1
Amino-acids

BY G. C. BARRETT

1 Introduction

No substantial new emphasis on some aspect of amino-acid science has arisen in the recent literature, and the present Chapter, reviewing the literature of 1974, is subdivided as in previous Volumes of this series. As before, the coverage is intended to be thorough, but excludes most of the biological literature dealing with biosynthetic, metabolic, physiological, and microbiological aspects.

Textbooks and Reviews.—The laboratory synthesis [1-3] and large-scale production [2, 3] of amino-acids, and their technological applications,[1, 2] have been surveyed. Other more specific reviews are cited in the appropriate sections.

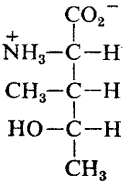
2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—Increasing attention is being given to the identification of organic compounds in geological samples, and the analysis of ancient cyanite schists from the Kola peninsula [4] (six free amino-acids and seven in a bound form) uses routine techniques; more information can be inferred from the degree of racemization of amino-acids present in fossils (see p. 21).

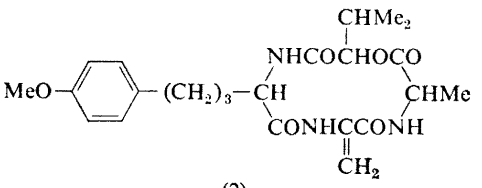
Aspects of the distribution of non-protein amino-acids in plants have been reviewed.[5] Among the more notable reports of the appearance of known amino-acids in plant sources are the presence of *cis*-4-hydroxy-L-proline in three genera (four species) of *Santalaceae*,[6] suggesting a useful taxonomic index for the species; also, the isolation from *Crotalaria juncea* seeds of δ-hydroxy-norleucine (5-hydroxy-2-aminohexanoic acid),[7] previously noted to be a constituent of the ilamycins. Partly racemized (*R*)-2-aminobut-3-enoic acid ('D-vinylglycine') isolated from *Rhodophyllus nidorosus* [8] is shown to exist in the plant in optically impure form. Tissues of *Medicago sativa* contain several amino-acid betaines, including stachydrine and homostachydrine (*NN*-dimethylproline and *NN*-dimethylpipecolic acid betaines, respectively),[9] and a careful study has established links between betaine content and growth rate.

[1] V. M. Belikov, *Vestnik Akad. Nauk S.S.S.R.*, 1973, 33.
[2] 'Synthetic Production and Utilization of Amino-acids', ed. T. Kaneko, Y. Izumi, I. Chibata, and T. Itoh, Kodansha, Tokyo, and Wiley, New York, 1974.
[3] E. N. Safonova and V. M. Belikov, *Uspekhi Khim.*, 1974, **43**, 1575.
[4] I. Z. Sergienko, M. I. Bobyleva, S. A. Sidorenko, and I. A. Egorov, *Doklady Akad. Nauk S.S.S.R.*, 1974, **215**, 474.
[5] L. Fowden, *Ann. Proc. Phytochem. Soc.*, 1972, **9**, 323.
[6] R. Kuttan, K. S. V. Pattabhiraman, and A. N. Radhakrishnan, *Phytochemistry*, 1974, **13**, 453.
[7] R. Pant and H. M. Fales, *Phytochemistry*, 1974, **13**, 1626.
[8] G. Dardenne, J. Casimir, M. Marlier, and P. O. Larsen, *Phytochemistry*, 1974, **13**, 1897.
[9] J. K. Sethi and D. P. Carew, *Phytochemistry*, 1974, **13**, 321.

Chirality at side-chain asymmetric centres may differ from species to species. The demonstration [10] that enniatin A is a mixture of diastereoisomers containing both *N*-methyl-L-isoleucine and *N*-methyl-L-alloisoleucine residues is incorrectly claimed (see refs. 12, 31, 32) to be the first report of the co-occurrence of both epimers of an L-amino-acid with two chiral centres in the same group of natural products. γ-L-Glutamyl-*S*-(*trans*-prop-1-enyl)-L-cysteine sulphoxide isolated from *Santalum album* leaves has the opposite configuration at sulphur from that in the same dipeptide isolated from onion.[11] The γ-hydroxyisoleucine residue in γ-amanatin is shown by *X*-ray analysis of its lactone hydrobromide [12] to be (2*S*,3*R*,4*S*)-2-amino-3-methyl-4-hydroxyvaleric acid (1), from which there follows a re-formulation of the absolute configuration of γδ-dihydroxyisoleucine present in the α- and β-amanatins to (2*S*, 3*R*, 4*R*)-2-amino-3-methyl-4,5-dihydroxyvaleric acid on the basis of chemical correlation.[12] Revision of the



(1)



(2)

configurational assignments to the γ-hydroxyisoleucine diastereoisomers found recently (in unequal amounts) in plants, for the first time (see Volume 6, p. 2), may now be necessary. Alternariolide (2), a host-specific toxin produced by *Alternaria mali* (responsible for apple blotch), contains two non-protein amino-acids;[13] structure (2) is assigned [13] to the toxin on the basis of spectroscopic data, but no evidence for absolute configuration was obtained.

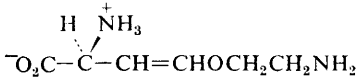
N-Methyl amino-acids of various types are represented for this Section by *N*-methyl-L-methionine-*S*-sulphoxide which is found, together with the corresponding primary amino-acid, in the red alga *Grateloupia turuturu*;[14] the proposal [15] that promine and retine, from calf liver and thymus, are N^{ε}-trimethyllysine and N^{G}-dimethyl-arginine respectively is not borne out by the physical and chemical properties of the compounds.[16, 17]

Where appropriate, mention is made in this Chapter of β- and γ-amino-acids, although most amino-acids mentioned in the literature are of the α-series. γ-Amino-L-α-hydroxybutyric acid has been established as a component of 4′-deoxybutirosins.[18]

[10] T. K. Audhya and D. W. Russell, *J.C.S. Perkin I*, 1974, 743.
[11] R. Kuttan, N. G. Nair, A. N. Radhakrishnan, T. F. Spande, H. J. Yeh, and B. Witkop, *Biochemistry*, 1974, **13**, 4394.
[12] A. Gieren, P. Narayanan, W. Hoppe, M. Hasan, K. Michl, T. Wieland, H. O. Smith, G. Jung, and E. Breitmayer, *Annalen*, 1974, 1561.
[13] T. Okuno, Y. Ishita, K. Sawai, and T. Matsumoto, *Chem. Letters*, 1974, 635.
[14] K. Miyazawa and K. Ito, *Nippon Suisan Gakkaishi*, 1974, **40**, 655.
[15] E. Tyihak and A. Patthy, *Acta Agron. Acad. Sci. Hung.*, 1973, **22**, 445.
[16] C. Marmasse, *Acta Agron. Acad. Sci. Hung.*, 1974, **23**, 216.
[17] T. Nakajima, *Acta Agron. Acad. Sci. Hung.*, 1974, **23**, 236.
[18] M. Konishi, K. Numata, K. Shimoda, H. Tsukiura, and H. Kawaguchi, *J. Antibiotics*, 1974, **27**, 471.

