment of the L-configuration to the chiral centre in the glutamyl moiety, but the opportunity was not taken to interpret the negative Cotton effect at 280 nm for the assignment of absolute configuration to the other chiral centre in this compound;[30] the (*S*)-configuration for this chiral centre shown in (3) might be assigned on the basis of the octant rule, but support is required from data for closely similar compounds when semi-empirical assignments are made to acyclic ketones.

The alternative to the use of the 215 nm carboxylate Cotton effect for the assignment of absolute configuration to an α-amino-acid is the introduction of an appropriate chromophore, usually by *N*-substitution, which results in diagnostic Cotton effect behaviour, and further advocacy of the use of fluorescamine derivatives for this purpose (see also Vol. 8, p. 19) has been published.[201] *N*-(3-Methyl-2-quinoxaloyl)amino-acids are new representatives of chromophore-substituted amino-acids, but these appear to lack a consistent relationship between sign of Cotton effect and absolute configuration.[202]

An investigation of the chirospectroscopic properties of the selenide chromophore has been made, in the framework of a c.d. study of seleno-amino-acids.[203]

Chromophore–chromophore interaction effects arise in studies of the extrinsic Cotton effect contribution to the c.d. of 3,5-di-iodo-L-tyrosine near 320 nm induced by binding to bovine serum albumin,[204] and in the c.d. of adenin-(5′-*N*)-yl derivatives of phenylalanine, tyrosine, and tryptophan methyl esters,[199] interpreted in the latter case to show coplanarity of adenine and aromatic moieties in these compounds.

There are few examples of the effect of isotopic substitution on optical rotation data, but a new result, that ^{15}N-alanine shows lower o.r.d. amplitudes at corresponding wavelengths than its ^{14}N counterpart, implies surprisingly large differences.[205]

Mass Spectrometry.—Chemical ionization techniques continue to show their relative advantages in mass spectrometric analysis of amino-acids, with H_2 as a more suitable reactant gas than CH_4 since it gives lower abundance of M + 1 ions and increased abundance of fragment ions with advantages, therefore, in structure determination.[206] This study included β-, γ-, and higher homologous amino-acids as well as 14 common α-amino-acids.[206] Free amino-acids have

[199] B. V. Tyaglov, S. V. Zenin, E. S. Gromova, G. B. Sergeev, and Z. A. Shabarova, *Mol. Biol. (Moscow)*, 1976, **10**, 347.
[200] V. I. Bruskov and V. N. Bushuev, *Bio-org. Khim.*, 1975, **1**, 1606.
[201] V. Toome, B. Wegrzynski, and G. Reymond, *Biochem. Biophys. Res. Comm.*, 1976, **69**, 206.
[202] M. M. El-Abadelah, S. S. Sabri, M. Z. Nazer, and M. F. Za'ater, *Tetrahedron*, 1976, **32**, 2931.
[203] J. C. Craig, S. Y. C. Lee, G. Zdansky, and A. Fredga, *J. Amer. Chem. Soc.*, 1976, **98**, 6456.
[204] N. Okabe, N. Manabe, R. Tokuoka, and K. Tomita, *J. Biochem. (Tokyo)*, 1976, **80**, 455.
[205] W. Darge, I. Laczko, and W. Thiemann, *J. Radioanalyt. Chem.*, 1976, **30**, 521.
[206] C. W. Tsang and A. G. Harrison, *J. Amer. Chem. Soc.*, 1976, **98**, 1301.

been studied also by secondary ion mass spectrometry, which leads to high abundance secondary ion–parent peaks in mass spectra.[207] Parallel studies of chemical ionization mass spectrometry in this area [208, 209] and of field desorption [210] and negative ion [211] techniques are mostly concerned with derivatives of amino-acids (thiohydantoins [209, 210] and *N*-nitrobenzoyl amino-acid methyl esters [211]).

Derivativization has, of course, been the first stage in nearly all previous studies of amino-acids, and the more routine papers in the 1976 literature continue this tradition with studies of methylthiohydantoins,[212] fluorescamine derivatives,[213] *N*-acetyl amino-acid *p*-nitrobenzyl esters,[214] *N*-dimethylamino-methylene amino-acid methyl esters,[215] and *N*-pentafluoropropionyl amino-acid hexafluoropropyl esters.[216]

Further illustration of the sensitivity of the g.c.–m.s. combination is provided [216] by a study of the determination of glutamic acid and γ-aminobutyric acid in rat brain nuclei, based upon *ca.* 50 μg protein samples (see also ref. 318).

Other Physical and Theoretical Studies.—A study of crystalline amino-acids by photoelectron spectroscopy has been reported,[217] indicating extensive intermolecular hydrogen bonding between zwitterionic tautomers; i.r. spectra of α-amino-acids are also usually interpreted in terms of zwitterionic structures, but γ-aminobutyric acid has been reported [218] to be capable of adopting the alternative uncharged form in the solid state. Far i.r. spectra (10—150 cm^{-1}) of α-amino acids at temperatures 80 and 300 K contain characteristic peaks for all common amino-acids except glycine.[219] Raman study of ^{15}N-glycine has been described.[220]

Gas-phase ionization energies of simple molecules are readily determined by He^{I} photoelectron spectroscopy, but only those amino-acids with alkyl side-chains were found to be sufficiently stable for study by this technique.[221]

Semi-empirical m.o. calculations indicate non-planar structures for *N*-acetyl-L-alanine *N*-methylamide and MeCONHMe in the solid state,[222] and comparable studies [223] for nineteen α-amino-acids predict three-dimensional structures corresponding closely with those determined by *X*-ray crystal structure and n.m.r. analysis.

[207] A. Benninghoven, D. Jaspers, and W. Sichtermann, *Appl. Phys.*, 1976, **11**, 35.
[208] D. Issacher and J. Yinon, *Clinica Chim. Acta*, 1976, **73**, 307.
[209] T. Suzuki, K.-D. Song, Y. Hagaki, and K. Tuzimura, *Org. Mass Spectrom.*, 1976, **11**, 557.
[210] H. R. Schulten and B. Wittmann-Liebold, *Analyt. Biochem.*, 1976, **76**, 300.
[211] B. J. Stapleton and J. H. Bowie, *Org. Mass Spectrom.*, 1976, **11**, 429.
[212] J. Lindeman and R. E. Lovins, *Analyt. Biochem.*, 1976, **75**, 682.
[213] J.-J. Shieh, K. Leung, and D. M. Desiderio, *Org. Mass Spectrom.*, 1976, **11**, 479.
[214] C. L. Brown and C. L. Chan, *J. Amer. Chem. Soc.*, 1976, **98**, 2682.
[215] I. Horman and F. J. Hesford, *Biomed. Mass Spectrom.*, 1974, **1**, 115.
[216] L. Bertilsson and E. Costa, *J. Chromatog.*, 1976, **118**, 395.
[217] D. T. Clark, J. Peeling, and L. Colling, *Biochim. Biophys. Acta*, 1976, **453**, 533.
[218] P. V. Huong and J. C. Cornut, *J. Chem. Phys.*, 1976, **65**, 4748.
[219] X. Gerbaux and A. Hadni, *Compt. rend.*, 1976, **282**, 181, 397.
[220] H. Steinback, *J. Raman Spectroscopy*, 1976, **5**, 49.
[221] L. Klasinc, *J. Electron. Spectrosc. Related Phenomena*, 1976, **8**, 161.
[222] V. Renugopalakrishnan and R. Rein, *Biochim. Biophys. Acta*, 1976, **434**, 164.
[223] A. A. Akhrem, V. P. Golubovich, S. G. Galaktionov, and G. V. Nikiforovich, *Vestsi Akad. Navuk Belarusk. S.S.R., Ser. khim. Navuk*, 1976, 77.
[224] H. D. Belitz and H. Wieser, *Z. Lebensm.-Untersuch.*, 1976, **160**, 251.

One of the simplest possible physical studies forms the basis of the report [224] that the sweet or bitter taste of amino-acids and peptides may be related to their three-dimensional arrangement of polar and hydrophobic groups.

5 Chemical Studies of Amino-acids

Racemization.—The application of adventitious racemization for dating fossils and corresponding biological samples has been surveyed in this section in preceding volumes of this report. The optical purity of amino-acids existing in these samples, either in the free state or as constituents of residual collagen, is related to the age of the sample, and confident use of this fact has been made after racemization rate constants appropriate for the particular site have been determined. Further application of the method has been reported for 4220-year-old Bristlecone pine and fossil wood from Kalambo Falls, Zambia, with data obtained on 13 amino-acids extracted from the samples.[225] Racemization rate constants derived from these data, for proline and hydroxyproline, lead to a date >110 000 years for the Acheulian–Sangoan transition at the site, based on the optical purity of amino-acids found in contemporary samples.[225] The possible sources of error in the method have been well appreciated, and studies of fossil coral samples of known age from Pleistocene sites [226] indicate the inadequacy of the method, based on epimerization and racemization of L-isoleucine, when leaching of free amino-acids from samples, or later contamination, or variations in the mineral content leading to variable racemization rates, can be suspected to have occurred. Meanwhile, several laboratories are using the method, a further example being a report of preliminary studies of the state of the amino-acids comprising the collagen of mammoth bone.[227]

Novel systems which are effective in the racemization of L-alanine in alkaline aqueous solutions are copper(II) salts with nitrosophenols [228] and copper(I) oxide.[229] In the latter case, copper(II) ions are probably also the effective species, formed from Cu_2O during aerial oxidation of the amino acid.