Microbial synthesis of amino-acids continues to provide an expanding literature, and only representative papers can be cited here. L-Amino-acids produced through biosynthesis include isoleucine[19] and *cyclo*-isoleucylisoleucine,[20] threonine,[21] *O*-alkyl-homoserines,[22] arginine and citrulline,[23] indole-substituted tryptophans,[24] phenylalanine,[25] histidine,[26] dopa[27] and *N*-Z, *N*-Boc, and *N*-formyl derivatives of dopa,[28] and azetidine-2-carboxylic acid.[29]

New Natural Free Amino-acids.—Further details have been provided[30] of the acetylenic amino-acids present in *Tricholomopsis rutilans*. Both *threo* and *erythro* diastereoisomers of L-2-amino-3-hydroxyhex-4-ynoic acid are present, adding a further example to those reported in the past two years[10, 12, 31, 32] of the occurrence of epimeric amino-acids in the same species. A number of other unsaturated amino-acids have been isolated from plant sources, and from bacterial and fungal cultures, and reported during the year under review. (2*S*,3*S*)-3-Hydroxy-4-methylene-glutamic acid is present in seeds of *Gleditsia caspica* [the known amino-acids (2*S*,4*R*)-4-methyl-glutamic acid and its (2*S*,3*S*,-4*R*)-3-hydroxy analogue are also present],[33] and L-2-amino-4-chloropent-4-enoic acid (from *Amanita pseudoporphyria*)[34] and L-2-amino-4-(2-aminoethoxy)-*trans*-but-3-enoic acid (3) (from an unidentified *Streptomycete*)[35] are further



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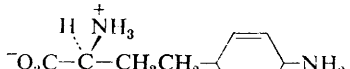


(4)

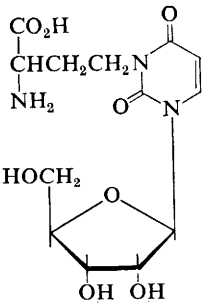
acyclic examples, with an unusual alicyclic derivative, L-2-amino-4-(4′-amino-2′,5′-cyclohexadienyl)butyric acid (4), being a new amino-acid antibiotic.[36] The stereochemistry of the cyclohexadienyl moiety in (4) is not yet established.

[19] H. Matsushima, K. Murata, and Y. Mase, *Hakko Kogaku Zasshi*, 1974, **52**, 20.
[20] Y. Yamada, S. Sawada, and H. Okada, *Hakko Kogaku Zasshi*, 1974, **52**, 143.
[21] T. Hirakawa and K. Watanabe, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 77.
[22] N. Ogasawara, T. Sato, M. Kato, and K. Sakaguchi, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 515.
[23] K. Kubota, T. Onoda, H. Kamijo, F. Yoshinaga, and S. Okumura, *J. Gen. Appl. Microbiol.*, 1973, **19**, 339.
[24] M. Wilcox, *Analyt. Biochem.*, 1974, **59**, 436.
[25] H. Hagino and K. Nakayama, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 157.
[26] K. Araki, F. Kato, Y. Arai, and K. Nakayama, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 189.
[27] H. Yoshida, Y. Tanaka, and K. Nakayama, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 455, 633.
[28] J. Rosazza, P. Foss, M. Lemberger, and C. J. Sih, *J. Pharm. Sci.*, 1974, **63**, 544.
[29] E. Leete, G. E. Davis, C. R. Hutchinson, K. W. Woo, and M. R. Chedekel, *Phytochemistry*, 1974, **13**, 427.
[30] Y. Niimura and S. Hatanaka, *Phytochemistry*, 1974, **13**, 175.
[31] G. A. Dardenne, J. Casimir, E. A. Bell, and J. R. Nulu, *Phytochemistry*, 1972, **11**, 787.
[32] G. A. Dardenne, E. A. Bell, J. R. Nulu, and C. Cone, *Phytochemistry*, 1972, **11**, 791.
[33] G. A. Dardenne, J. Casimir, and H. Sorensen, *Phytochemistry*, 1974, **13**, 2195.
[34] S. Hatanaka, S. Kaneko, Y. Niimura, F. Kinoshita, and G. Soma, *Tetrahedron Letters*, 1974, 3931.
[35] D. L. Pruess, J. P. Scannell, M. Kellett, H. A. Ax, J. Janecek, T. H. Williams, A. Stempel, and J. Berger, *J. Antibiotics*, 1974, **27**, 229.
[36] Y. Okami, T. Kitihara, M. Hamada, H. Naganawa, S. Kondo, K. Maeda, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 656.

α-Amino-γ-(isoxazolin-5-on-2-yl)butyric acid has been isolated from *Lathyrus odoratus*,[37] together with β-(isoxazolin-5-on-2-yl)alanine and β-(2-β-D-glucopyranosyl-isoxazolin-5-on-4-yl)alanine which were previously found in *Pisum sativum* seedlings. 4-(4-Hydroxy-3-methyl-Δ^2-butenyl)tryptophan has been isolated from cultures of *Claviceps purpurea*, the structural assignment resting on mass spectrometric study of its *N*-trifluoroacetyl methyl ester so that no configurational assignment could be made.[38] A further new heterocyclic amino-acid, of particular interest, is 3-(3-amino-3-carboxypropyl)uridine (5), a novel modified nucleoside from *E. coli* tRNA representing the site of reaction with phenoxyacetic acid.[39, 40]

N-(3-Aminopropyl)-4-aminobutyric acid, $NH_2(CH_2)_3NH(CH_2)_3CO_2H$, appears in rabbit urine as a metabolite of bleomycin A_5.[41]

New Amino-acids from Hydrolysates.—Peptide antibiotics continue to provide novel amino-acids, often closely related in structure to the protein amino-acids. Hydrolysates of longicatenamycin contain 5-chloro-D-tryptophan,[42] and antibiotic SF-1293 contains an L-2-amino-4-(methylphosphino)butyric acid residue (6).[43] The structure of SF-1293, the tripeptide (6)-L-Ala-L-Ala,[43a] has an extra-



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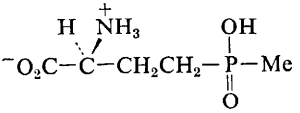

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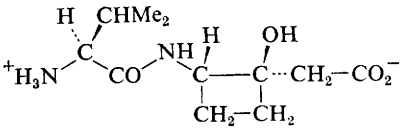

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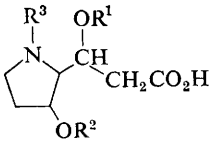


(8) R^1 = (+)-(*S*)-2-methylbutyryl-L-phenylalanine
R^2 = Ac, EtCO, Pr^{i}CO, Pr^{n}CO, Me_2CHCH_2CO
R^3 = L-valyl

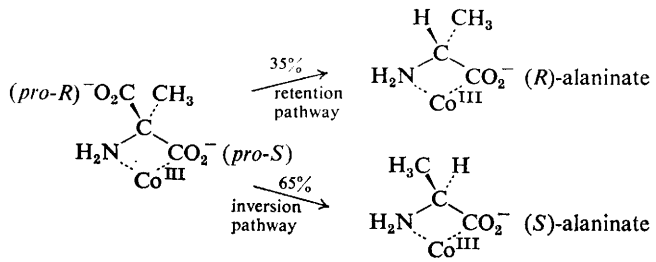
[37] F. Lambien and R. Van Parijs, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 155.
[38] J. A. Anderson and M. S. Saini, *Tetrahedron Letters*, 1974, 2107.
[39] Z. Ohashi, M. Maeda, J. A. McCloskey, and S. Nishimura, *Biochemistry*, 1974, **13**, 2620.
[40] S. Friedman, H. J. Li, K. Nakanishi, and G. Van Lear, *Biochemistry*, 1974, **13**, 2932.
[41] S. Hori, T. Sawa, T. Yoshioka, T. Takita, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 489.
[42] T. Shiba, Y. Mukunoki, and H. Akiyama, *Tetrahedron Letters*, 1974, 3085.
[43] (*a*) Y. Ogawa, T. Tsuruoka, S. Inoue, and T. Niida, *Meiji Seika Kenkyu Nempo*, 1973, No. 13, 42; (*b*) Y. Ogawa, H. Yoshida, S. Inoue, and T. Niida, *ibid.*, p. 49; (*c*) N. Ezaka, S. Amano, K. Fukushima, S. Inoue, and T. Niida, *ibid.*, p. 60 (*Chem. Abs.*, 1974, **81**, 37 806, 37 788, and 37 805).