Reactions of Amino-acids Involving Amino- and Carboxy-groups.—There is still scope for the formation of novel functional derivatives in the amino-acid series, current examples being the synthesis of DL-phenylalanine ortho-esters [$Ph(CH_2)_3CN \rightarrow PhCH_2CH_2C(=NCl)OEt \rightarrow NH_2CH(CH_2Ph)C(OEt)_3$],[230] and formation of *NN*-dichloro-amino-acids.[231] In the latter example, a quantitative yield of $Cl_2NCH_2CO_2H$ is obtained from the amino-acid by treatment with Bu^tOCl in MeOH at 0 °C (an analogous product is formed from β-alanine), but the tendency for these derivatives to explode at temperatures not much above room temperature should be noted. Factors determining the stability of chloro-derivatives of amino-acids have been assessed.[232]

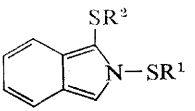
[225] C. Lee, J. L. Bada, and E. Peterson, *Nature*, 1976, **259**, 183.
[226] J. F. Wehmiller, P. E. Hare, and G. A. Kujala, *Geochim. Cosmochim. Acta*, 1976, **40**, 763.
[227] G. Dungworth, A. W. Schwartz, and L. Van De Leemput, *Comp. Biochem. Physiol.*, 1976, **53**(4B), 473.
[228] K. Hirota, H. Koizumi, Y. Hironaka, and Y. Izumi, *Bull. Chem. Soc. Japan*, 1976, **49**, 289.
[229] A. Tai, K. Okada, T. Masuda, and Y. Izumi, *Bull. Chem. Soc. Japan*, 1976, **49**, 310.
[230] J. Zemlicka and M. Murata, *J. Org. Chem.*, 1976, **41**, 3317.
[231] J. Vit and S. J. Barer, *Synth. Comm.*, 1976, **6**, 1.
[232] J. J. Kaminski, N. Bodor, and T. Higuchi, *J. Pharm. Sci.*, 1976, **65**, 553.

Quaternization of amino-acids may be achieved under mild conditions using MeI and $KHCO_3$ in MeOH.[233]

Thermal degradation of amino-acids is the subject of continuing studies, with recent emphasis on aspartic acid[234] and sulphur-containing amino-acids.[235, 236] Under N_2 at temperatures between 350—650 °C, succinic acid is the major condensable product (12%) with dimethylmaleic anhydride, from aspartic acid,[234] while S is lost as CS_2 or COS when the common sulphur-containing amino-acids are heated at 850 °C under N_2 to give degradation products of a similar type to those obtained from simple aliphatic amino-acids.[235] A variety of sulphur-containing products is formed from cysteine derivatives heated in soya bean oil at 200 °C.[236]

Strecker degradation of α-amino-acids with phenalene-1,2,3-trione hydrate, giving the corresponding aldehyde, NH_3, and CO_2, has been studied from the structure–reactivity viewpoint.[237] A similarly well-known reaction of amino-acids, nitrous acid deamination, has been studied for its stereochemical consequences with isoleucine derivatives.[238]

Colour reactions of amino-acids of particular current interest, formation of fluorescent products with fluorescamine,[239] and with *o*-phthalaldehyde and a thiol,[240] have been discussed. In the latter case, the 1-(alkylthio)-2-substituted iso-indoles (22) are the surprising products whose intense fluorescence allows the picomole level determination of α-amino-acids.[240]



(22)

L-Amino-acids and derivatives employed in asymmetric synthesis and in stereoselective synthesis are: L-amino-acid t-butyl esters used in asymmetric transamination with ketones,[241] aldehydes,[41] and keto-esters,[41] boron hydride–L-leucine methyl ester complex for the asymmetric reduction of ketones,[242] L-glutamic acid in the enantioselective synthesis of epoxy-ketones,[243] and similar use of L-lysine in new syntheses of L-pipecolic acid and of (*S*)-(+)-coniine.[244]

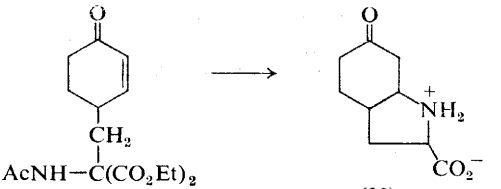
Reactions Involving Side-chains of α-Amino-acids.—In this section, papers are brought together covering reactions of side-chain functional groups in amino-

[233] F. C. M. Chen and N. L. Benoiton, *Canad. J. Chem.*, 1976, **54**, 3310.
[234] A. W. Fort, J. M. Patterson, R. Small, B. K. Bandlish, and W. T. Smith, *J. Org. Chem.*, 1976, **41**, 3697.
[235] J. M. Patterson, C.-Y. Shille, and W. T. Smith, *J. Agric. Food Chem.*, 1976, **24**, 988.
[236] F. Ledl, *Z. Lebensm.-Untersuch.*, 1976, **161**, 125.
[237] W. I. Awad, S. Nashed, S. S. M. Hassan, and R. F. Zakhary, *J.C.S. Perkin II*, 1976, 128.
[238] W. Kirmse and G. Rauleder, *Annalen*, 1976, 1333.
[239] H. Nakamura and J. J. Pisano, *J. Chromatog.*, 1976, **121**, 33, 79.
[240] S. S. Simons and D. F. Johnson, *J. Amer. Chem. Soc.*, 1976, **98**, 7098.
[241] S. Yamada, N. Ikota, and K. Achiwa, *Tetrahedron Letters*, 1976, 1001.
[242] M. F. Grundon, D. G. McCleery, and J. W. Wilson, *Tetrahedron Letters*, 1976, 295.
[243] S. Yamada, N. Oh-hashi, and K. Achiwa, *Tetrahedron Letters*, 1976, 2557, 2561.
[244] K. Aketa, S. Terashima, and S. Yamada, *Chem. Pharm. Bull.*, 1976, **24**, 621.

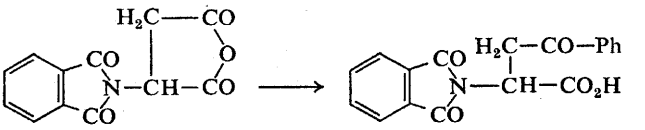
acids. An attempted synthesis of a β-cyclohexenonyl-alanine *via* the acetamidomalonate route led instead to the indolinonecarboxylate (23).[245]

Alkylation rates for methionine derivatives and related model compounds indicate the high nucleophilicity of the methionine sulphur atom; among common nucleophiles, only the thiolate anion has higher nucleophilicity.[246]

Friedel–Crafts acylation of benzene with phthalylaspartic anhydride and $AlCl_3$ gives 3-benzoyl-2-phthalimidopropionic acid (24), consistent with anhydride



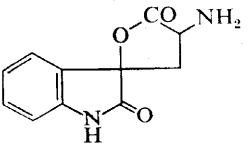
(23)



(24)

ring-opening in the predicted direction (opposition by the phthalimido-group of build-up of charge at the neighbouring carbon atom),[247] although the isomeric structure had been assigned earlier to the reaction product.

Reactions of tryptophan derivatives involving the indole grouping include the formation of β-3-oxindolylalanine during the hydrolysis of proteins in 6M hydrochloric acid, if dithioglycollic acid or cystine is present,[248] the same product is formed from tryptophan itself.[248] L-Tryptophan gives two diastereoisomers of the spirolactone (25) on treatment with t-butyl hydroperoxide and $FeSO_4$.[249]



(25)

Inorganic radical anion oxidation of tryptophan has been studied.[250] Condensation products are formed from tryptophan and α-keto-acids *via* 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and its homologues.[251]

[245] P. Sawlewicz, I. Barska, M. Smulkowski, J. Gumieniak, T. Czarnomska, M. Dzieduszycka, and E. Borowski, *Roczniki*, 1976, **50**, 1005.

[246] G. A. Rogers, N. Shaltiel, and P. D. Boyer, *J. Biol. Chem.*, 1976, **251**, 5711.

[247] W. G. Reifenrath, D. J. Bertelli, M. J. Micklus, and D. S. Fries, *Tetrahedron Letters*, 1976, 1959.

[248] T. Nakai and T. Ohta, *Biochim. Biophys. Acta*, 1976, **420**, 258.

[249] G. Stoehrer, *J. Heterocyclic Chem.*, 1976, **13**, 157.

[250] M. L. Posener, G. E. Adams, P. Wardman, and R. B. Cundall, *J.C.S. Faraday I*, 1976, **72**, 2231.

[251] N. T. Chu and F. M. Clydesdale, *J. Food Sci.*, 1976, **41**, 891.

Specific Reactions of Amino-acids Related to Biochemical Processes.—The decarboxylation of L-threonine by a di-aquo-cobyric acid–thiol complex has been investigated.[252] Continuing studies of the condensation of pyridoxal with amino-acids to form Schiff bases provide comparisons of the reactivity of α-amino-acids, α,ω-di-amino acids, and ω-amino-acids.[253]

Effects of Electromagnetic Radiation on Amino-acids.—A larger number of references than usual has been collected for this section, and rather than leave particular areas of current study unrepresented, there has been even greater restraint on the amount of space used for each reference. Except for a study of the formation of methyl radicals by u.v. photolysis of *N*-methyl- and *N*-acetyl-amino-acids at 77 K,[254] the photochemical studies in the recent literature are concerned with the aromatic amino-acids. The effect of sodium alginate on the rates of dye-sensitized photo-oxidation of tyrosine and tyramine has been studied,[255] and the products of irradiation of phenylalanine at 253.7 nm in H_2O_2 have been shown to be aspartic acid, serine, alanine, ammonia, and a trace of lysine.[256] Effects of solvent polarity, metal ions, and sulphur compounds on the phosphorescence and fluorescence of tryptophan at 77 K have been assessed.[257] Decay rates and spin-lattice relaxation rates for the lowest excited triplet states of tyrosine and tryptophan at 1.34 K have been measured;[258] and flash photolysis products of aqueous solutions of tryptophan and tryptophan-containing peptides have been studied.[259]

γ-Irradiation of aqueous solutions of amino-acids [260–263] gives products of H-abstraction resulting from secondary effects of the formation of hydroxyl radicals;[260] penicillamine gives thiyl radicals in de-aerated 1M $HClO_4$,[261] and proline gives products of de-amination or ring cleavage together with hydroxy-proline,[262] while peroxyglycine radicals are formed in irradiated glycine solutions under O_2.[263] γ-Irradiated solid glycine [264] and L-valine [265] undergo partial degradation. These results are supplemented by results from *X*-irradiation of cysteine hydrochloride [266] and aspartic acid hydrochloride [267] leading to products from primary radical formation; the e.s.r. spectra of *X*-irradiated solid L-amino-acids differ in nearly every case from those of corresponding DL-amino-acids.[268] M.o. calculations show that loss of an electron under ionizing radiation would be expected to occur from the carboxy-grouping of aliphatic amino-acids, while for tyrosine and tryptophan the source of the excited electron is the aromatic