ordinary similarity with an L-glutamine antimetabolite, (X)-L-Ala-L-Ala[where (X) = L-(N^5-phosphono)methionine-*S*-sulphoximine residue], mentioned in last year's review (Volume 6, p. 7). Antibiotic LL-AV 290 contains 3-chloro-4-hydroxyphenylglycine and *p*-hydroxyphenylsarcosine residues.[44]

New β-amino-acids and higher homologues have been reported. γ-Hydroxy-β-lysine is a new basic amino-acid from hydrolysates of tuberactinomycins A and N;[45] a metabolite from an unclassified *Streptomycete* is a dipeptide (7) containing a 2-aminocyclobutane-1-acetic acid moiety.[46] The novel amino-acid detoxinine (8; $R^1 = R^2 = R^3 = H$) is a constituent of a group of depsipeptide antibiotics, the detoxins.[47]

3 Chemical Synthesis and Resolution of Amino-acids

Asymmetric Synthesis.—Decarboxylation of α-amino-α-methylmalonic acid after binding to $\Lambda(-)_{436}$-α-[(2*S*,9*S*)-2,9-diamino-4,7-diazadecanecobalt(III) dichloride] cation leads to the corresponding (*R*,*S*)-alanine complex in which the (*S*)-enantiomer is present in 30% excess.[48] This is the first example of the absolute chiral recognition of a prochiral centre by a small molecule – the process is otherwise well illustrated in enzymic reactions. The crystal structure of $\Lambda(-)_{436}$-β_2-[(2*S*,9*S*)-2,9-diamino-4,7-diazadecanecobalt(III) α-amino-α-methylmalonate] perchlorate monohydrate[49] shows that a Λ-β-*R*-conformation is adopted, with the pro-*S*-carboxy-group of the malonate moiety co-ordinated to cobalt, rather than the pro-*R*-carboxy-group, and the considerable asymmetric induction caused by the dissymmetric cobalt centre in favour of inversion accompanying decarboxylation (Scheme 1) is due to a less obstructed pathway



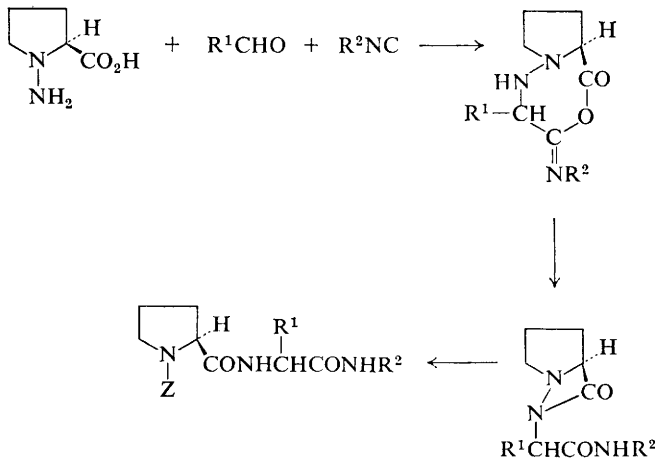
Scheme 1

for the incoming proton in this direction.[49] A late stage in the classical malonic ester synthesis of α-amino-acids is represented in these decarboxylation studies, and the opportunity has now been created for developing a new asymmetric synthesis based on otherwise well-established reactions.

[44] J. J. Hlavka, P. Bitha, J. H. Boothe, and G. Morton, *Tetrahedron Letters*, 1974, 175.
[45] T. Wakamiya, T. Teshima, I. Kubota, T. Shiba, and T. Kaneko, *Bull. Chem. Soc. Japan*, 1974, **49**, 2292.
[46] D. L. Pruess, J. P. Scannell, J. F. Blount, H. A. Ax, M. Kellett, T. H. Williams, and A. Stempel, *J. Antibiotics*, 1974, **27**, 754.
[47] N. Otake, K. Furihata, K. Kakinuma, and H. Yonehara, *J. Antibiotics*, 1974, **27**, 484.
[48] R. C. Job and T. C. Bruice, *J. Amer. Chem. Soc.*, 1974, **96**, 809.
[49] J. P. Glusker, H. L. Carrell, R. Job, and T. C. Bruice, *J. Amer. Chem. Soc.*, 1974, **96**, 5741.

Treatment of a Schiff base derived from (−)-(*S*)-1-(4-pyridyl)ethylamine and an α-keto-ester with base in Bu^{t}OH solution gives the rearranged (*S*)-α-amino-acid ester Schiff base (see Scheme 6, p. 23). This stereospecific (suprafacial) proton transfer depends on the presence of bulky substituents to sustain the geometry of the starting material through the transition state. In Bu^{t}OD, the α-deuteriated (*S*)-α-amino-acid is formed. Efficient asymmetric hydrogenation of α-acetamidocinnamic acids is catalysed by chiral phosphine–rhodium complexes;[50] in a partial asymmetric synthesis, chiral isocyanides are converted into their lithium aldimine homologues, *e.g.* PhCMe(Et)N=CRLi, followed by carboxylation or ethoxycarbonylation.[51]

A novel procedure favouring the formation of D-amino-acids[52] based on *N*-amino-L-proline and an isocyanide is displayed in Scheme 2.

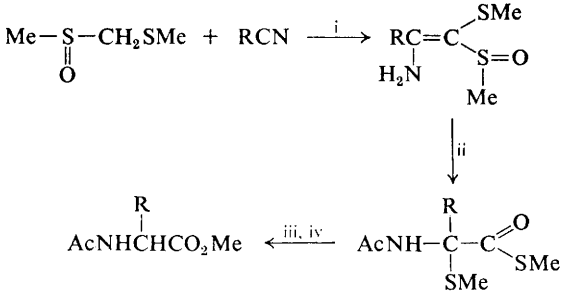


Scheme 2

General Methods of Synthesis.—Further examples of the Ugi reaction have been provided, illustrating a synthesis of L-prolyl-D-amino-acids[52] and a synthesis of 1,4-dihydrophenylalanine,[53] for which a conventional Strecker synthesis was inappropriate.[53] A review has appeared[54] of the uses of α-metallated isocyanides in organic synthesis, including the synthesis of β-functional α-amino-acids (see Volume 6, p. 9). An outstanding new synthesis of α-amino-acids from nitriles (Scheme 3)[55] involves a rearrangement step whose characteristics are not yet fully understood.

Full details of the use of malonic acid half-esters in a modified Curtius reaction (diphenylphosphoryl azide) for amino-acid synthesis are available,[56] supplement-

[50] W. S. Knowles, M. J. Sabacky, and B. D. Vineyard, in 'Homogeneous Catalysis—II', Advances in Chemistry Series No. 132, American Chemical Society, 1974, p. 274.
[51] N. Hirowatari and M. H. Walborsky, *J. Org. Chem.*, 1974, **39**, 604.
[52] K. Achiwa and S. Yamada, *Tetrahedron Letters*, 1974, 1799.
[53] D. Scholz and U. Schmidt, *Chem. Ber.*, 1974, **107**, 2295.
[54] D. Hoppe, *Angew. Chem. Internat. Edn.*, 1974, **13**, 789.
[55] K. Ogura and G. Tsuchihashi, *J. Amer. Chem. Soc.*, 1974, **96**, 1960.
[56] K. Ninomiya, T. Shioiri, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1398.



Reagents: i, NaH; ii, Ac_2O; iii, MeOH; iv, desulphurization

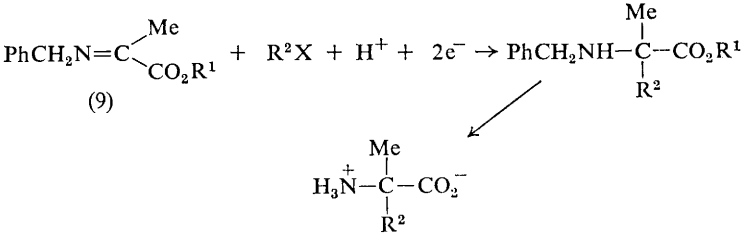
Scheme 3

ing the preliminary communication mentioned in Volume 6 (p. 11). The use of the α-acylamino-malonic ester route is exemplified in many papers, as usual, for the synthesis of specific α-amino-acids,[34, 43, 57–59] and the hydantoin synthesis [60–64] and azlactone synthesis,[65–68] Strecker synthesis,[69, 70] and the α-halogeno-acid amination procedure,[64, 70–72] have been employed.

Schiff bases are already counted among the more valuable starting materials for amino-acid synthesis, and further such applications have been devised. Electroreductive coupling with an alkyl halide using constant potential electrolysis can give 38—86% yields of α-methyl-α-amino-acids from a pyruvate ester Schiff base (9; see Scheme 4).[73] β-Amino-acid amides $R^1NHCHR^2CH_2CONR^3_2$ and corresponding esters may be prepared from Schiff bases through the Reformatzky reaction.[74]

General methods for the synthesis of β-carboxy-α-aminosulphonic acids [75] and αβ-unsaturated α-amino-acids [76] have been reported; *N*-trimethylsilylmethylglycinamide, $Me_3SiCH_2NHCH_2CONH_2$, has been synthesized from $Me_3SiCH_2NH_2$ and $ClCH_2CONH_2$ in a method suitable for general application.[72]

[57] K. Matsumoto, T. Miyahara, M. Suzuki, and M. Miyoshi, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1097.
[58] H. Maehr and M. Leach, *J. Org. Chem.*, 1974, **39**, 1166.
[59] L. Pichat and J. P. Beaucourt, *J. Labelled Compounds*, 1974, **10**, 103.
[60] A. Arendt, A. Kolodziejczyk, T. Sokolowska, and M. Mrozowski, *Roczniki Chem.*, 1974, **48**, 883.
[61] J. J. Ellington and I. L. Honigberg, *J. Org. Chem.*, 1974, **39**, 104.
[62] M. M. Abdel-Monem, N. E. Newton, and C. E. Weeks, *J. Medicin. Chem.*, 1974, **17**, 447.
[63] M. M. Ames and N. Castagnoli, *J. Labelled Compounds*, 1974, **10**, 195.
[64] J. Mizon and C. Mizon, *J. Labelled Compounds*, 1974, **10**, 229.
[65] R. T. Coutts and J. L. Malicky, *Canad. J. Chem.*, 1974, **52**, 390.
[66] M. L. Anhoury, P. Crooy, R. De Neys, and J. Eliaers, *Bull. Soc. chim. belges*, 1974, **83**, 117.
[67] T. S. T. Wang and J. A. Vida, *J. Medicin. Chem.*, 1974, **17**, 1120.
[68] G. W. Kirby and M. J. Varley, *J.C.S. Chem. Comm.*, 1974, 833.
[69] D. J. Aberhardt and L. J. Lin, *J.C.S. Perkin I*, 1974, 2320.
[70] P. Friis, P. Helboe, and P. O. Larsen, *Acta Chem. Scand. (B)*, 1974, **28**, 317.
[71] C. Eguchi and A. Kakuta, *Bull. Chem. Soc. Japan*, 1974, **47**, 1704.
[72] W. Fink, *Helv. Chim. Acta*, 1974, **57**, 1042.
[73] T. Iwasaki and K. Harada, *J.C.S. Chem. Comm.*, 1974, 338.
[74] F. Dardoize and M. Gaudemar, *Bull. Soc. chim. France*, 1974, 939.
[75] A. Le Berre, A. Etienne, and J. Coquelin, *Bull. Soc. chim. France*, 1974, 221.
[76] D. H. Rich, J. Tam, P. Mathiaparanam, J. A. Grant, and C. Mabuni, *J.C.S. Chem. Comm.*, 1974, 897.



Scheme 4

Prebiotic Synthesis; Model Reactions.—Electrical discharge studies with CH_4–CO_2–NH_3 [77] and CH_4–NH_3–H_2O [78] mixtures continue to demonstrate the formation of amino-acid mixtures, and the synthesis of amino-acids and high molecular weight proteins under radiofrequency cold plasma conditions has been reported.[79] Polymeric material obtained from aqueous methylammonium bicarbonate after n,γ-irradiation gave glycine, alanine, and lysine on hydrolysis;[80] trimethylammonium bicarbonate gave in addition γ-aminobutyric acid and valine, and n-pentylammonium bicarbonate gave norleucine, γ-aminobutyric acid, alanine, and 6-aminohexanoic acid, on similar treatment. A related study, but with some preparative value, has shown that aliphatic carboxylic acids subjected to contact glow discharge electrolysis in concentrated aqueous ammonia give a wide variety of amino-acids in yields up to 13%.[81] Propionic acid, for example, under these conditions (75 mA at 15 °C for 3 h) gives 6.9% alanine, 5.3% β-alanine, and 1% glycine.

Exposure to sunlight of solutions of formaldehyde, ammonium molybdate, ammonium phosphate, and mineral salts gives appreciable amounts of amino-acids after 80 h,[82] with some dependence of relative proportions of the different amino-acids upon the concentrations of formaldehyde and ammonium molybdate.

Hydrogen cyanide oligomers have been shown earlier to be a source of amino-acids on hydrolysis, even though the oligomers themselves do not appear to be closely related to polypeptides. Fractionation of the oligomers into acidic, neutral, and basic components, followed by hydrolysis and analysis by g.l.c. and mass spectrometry, shows [83] that a wider range of protein amino-acids is available from this source than previously supposed. Glutamic acid is obtained by hydrolysis of the neutral oligomers, but not from the acidic and basic fractions which give glycine, aspartic acid, and *meso*- and DL-diaminosuccinic acids, with smaller amounts of alanine, isoleucine, and α-aminoisobutyric acid.[83] In comparison with the somewhat disappointing earlier evidence that only the more esoteric amino-acids could be generated by hydrolysis of hydrogen cyanide oligomers,

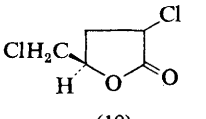
[77] E. F. Simonov, V. B. Lukyanov, and E. R. Roshal, *Vestnik Muskov Univ., Khim.*, 1974, **15**, 365.
[78] D. Stefanescu, *Stud. Cercet. Biochim.*, 1974, **12**, 205.
[79] C. I. Simionescu, F. Denes, and M. Dragnea, *Compt. rend.* 1974, **278**, *C*, 29; C. I. Simionescu, F. Denes, D. Onac, and G. Bloos, *Biopolymers*, 1974, **13**, 943.
[80] L. N. Zhigunova, G. V. Manuilova, and E. P. Petryaev, *Vestsi Akad. Navuk B.S.S.R., Ser. Fiz. Energ. Navuk*, 1974, 33 (*Chem. Abs.*, 1974, **81**, 152 607).
[81] K. Harada and T. Iwasaki, *Nature*, 1974, **250**, 426.
[82] K. Bahadur, M. L. Verma, and Y. P. Singh, *Z. allg. Mikrobiol.*, 1974, **14**, 87.
[83] J. P. Ferris, J. D. Wos, D. W. Nooner, and J. Oro, *J. Mol. Evol.*, 1974, **3**, 225.

these results will enliven the arguments of those who advocate the origin of life within the chemistry of hydrogen cyanide.