[252] S. H. Ford and H. C. Friedmann, *Biochem. Biophys. Res. Comm.*, 1976, **72**, 1077.
[253] A. M. Der Garabedian and M. A. Der Garabedian, *F.E.B.S. Letters*, 1976, **72**, 87.
[254] G. H. Schepel and H. Neubacher, *Radiation Environ. Biophys.*, 1976, **13**, 49.
[255] G. R. Seely and R. L. Hart, *Photochem. Photobiol.*, 1976, **23**, 1, 7.
[256] A. S. Ansari, S. Tahib, and R. Ali, *Experientia*, 1976, **32**, 573.
[257] L. A. King and J. N. Miller, *Biochim. Biophys. Acta*, 1976, **446**, 206.
[258] K. W. Rousslang and A. L. Kwiram, *Chem. Phys. Letters*, 1976, **39**, 226.
[259] H. Templer and P. J. Thistlethwaite, *Photochem. Photobiol.*, 1976, **23**, 79.
[260] T. Masuda, S. Nakano, K. Yoshihara, and M. Kondo, *J. Radiation Res.*, 1976, **17**, 63.
[261] G. C. Goyal and D. A. Armstrong, *J. Phys. Chem.*, 1976, **80**, 1848.
[262] N. A. Duzhenkova and J. Kopoldova, *Khim. vysok. Energii*, 1976, **10**, 351.
[263] S. Abramovitch and J. Rabani, *J. Phys. Chem.*, 1976, **80**, 1562.
[264] P. R. Crippa, C. Giori, and A. Vacli, *Radiat. Eff.*, 1976, **29**, 143.
[265] D. G. Howitt, R. M. Glaeser, and G. Thomas, *J. Ultrastruct. Res.*, 1976, **55**, 457.
[266] W. W. H. Kon and H. C. Box, *J. Chem. Phys.*, 1976, **64**, 3060.
[267] S. M. Adams, E. E. Budzinski, and H. C. Box, *J. Chem. Phys.*, 1976, **65**, 998.
[268] H. Shields and P. J. Hamrick, *J. Chem. Phys.*, 1976, **64**, 263.

moiety;[269] for phenylalanine, there is roughly equal probability of electron abstraction from carboxy and phenyl groupings.[269]

Reference has been made in the preceding paragraph to the selective radiolysis of L- and DL-amino-acids, and for solid samples the differences in crystal structures account for these results. However, the interest which has been stimulated recently (Vol. 7, p. 25; Vol. 8, p. 16) in the enantioselective destruction of racemic amino-acids by irradiation of solutions has been expressed by the appearance of papers from workers new to the field. While no support for the notion that positrons react at different rates with D- and L-tryptophan has been secured, since positronium yields from the interaction of ^{22}Na positrons with each enantiomer are identical,[270] a relative enrichment of *ca.* 19% has been reported for the D-enantiomer as a result of 12 weeks' irradiation of a 0.005% aqueous solution of DL-tryptophan by ^{32}P β-radiation (0.57 MeV) resulting in 33% decomposition of the sample and indicating preferential decomposition of the L-enantiomer.[271] γ-Radiolytic decarboxylation of D-phenylalanine-1-^{14}C occurs *ca.* 2.7 times faster than for the L-enantiomer,[272] an extraordinary example of the selective destruction of one enantiomer by non-polarized radiation.

6 Analytical Methods

Reviews of sample preparation [273] and determination [273, 274] of amino-acids in biological samples have been published.

Gas–Liquid Chromatography.—Quantitative analysis of amino-acids by g.l.c. has been reviewed.[275]

Conversion of amino-acids into volatile derivatives is the unavoidable preliminary stage in their analysis by g.l.c., and perfluoroalkyl derivatives remain the most widely used, with, as usual, strong advocacy of alternative new types. *N*-Trifluoroacetyl amino-acid n-butyl esters [276–279] remain in wide use, together with analogous ethyl [14c] and trimethylsilyl [280, 281] esters. *N*-Heptafluorobutyryl amino-acid isobutyl esters of fifty amino-acids have been studied by g.l.c.;[282] analogous iso-amyl esters have been included in a broad study.[276] *N*-Pentafluoropropionyl-γ-aminobutyric acid hexafluoroisopropyl ester and the analogous