Protein and Other Naturally Occurring Amino-acids.—New syntheses described in the preceding sections have employed some of the well-known protein amino-acids as synthetic objectives. This section reports specific syntheses which are interesting in their own right, and also capable of being developed into routes to close analogues of natural products.

A synthesis of lysine from butadiene[84] involves conversion with nitrogen pentoxide into 1-nitrobuta-1,3-diene followed by addition to ethyl nitroacetate or diethyl 2-nitromalonate, and hydrogenation and acid hydrolysis.

A simple synthesis of L-proline from L-pyroglutamic acid[85] (2-oxopyrrolidine-5*S*-carboxylic acid) employs the method used by the same author in a cucurbitine synthesis described in Volume 6 (p. 15), in which the amide grouping is converted into an imidate ester with triethyloxonium fluoroborate [—CO—NH— → —C(OEt)=N—], which on reduction ($NaBH_4$) gives the secondary amine —CH_2—NH—. D-Glutamic acid gives a mixture of L-hydroxyproline and D-allohydroxyproline through a route involving the butyrolactone (10);[71] amination of (10) followed by hydrolysis of the resulting amide gives a mixture of the diastereoisomeric hydroxyprolines from which an enhanced yield of L-hydroxyproline may be obtained[86] by equilibration of the cyclic dipeptide of the D-allo-isomer, followed by acid hydrolysis.



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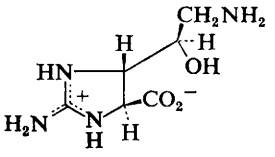

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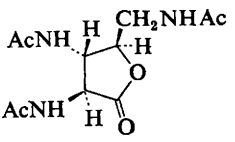


(12)

Specific examples of syntheses of less-common naturally occurring amino-acids, using well-established methods, are αα′-diaminopimelic acid,[60] 3-(3-amino-3-carboxypropyl)uridine,[39] and 4-methylphosphino-L-butyrine.[43]

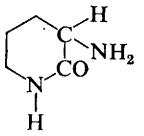
Elegant syntheses[87, 88] of roseonine [(11) *alias* streptolidine or geamine] starting from D-ribose have been reported, involving the lactone (12) as intermediate.

Mention has already been made (p. 3) of microbiological syntheses of natural amino-acids and close relatives, and the possibilities are intriguing when the continuous production implicit in the use of *E. coli* cells immobilized in polyacrylamide gel is taken into account; the feasibility of this has been demonstrated[89] for the synthesis of L-aspartic acid from ammonium fumarate.

[84] T. I. Samoilovich, A. S. Polyanskaya, and V. V. Perekalin, *Doklady Akad. Nauk S.S.S.R.*, 1974, **217**, 1335.
[85] H. G. Monteiro, *Synthesis*, 1974, 137.
[86] C. Eguchi and A. Kakuta, *Bull. Chem. Soc. Japan*, 1974, **47**, 2277.
[87] T. Goto and T. Ohgi, *Tetrahedron Letters*, 1974, 1413.
[88] S. Kusumoto, S. Tsuji, and T. Shiba, *Tetrahedron Letters*, 1974, 1417.
[89] T. Tosa, T. Sato, T. Mori, and I. Chibata, *Appl. Microbiol.*, 1974, **27**, 886.

α-Alkyl and αα-Dialkyl Amino-acids.—A novel synthesis of a chloroalkyl amino-acid in which L-methionine methyl ester is converted in 28% yield into L-2-amino-4,4,4-trichlorobutanoic acid, $Cl_3CCH_2CH(NH_2)CO_2H$, by chlorine in chloroform, followed by hydrolysis,[90] should be more generally applicable where amino-acid side-chains carry functional groups capable of activating an adjacent saturated carbon centre towards halogenation.

Syntheses of α-methylproline[61] and α-methylornithine[61, 62] by the hydantoin route have been reported. An alternative synthesis[62] of α-methylornithine from the parent amino-acid involves treatment of the derived amide (13) with phenyllithium followed by methyl iodide; less direct methods are usually employed in the synthesis of α-alkyl-α-amino-acids.[91–93]



(13)

Amino-acids with Unsaturated Functional Groups in Side-chains.—α-Cyanoglycine is a reactive amino-acid obtained in the past by enzymic deacylation of acetamidocyanoacetic acid; an alternative method[94] involves careful hydrolysis of its *N*-carboxyanhydride prepared from ethyl aminocyanoacetate. Syntheses of DL-vinylglycine (2-aminobut-3-enoic acid)[8, 70] and its enzymic resolution[70] have been reported; 2*S*-amino-4-chloropent-4-enoic acid[34] and diastereoisomers of 2-amino-3-hydroxyhex-4-ynoic acid[30] have been synthesized by standard methods.

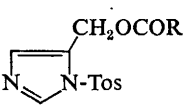
β-Bromo-αβ-unsaturated-α-amino-acids, $RCBr{=}C(NH_2)CO_2H$, are accessible (in low yield) from α-hydroxyaminoalkanoic esters by reaction with bromoacetyl bromide, or in quantitative yield from *N*-acyl-αβ-unsaturated amino-acids by reaction with *N*-bromosuccinimide.[95] *N*-Bromoamide intermediates are involved in these processes.

Amino-acids with Hydroxyalkyl Side-chains.—Syntheses of β-hydroxy-α-amino-acids by standard methods are illustrated in the 1974 literature for 2-amino-3-hydroxyhex-4-ynoic acid[30] and β-hydroxy-methionine and -homomethionine.[96] Nucleophilic addition of the corresponding aldehydes to cupric glycinate in alkaline solution[96] is one of several minor variations of a standard procedure.

γ-Hydroxy-β-lysine, a component of tuberactinomycins A and N, has been synthesized[45] by the Arndt–Eistert route from β-hydroxyornithine. A biogenetically modelled synthesis has been described for (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methyl-n-valeric acid.[97]

[90] Y. Urabe, T. Okawara, K. Okumura, M. Miyoshi, and K. Matsumoto, *Synthesis*, 1974, 440.
[91] J. G. Cannon, J. P. O'Donnell, J. P. Rosazza, and C. R. Hoppin, *J. Medicin. Chem.*, 1974, **17**, 565.
[92] N. Zenker, V. H. Morgenroth, and J. Wright, *J. Medicin. Chem.*, 1974, **17**, 1223.
[93] M. Suzuki, T. Miyahara, R. Yoshioka, M. Miyoshi, and K. Matsumoto, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1709.
[94] C. B. Warren, R. D. Minard, and C. N. Matthews, *J. Org. Chem.*, 1974, **39**, 3375.
[95] C. Shin, K. Nanjo, and J. Yoshimura, *Tetrahedron Letters*, 1974, 521.
[96] T. T. Otani and M. R. Briley, *J. Pharm. Sci.*, 1974, **63**, 1253.
[97] T. Yoshioka, T. Hara, T. Takita, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 356.