[269] D. A. Dixon and W. N. Lipscomb, *J. Biol. Chem.*, 1976, **251**, 5992.
[270] W. Brandt and T. Chiba, *Phys. Letters*, 1976, **57A**, 395.
[271] W. Darge, I. Laczko, and W. Thiemann, *Nature*, 1976, **261**, 522.
[272] O. Merwitz, *Radiat. Environment. Biophys.*, 1976, **13**, 63.
[273] E. Reid, *Analyst*, 1976, **101**, 1.
[274] J. E. Hammond and J. Savory, *Ann. Clin. Lab. Sci.*, 1976, **6**, 158.
[275] S. V. Vitt, M. B. Saporovskaya, G. V. Avvakumov, and V. M. Belikov, *Uspekhi. Khim.*, 1976, **45**, 548.
[276] G. E. Otter and L. Taylor, *J. Inst. Brew., London*, 1976, **82**, 264.
[277] L. A. Appelqvist and B. M. Nair, *J. Chromatog.*, 1976, **124**, 239.
[278] K. Samukawa, T. Nagai, and S. Takahashi, *Radioisotopes*, 1976, **25**, 135.
[279] V. Amico, G. Oriente, and C. Tringali, *J. Chromatog.*, 1976, **116**, 439.
[280] M. Schwarz and G. Michael, *J. Chromatog.*, 1976, **118**, 101.
[281] M. Donike, *J. Chromatog.*, 1975, **115**, 591.
[282] R. J. Siezen and T. H. Mague, *J. Chromatog.*, 1977, **130**, 151.
[283] P. Felker, *Anal. Biochem.*, 1976, **76**, 192.
[284] I. M. Moodie and R. D. George, *J. Chromatog.*, 1976, **124**, 315.
[285] M. Makita, S. Yamamoto, and M. Kono, *J. Chromatog.*, 1976, **120**, 129; M. Makita, S. Yamamoto, K. Sakai, and M. Shiraishi, *ibid.*, 1976, **124**, 92; M. Makita and S. Yamamoto, *Yakugaku Zasshi*, 1976, **96**, 777; *ibid.*, 1976, **96**, 813.

L-glutamic acid derivative form the basis of a g.c.–m.s. analysis of amino-acid transmitters in rat brain nuclei.[216]

N-Acetyl amino-acid methyl [283] and n-propyl esters [284] are also advocated as volatile derivatives for the g.l.c. analysis of amino-acids, and several papers have been published [285] describing the use of *N*-isobutyloxycarbonyl amino-acid methyl esters for the same purpose.

Husek introduced 1,3-dichloro-1,1,3,3-tetrafluoropropanone as a reagent in 1974 for derivatization of amino-acids for g.l.c. analysis (Vol. 7, p. 26), and has reported further [286] on the merits of the resulting cyclic stable derivatives. Ongoing study of g.l.c. separation of *N*-phenylthiohydantoins is represented by independent reports [287] establishing conditions for resolution of leucine and isoleucine PTH's.

Mention has been made in a preceding section of the use of g.l.c. for the determination of the optical purity of amino-acids, in which volatile derivatives are separated on optically-active stationary phases;[149–152] the alternative approach, in which diastereoisomeric derivatives of the amino-acid are formed, is illustrated for aspartic acid, for which *N*-trifluoroacetylation followed by esterification with (+)-3-methyl-2-butanol gives a pair of diastereoisomeric derivatives which may be separated on normal g.l.c. stationary phases.[288]

Ion-exchange Chromatography.—The main categories into which papers could be located under this heading are advances in instrumentation, and improvements in ion-exchange separation of particular amino-acids as part of a quantitative analysis routine. Opportunities to reduce the time taken in automated amino-acid analysis of mixtures have been studied;[289, 290] in the determination of phenylalanine and tyrosine, the use of anisylalanine as internal standard has been proposed,[290] and a similar use of 5-methyltryptophan in quantitative analysis of tryptophan has been suggested.[291]

Attention has been given to the separation of asparagine and glutamine from other amino-acids in the analysis of biological fluids.[292] A broad study has been made [293] of the automated amino-acid analysis of sulphur-containing amino-acids and their derivatives, and conditions for separation of *S*-carboxymethyl cysteine from its Se analogue have been established.[294]

Further illustration of the potential of the *o*-phthalaldehyde–mercaptoethanol system for automated fluorimetric amino-acid analysis has been provided in the determination of nanomole quantities of amino-acids in blood plasma samples.[295]

High Performance Liquid Chromatography.—In terms of cost, equipment for h.p.l.c. can compete with that for g.l.c., but in terms of speed in the provision

[286] P. Husek, *Ergeb. Exp. Med.*, 1976, **20**, 24 (*Chem. Abs.*, **85**, 58973).
[287] P. D. Van Wassenaar and R. B. Iyengar, *J. Chromatog.*, 1976, **118**, 99; J. P. Van Eerd, *Anal. Biochem.*, 1976, **71**, 612.
[288] W. Rahn, H. Eckstein, and W. A. Koenig, *Z. physiol. Chem.*, 1976, **357**, 1223.
[289] R. S. Ersser, *Med. Lab. Sci.*, 1976, **33**, 257.
[290] R. S. Ersser, *Med. Lab. Sci.*, 1976, **33**, 57.
[291] M. Wilkinson, G. A. Iacobucci, and D. V. Myers, *Analyt. Biochem.*, 1976, **70**, 470.
[292] S. Gross and M. L. Maskaleris, *Clin. Chem.*, 1976, **22**, 1233.
[293] L. Bowie, J. C. Crawhall, N. Gochman, K. Johnson, and J. A. Schneider, *Clin. Chim. Acta*, 1976, **68**, 349.
[294] A. Rinaldi, P. Cossu, and C. DeMarco, *J. Chromatog.*, 1976, **120**, 221.
[295] M. Roth, *J. Clin. Chem. Clin. Biochem.*, 1976, **14**, 361.