Aromatic and Heteroaromatic Amino-acids.—Important new approaches to the synthesis of β-aryl- and β-heteroaryl-alanines, $ArCH_2CH(NH_2)CO_2H$,[57] and α-methyl analogues[93] have been described. In an improved synthesis of DL-histidine[57] an N-protected acyloxymethyl-imidazole (14) is condensed with diethyl acetamidomalonate; and the same intermediate is used in a synthesis of α-methylhistidine by condensation with ethyl isocyanopropionate.[93] α-Methyl-tryptophan and α-methyl-dopa syntheses have also been recorded,[93] employing



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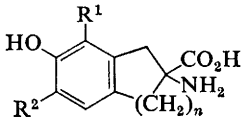


(15) n = 1 or 2; R^1 = H, R^2 = OH or R^1 = OH, R^2 = H

isocyanopropionate esters and gramine methiodide and O-protected 3,4-dihydroxybenzyl bromides, respectively. Cyclic analogues (15) of α-methyl-dopa have been synthesized[91] and employed in studies of the mode of action of the amino-acid.

Synthetic analogues of L-dopa and their biological evaluation have been reviewed.[98]

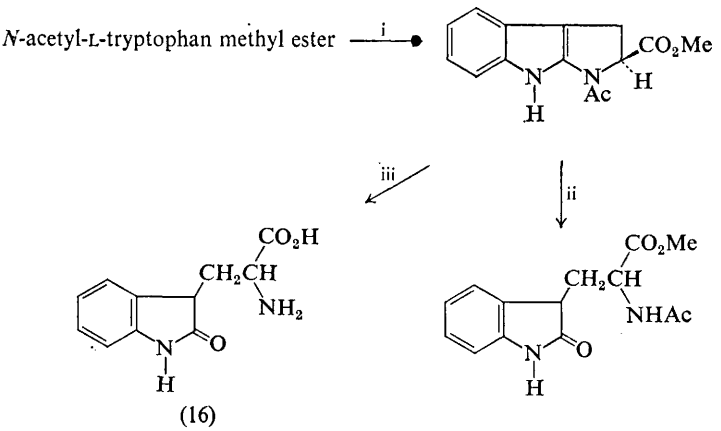
A new synthesis of 2′-mercapto-DL-histidine[99] and syntheses of DL-α-(2-thiazolyl)-glycines[100] illustrate continuing minor improvements in synthetic methods.

Most of the new analogues of aromatic and heteroaromatic amino-acids reported this year, as in previous years, have been prepared by substitution and other elaboration reactions of the protein amino-acids. Ring substitution of phenylalanine is readily brought about through straightforward procedures, but mixtures of products are often troublesome to separate. Pure *p*-chlorophenylalanine is best obtained[101] through a roundabout route; nitration gives a mixture of 55% *p*-nitro-, 25% *m*-nitro-, and 20% *o*-nitro-phenylalanines from which the *p*-isomer is conveniently separated, reduced, and the product obtained through application of the Sandmeyer reaction. *N*-Benzyloxycarbonyl-L-tyrosine and -DL-α-methyltyrosine have been converted into their 3-(hydroxymethyl) derivatives through reaction with formaldehyde;[102] a quite different approach to DL-3-(hydroxymethyl)tyrosine[67] uses 3-hydroxymethyl-4-hydroxybenzaldehyde as starting material for a conventional azlactone synthesis. Thyroxine analogues annelated between positions 3 and 4 of the 'outer' ring have been prepared.[103]

Tryptophan analogues of particular interest have been described by Witkop[104a] and by Wieland.[104b] 2-Hydroxy-L-tryptophan (16) is now more realistically

[98] A. Brossi, W. Pool, H. Sheppard, J. J. Burns, A. Kaiser, R. Bigler, G. Bartholini, and A. Pletscher, *Adv. Neurol.*, 1974, **5**, 291.
[99] J. Fernandez-Bolanos, D. Martinez Ruiz, and M. Menendez Gallego, *An. Quim.*, 1974, **70**, 94.
[100] M. Hatanaka and T. Ishimaru, *Bull. Chem. Soc. Japan*, 1973, **46**, 3600.
[101] R. A. Houghten and H. Rapoport, *J. Medicin. Chem.*, 1974, **17**, 556.
[102] M. Atkinson, D. Hartley, L. H. C. Lunts, and A. C. Ritchie, *J. Medicin. Chem.*, 1974, **17**, 248.
[103] M. T. Cox, W. G. Bowness, and J. J. Holohan, *J. Medicin. Chem.*, 1974, **17**, 1125.
[104] (*a*) M. Ohno, T. F. Spande, and B. Witkop, *J. Org. Chem.*, 1974, **39**, 2635; (*b*) T. Wieland, M. P. Jordan de Urries, H. Indest, H. Faulstich, A. Gieren, M. Sturm, and W. Hoppe, *Annalen*, 1974, 1570.

available through reactions shown in Scheme 5,[104a] and the 2-ethylsulphinyl-L-tryptophan residue present in a toxic phalloidin has the (*R*)-configuration at sulphur, as shown through synthesis from tryptophan by treatment with ethanesulphenyl chloride, oxidation using hydrogen peroxide, and separation of the



(16)

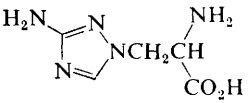
Reagents: i, Bu^t OCl; ii, 20% aq. AcOH, room temperature; iii, 6N-HCl, 110 °C

Scheme 5

diastereoisomeric sulphoxides; that which was identical with the natural amino-acid (comparison of c.d. curves) was shown by *X*-ray analysis to be the (*R*)-sulphoxide.[104b]

α-Methylphenylalanine has been used as starting material for the synthesis of DL-3-(5-benzimidazolyl)-2-methylalanine;[92] *N*-acetyl-L-aspartic-α-thioamide β-methyl ester, on condensation with diethyl bromoacetal, gives L(+)-β-(2-thiazolyl)-β-alanine, identical with a component of bottromycin.[105]

Further studies of the use of enzyme preparations from *Pisum sativum* (and similar extracts from water melon and *Leucaena leucoephala*) for the conversion of *O*-acetyl serine and a five-membered nitrogen heterocycle into a (1-heteroaryl)-alanine have been reported,[106] 3-amino-1,2,4-triazole giving (17).



(17)

N-Substituted Amino-acids.—This section deals with *N*-methyl and *N*-hydroxy-amino-acids synthesized for their importance as natural products; N-protected amino-acids as intermediates in peptide synthesis are excluded.

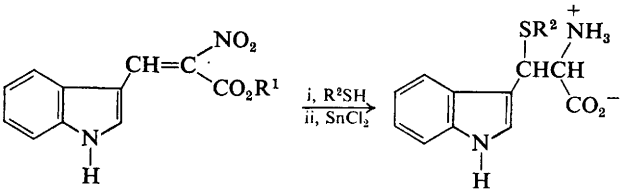
[105] Y. Seto, K. Torii, K. Bori, K. Inabata, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Japan*, 1974, **47**, 151.

[106] I. Murakoshi, F. Kato, and J. Haginiwa, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 480.

Condensation of L-ornithine with *S*-methyl-iso-*N*-methylthiourea gives N^7-methyl-L-arginine.[107]

The announcement last year (Volume 6, p. 5) that N^5-hydroxy-L-arginine from natural sources possesses antimicrobial properties has been followed by descriptions of methods for its synthesis.[58, 108] Methyl 2-acetamido-5-iodovalerate can be converted into the 5-(α-phenylnitrone) which, by selective hydrolysis, gives N^2-acetyl-N^5-hydroxyornithinamide; reaction with *S*-methyl isothiourea gives N^5-hydroxy-DL-arginine.[58] The relatively limited range of alternative methods available for the synthesis of hydroxylamines has been well tried in recent years, and further examples are provided this year for the synthesis of N^4-hydroxy-L-lysine (a component of mycobactins),[109] and O-protected α-hydroxylamino-acids.[110]

Amino-acids containing Sulphur.—An entry into series of β-mercapto analogues of some of the protein amino-acids is provided by the sequence (18) → (19),[111] illustrating the synthesis of tryptophan analogues.



(18) (19) R^2 = Ac, $PhCH_2$, or H

S-(Pyrimidin-2-yl)-L-cysteine may be synthesized by reaction of *N*-benzyloxycarbonyl-*O*-toluene-*p*-sulphonyl-L-serine *p*-nitrobenzyl ester with the sodium salt of 2-mercaptopyrimidine, though a partly racemized protected product is obtained;[112] 2-chloro-L-alanine gives racemic product with the mercaptopyrimidine but enzymic combination of these reactants gives optically pure material.[112]

A List of α-Amino-acids which have been Synthesized for the First Time

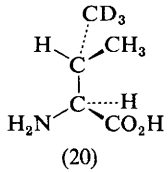
Compound [a]	*Ref.*
L-*threo*-2-Amino-3-hydroxyhex-4-ynoic acid	30
L-*erythro*-2-Amino-3-hydroxyhex-4-ynoic acid	30
(2*S*,3*S*,4*S*)-3-Hydroxy-4-methylglutamic acid	33
L-2-Amino-4-chloropent-4-enoic acid	34
3-(3-Amino-3-carboxypropyl)uridine	39
L-2-Amino-4-(methylphosphino)butyric acid	43
3-(2,5-Dimethoxyphenyl)alanine	65
3-(2,5-Dimethoxy-4-methylphenyl)alanine	65
3-(4-Bromo-2,5-dimethoxyphenyl)alanine	65

[107] J. L. Corbin and M. Reporter, *Analyt. Biochem.*, 1974, **57**, 310.
[108] K. Widmer and W. Keller-Schierlein, *Helv. Chim. Acta*, 1974, **57**, 657.
[109] Y. Isowa and M. Ohmori, *Bull. Chem. Soc. Japan*, 1974, **47**, 2672.
[110] T. Polonski and A. Chimiak, *Tetrahedron Letters*, 1974, 2453; T. Kolasa and A. Chimiak, *Tetrahedron*, 1974, **30**, 3591.
[111] L. K. Vinograd, O. D. Shalygina, N. P. Kostyuchenko, and N. N. Suvorov, *Khim. geterotsikl. Soedinenii*, 1974, 1236.
[112] A. Holy, I. Votruba, and K. Jost, *Coll. Czech. Chem. Comm.*, 1974, **39**, 634.

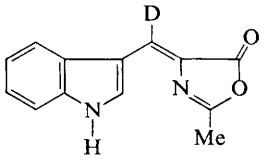
2,6-Dibromo-L-dopa	66
5,6-Dibromo-L-dopa	66
2,3,5-Tribromo-L-dopa	66
3,5,3′-Trimethyl-L-thyronine	114
3,5-Dimethyl-3′-iodo-L-thyronine	114
3,5′-Dimethyl-3′-isopropyl-L-thyronine	114
S-Adenosyl-L-homocysteine sulphoxide [b]	113*a*
S-Adenosyl-L-homocysteine sulphone	113*a*
S-4-Chloro[(β-D-ribofuranosyl)imidazo(4,5-*c*)pyrid-5′-yl]-L-homocysteine	113*b*
S-4-Amino[(β-D-ribofuranosyl)imidazo(4,5-*c*)pyrid-5′-yl]-L-homocysteine	113*b*
S-4-Methylamino[(β-D-ribofuranosyl)imidazo(4,5-*c*)pyrid-5′-yl]-L-homocysteine	113*b*
S-4-Dimethylamino[(β-D-ribofuranosyl)imidazo(4,5-*c*)pyrid-5′-yl]-L-homocysteine	113*b*
4-(1,4-Benzodioxan-6-yloxy)-3,5-di-iodo-L-phenylalanine	103

[a] Other new amino-acids, and labelled analogues of known amino-acids, mentioned elsewhere in this Chapter, are not repeated in this Table. [b] Both (*R*) and (*S*) sulphoxides synthesized and separated.

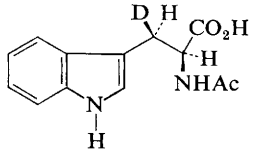
Labelled Amino-acids.—Full details have been published [69] of a route to labelled valines, described last year for the synthesis of (2*RS*,3*S*)-[4,4,4-$^2H_3$]-valine (Volume 6, p. 21) and now extended to the (2*RS*,3*S*)- and (2*RS*,3*R*)-[4-^{3}H]- and (2*RS*,3*S*)-[4-^{13}C]-analogues. A synthesis of (2*S*,3*S*)-[4,4,4-$^2H_3$]-valine (20) has been described [115] using the route established [116] for the [4-^{13}C]-analogue (Volume



(20)



(22)



(21)

6, p. 21) but with CD_3I used in place of $^{13}CH_3I$. (3*R*) and (3*S*) forms of [3-^{2}H]-tryptophan [*e.g.* (21), the 2*S*,3*R*-isomer] have been synthesized [68] by hydrogenation of the *Z*-arylidene-oxazolone (22), followed by hydrolysis. The hydrogenation step proceeds with greater than 95% *cis*-stereospecificity. The corresponding [3-^{3}H]tryptophans have been prepared by the same route.[68]

Deuteriation (with D_2–Pd or $NaBD_4$) of 2-bromo-, 4-bromo-, 2,5-dibromo-, and 3,4-dibromo-phenylalanines gives the corresponding ring-labelled amino-acids.[117]

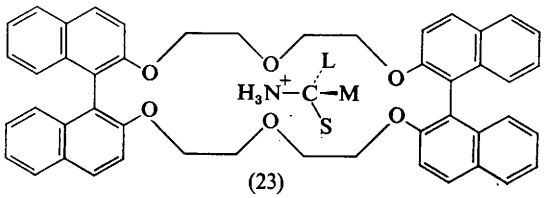
[113] (*a*) R. T. Borchardt and Y. S. Wu, *J. Medicin. Chem.*, 1974, **17**, 862; (*b*) R. T. Borchardt, J. A. Huber, and Y. S. Wu, *ibid.*, p. 868.
[114] E. C. Jorgensen, W. T. Murray, and P. Block, *J. Medicin. Chem.*, 1974, **17**, 434.
[115] H. Kleunder, F.-C. Huang, A. Fritzberg, H. Schnoes, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1974, **96**, 4054.
[116] H. Kleunder, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1973, **95**, 6149.
[117] H. Faulstich and H. Trischmann, *Analyt. Biochem.*, 1974, **62**, 615.