of data, the former technique is a clear leader; the scope for use of inexpensive components has been considered in a report [296] of the separation of taurine, γ-aminobutyric acid, and 5-hydroxytryptophan at <50 pmole levels from brain tissue samples, requiring between 3 and 7 minutes for each analysis. Dansyl-amino-acids may be readily separated by h.p.l.c.;[297] the literature on h.p.l.c. of PTH's has been boosted considerably during 1976,[298–302] and includes a reasoned comparison of g.l.c. and h.p.l.c. for the identification of PTH's.[302] The speedier h.p.l.c. technique should be considered to complement g.l.c. rather than replace it, since resolution of all amino-acids is not yet possible but those difficult to resolve by g.l.c. are readily resolved by h.p.l.c. (and *vice versa*).

Thin-layer Chromatography.—A new edition of a standard textbook includes an extensive survey of the chromatography of amino-acids and peptides.[303]

Analytical separation of particular classes of amino-acid by t.l.c. has been described for side-chain methylated lysines and arginines,[14d] and for the resolution of DL-tryptophan into its enantiomers.[304] The use of microcrystalline cellulose for separation of enantiomeric amino-acids is not new, but although several tryptophan analogues were also successfully resolved, this system is not suitable for other common amino-acids.[304]

Further experience in the use of fluorescamine [305] and the (cheaper) *o*-phthalaldehyde–mercaptoethanol [306, 240] spray reagents in quantitative t.l.c. of amino-acids has been described. The detection limit for fluorescent derivatives located through the *o*-phthalaldehyde procedure is in the range 50—100 pmoles.[306]

Improvements in technique have been reported for separations of dansylamino-acids,[307] methylthiohydantoins (two-dimensional t.l.c. of 23 derivatives),[308] and phenylthiohydantoins (use of formamide-impregnated paper,[309] separation of PTH's of basic amino-acids on polyamide layers [310]).

Other Analytical Methods.—Although many of the papers mentioned in this section have links with some of the preceding sections, their scope is rather broader. Analysis of actinomycin hydrolysates, which contain imino-acids as well as amino-acids, is best achieved through combinations of colorimetric and fluorimetric procedures.[311] The well-established fluorimetric assay for tryptophan

[296] J. L. Meck, *Analyt. Chem.*, 1976, **48**, 375.
[297] E. Bayer, E. Grom, B. Kaltenegger, and R. Uhmann, *Analyt. Chem.*, 1976, **48**, 1106.
[298] K. Muramoto, H. Kawauchi, Y. Yamamoto, and K. Tuzimura, *Agric. Biol. Chem. (Japan)*, 1976, **40**, 815.
[299] M. R. Downing and K. G. Mann, *Analyt. Biochem.*, 1976, **74**, 298.
[300] C. L. Zimmermann, E. Appella, and J. J. Pisano, *Analyt. Biochem.*, 1976, **75**, 77.
[301] C. Bollett and M. Caude, *J. Chromatog.*, 1976, **121**, 323.
[302] G. D. Lominac and H. S. Kingdon, *Arch. Biochem. Biophys.*, 1976, **173**, 320.
[303] A. Niederweiser, in 'Chromatography', ed. E. Heftmann, 3rd ed., Van Nostrand-Reinhold, New York, 1975, p. 393.
[304] R. L. Munier, A. M. Drapier, and C. Gervais, *Compt. rend.*, 1976, **282**, 1761.
[305] J. C. Touchstone, J. Sherma, M. F. Dobbins, and G. R. Hansen, *J. Chromatog.*, 1976, **124**, 111.
[306] E. Lindberg and G. Gunnar, *J. Chromatog.*, 1976, **117**, 439.
[307] M.-L. Lee and A. Safille, *J. Chromatog.*, 1976, **116**, 462.
[308] K. D. Kulbe and Y. M. Nogueira-Hattesohl, *Analyt. Biochem.*, 1976, **72**, 123.
[309] E. Soczewinski, J. Iskierko, and J. Klimek, *Chromatographia*, 1976, **9**, 323.
[310] S. Bose and H. B. Brewer, *Analyt. Biochem.*, 1976, **71**, 42; T. P. Hopp, *ibid.*, 1976, **74**, 638.
[311] A. M. Felix, J. W. Westley, and J. Meienhofer, *Analyt. Biochem.*, 1976, **73**, 70.

has been automated;[312] it has been found [313] that sucrose quenches the fluorescence generated in this assay, but since the effect is concentration-dependent it can be compensated. Comparative studies of dansyl-[1-^{14}C]-leucine with its mansyl analogue [mansyl = 6-(*N*-methylanilino)-2-naphthylsulphonyl] show that the latter derivative generates slightly greater fluorescence in some solvents, but not in others.[314]

A new electrophoretic method (omegaphoresis) has been applied to the quantitative analysis of amino-acids and peptides in mixtures, allowing the estimation of nmole to pmole levels in less than 5 minutes.[315]

Determination of Specific Amino-acids.—Reviews of the assay of hydroxy-L-proline in urine have been published.[316] Proline can be determined in samples containing hydroxyproline and other amino-acids using the isatin colour reaction and spectrophotometric (598 nm) quantitation.[317] Other aliphatic amino-acids for which appropriate chemical or physical techniques have been explored are: γ-amino-butyric acid in brain tissue (silylation [318] or *N*-pentafluoropropionylation and hexafluoropropyl ester formation [216] followed by m.s. determination at μg levels), L-canavanine (magenta colour reaction with photo-activated trisodium pentacyanoammonioferrate),[319] 2,6-di-aminopimelic acid,[320] and glycine and taurine at picomole levels (even in the presence of several thousand-fold excess of other amino-acids) using established [^{14}C]-dansyl chloride methods.[321] Fluorescence methods employed for the assay of aromatic amino-acids have been reported for tryptophan,[322] histidine and its derivatives,[323] and phenylalanine.[324] The fluorescamine derivative of histidine remains intensely fluorescent after heating in acid solutions, whereas the fluorescence of other correspondingly labelled compounds disappears;[323] exploitation of this fact allows the development of a method for the assay of imidazoles at *ca.* 0.01 nmole levels.[323] An unusual method developed for the assay of L-phenylalanine [324] in serum involves a specific fluorescence-forming reaction with ninhydrin in the presence of leucylalanine.[324]

Biochemical assays through which the amounts of γ-aminobutyric acid in brain tissue samples may be estimated have been reviewed.[325] Enzymic assays,

[312] A. J. Lewis, P. J. Holden, R. C. Ewan, and D. R. Zimmerman, *J. Agric. Food Chem.*, 1976, **24**, 1081.
[313] J. L. Woodcock and E. A. Khairallah, *Analyt. Biochem.*, 1976, **72**, 139.
[314] N. N. Osborne, W. L. Stahl, and V. Neuhoff, *J. Chromatog.*, 1976, **123**, 212.
[315] E. Schumacher and P. Ryser, *Chimia*, 1976, **30**, 105.
[316] N. L. Noble, in 'Methodology in Connective Tissue Research', ed. D. A. Hall, Joynson-Bruvvers, Oxford, 1976, p. 235; H. Burkhardt, R. Wepler, and K. Rommel, *Deut. Med. Wochenschr.*, 1976, **101**, 1394.
[317] R. J. Elliott and D. L. Gardner, *Analyt. Biochem.*, 1976, **70**, 268.
[318] F. Cattabeni, C. L. Galli, and T. Eros, *Analyt. Biochem.*, 1976, **72**, 1.
[319] G. A. Rosenthal, *Analyt. Biochem.*, 1976, **77**, 147.
[320] V. C. Mason and S. Bach-Andersen, *Z. Tierphysiol., Tierernaehr. Futtermittelkd.*, 1976, **36**, 221 (*Chem. Abs.*, **84**, 132218).
[321] E. Schulze and V. Neuhoff, *Z. physiol. Chem.*, 1976, **357**, 593.
[322] P. Pajot, *European J. Biochem.*, 1976, **63**, 263.
[323] H. Nakamura and J. J. Pisano, *Arch. Biochem. Biophys.*, 1976, **177**, 334.
[324] U. Grimm, *Zentralbl. Pharm., Pharmacother Laboratoriumsdiagn.*, 1976, **115**, 959 (*Chem. Abs.*, **86**, 13402); G. Machill, *ibid.*, 1976, **115**, 949 (*Chem. Abs.*, 86, 13458).
[325] S. J. Enna and S. H. Snyder, in 'Proceedings of the 6th International Congress of Pharmacology', ed. E. Klinge, Pergamon Press, Oxford, 1976.

including new examples of immobilized enzyme reactor electrodes for electrochemical techniques, have been established for L-leucine (L-amino-acid oxidase immobilized, with catalase, on glass),[326] L-glutamic acid,[327] L-arginine,[328] and L-lysine [328] (employing the respective decarboxylases and CO_3^{2-}-selective electrodes), and L-asparagine and L-arginine (immobilized asparaginase and arginase–urease electrodes, respectively, with NH_4^+-selective electrode).[329] L-Glutamine in serum may be estimated through use of an *Escherichia coli* glutamate synthase [330] or through use of an L-glutamine-binding protein from the same source.[331]

[326] G. Johansson, K. Edstrom, and L. Ogren, *Analyt. Chim. Acta*, 1976, **85**, 55.
[327] S. J. Yao, S. K. Wolfson, J. M. Tokarsky, and S. B. Weiner, *Bioelectrochem. Bioenerg.*, 1976, **3**, 106.
[328] S. L. Tong and G. A. Rachnitz, *Analyt. Letters*, 1976, **9**, 1.
[329] N. T. Tjien, *Canad. J. Biochem.*, 1976, **54**, 62.
[330] R. E. Miller, *Analyt. Biochem.*, 1976, **75**, 91.
[331] R. C. Willis and J. E. Seegmiller, *Analyt. Biochem.*, 1976, **72**, 66.