α-Methyldopa carrying a β-^{13}C label has been synthesized[63] from 3,4-dibenzyloxyphenyl-lithium by reaction with $^{13}CO_2$, and successive conversion into the aldehyde, 1-(3,4-dibenzyloxyphenyl)-2-nitropropene, and then the benzyl methyl ketone, from which the DL-amino-acid was prepared *via* the hydantoin route. 2-(Methylthio)[1-^{14}C]acetic acid has been converted into [3-^{14}C]methionine, *via* the correspondingly labelled 2-(methylthio)ethyl chloride and acetamidomalonate.[59] L-[3-^{14}C]Serine, on condensation with substituted indoles mediated by *E. coli* tryptophan synthetase, gives side-chain ^{14}C-labelled tryptophan analogues.[24]

The distribution of the ^{14}C label in lysine may be elucidated through permanganate oxidation to γ-aminopentanoic acid, γ-aminobutyric acid, β-alanine, and glycine, and assessing the ^{14}C content of these and the ninhydrin decarboxylation product of glycine formed in this way.[118] The ^{14}C content of methionine labelled in the methyl group may be determined through assay of $^{14}CH_3SCN$ formed by its treatment with CNBr.[119]

^{13}N-Labelled glutamic acid and glutamine have been prepared from $^{13}NH_3$ by enzymic synthesis.[120] DL-Lysine labelled either at N^{α} or N^{ϵ} with ^{15}N has been synthesized from potassium [^{15}N]phthalimide by reaction with $EtO_2CCHBr(CH_2)_4NHCOPh$ or with 5-(4-bromobutyl)hydantoin, respectively.[64]

Resolution of Amino-acids.—A larger number of papers than usual has appeared this year, partly due to progress in the design of chiral complexing agents capable of differentiation between enantiomers. Hexafluorophosphate salts of α-amino-acid esters have been shown to be resolvable by selective complexation with 3,3′-bis(hydroxymethyl)-2,2′-dihydroxy-1,1′-binaphthyl,[121] and pyridyl-bridged analogues.[122] The total optical resolution of an α-amino-acid ester by liquid–liquid chromatography based on the selective complexation principle has been further illustrated[123] using aqueous $NaPF_6$ or $LiPF_6$ on Celite as stationary phase, with a chloroform solution of the (*R*,*R*)-macrocycle (23) as mobile phase.



(23)

The mode of complexation of a chiral amine is shown in (23), and methyl *p*-hydroxyphenylglycinate is efficiently resolved by the technique;[123] the molecular architecture of the complexing agent has specific limitations if it is to recognize

[118] I. J. Christensen, P. O. Larsen, and B. L. Moller, *Analyt. Biochem.*, 1974, **60**, 531.
[119] B. R. Clark, H. Ashe, R. M. Halpern, and R. A. Smith, *Analyt. Biochem.*, 1974, **61**, 243.
[120] M. B. Cohen, L. Spolter, N. MacDonald, D. T. Masuoka, S. Laws, H. H. Neely, and J. Takahashi, 'Radiopharmacology of Labelled Compounds', I.A.E.A., Vienna, 1973, p.1.
[121] R. C. Helgeson, J. M. Timko, P. Moreau, S. C. Peacock, J. M. Mayer, and D. J. Cram, *J. Amer. Chem. Soc.*, 1974, **96**, 6762.
[122] M. Newcomb, G. W. Gokel, and D. J. Cram, *J. Amer. Chem. Soc.*, 1974, **96**, 6810.
[123] L. R. Sousa, D. H. Hoffmann, L. Kaplan, and D. J. Cram, *J. Amer. Chem. Soc.*, 1974, **96**, 7100.

one enantiomer preferentially, and the broader basis of complexation by chiral crown ether complexes has been reviewed.[124] To use the authors' words, 'a molecular basis has been provided by these studies for building an amino ester resolving machine'.[121] The principle is likely to be illustrated often in natural products, since there are indications that relatively simple structural requirements must be met; thus, *cyclo*-(L-Pro-Gly)$_n$ (n = 3 or 4) forms complexes with D- and L-amino-acid ester salts,[125] involving the carbonyl groups of the cyclic peptide and the protonated amino-group of the salt, and ^{13}C n.m.r. resonances for several carbon atoms of the complexed D-enantiomer are shifted relative to those of the L-enantiomer. This is taken as evidence for enantiomeric differentiation.[125]

Arising from a study of the transport of amino-acids through organic liquid membranes (toluene separating two aqueous phases),[126] the suggestion is made that chirospecific transport could be exploited in a novel resolution technique employing a chiral organic membrane.

A series of papers has appeared dealing with a more conventional ligand-exchange principle for the resolution of amino-acids.[127] Chloromethylated polystyrene treated with an L- or D-amino-acid ester, and hydrolysis of the product, provides a chiral phase which, after co-ordination to copper(II) ions, is suitable for column chromatographic resolution of amino-acids. Powdered paper is advocated for the column chromatographic resolution of DL-tryptophan-α-^{14}C,[128] and paper impregnated with alginic acid and silica gel provides an ion-exchange medium for the resolution of amino-acids.[129]

Leaving discussion of gas–liquid chromatographic resolution to Section 6 of this Chapter, standard techniques are illustrated in the use of *N*-carvomenthoxyacetyl derivatives for resolution by fractional crystallization,[130] differentiation by α-chymotrypsin between D- and L-*N*-acyl phenylalanine esters,[131] and resolution of diaminopimelic acid as the bis-benzyloxycarbonyl derivative by treatment with aniline in the presence of papain, to give the crystalline L,L-monoanilide.[132]

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids.—(See also Chapter 2, Part II.) Precision neutron diffraction studies of α-amino-acids continue to be reported, with definitive hydrogen locations and conformational features of L-valine hydrochloride,[133] hippuric acid,[134] and L-cystine dihydrochloride.[135]

[124] D. J. Cram and J. M. Cram, *Science*, 1974, **183**, 803.
[125] C. M. Deber and E. R. Blout, *J. Amer. Chem. Soc.*, 1974, **96**, 7566.
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[127] V. A. Davankov, S. V. Rogozhin, and A. V. Semechkin, *J. Chromatog.*, 1974, **91**, 493; I. Peslekas, S. V. Rogozhin, and V. A. Davankov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 174, 1872; *Zhur. obshchei Khim.*, 1974, **44**, 468.
[128] L. V. Handes, R. Kido, and M. Schmaeler, *Prep. Biochem.*, 1974, **4**, 47.
[129] A. M. El Din Awad and O. M. El Din Awad, *J. Chromatog.*, 1974, **93**, 393.
[130] K. Witkiewicz, F. Rulko, and Z. Chabudzinski, *Roczniki Chem.*, 1974, **48**, 651.
[131] M. S. Matta, J. A. Kelley, A. J. Tietz, and M. F. Rohde, *J. Org. Chem.*, 1974, **39**, 2291.
[132] A. Arendt, A. Kolodziejczyk, T. Sokolowska, and E. Szufler, *Roczniki Chem.*, 1974, **48**, 635.
[133] T. F. Koetzle, L. Golic, M. S. Lehmann, J. J. Verbist, and W. C. Hamilton, *J. Chem. Phys.*, 1974, **60**, 4690.
[134] M. Currie and A. L. MacDonald, *J.C.S. Perkin II*, 1974, 784.
[135] S. C. Gupta, A. Sequeira, and R. Chidambaram, *Acta Cryst.*, 1974, **B30**, 562.

X-Ray crystal analysis of other naturally occurring amino-acids reported during the year deal with DL-serine and anhydrous L-serine,[136] calcium L-glutamate trihydrate,[137] pyroglutamic acid,[138] 3,5,3′-tri-iodo-L-thyronine,[139] L-thyronine ethyl ester hydrochloride monohydrate,[140] and D-β-tyrosine hydrobromide and hydrochloride.[141] L-Cysteine is bound to methylmercury(II) *via* a deprotonated thiol group, whereas L-methionine is co-ordinated *via* nitrogen, in their respective 1 : 1 complexes.[142]

Proof of structure for (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methyl-n-valeric acid[143] (a component of bleomycins), and (2*S*,3*R*,4*S*)-2-amino-3-methyl-4-hydroxy-n-valeric acid[12] (a component of γ-amanatin) has been supplied by *X*-ray analysis, for the latter in the form of its lactone hydrobromide. The crystal structure of the potassium salt of *N*-(purin-6-ylcarbamyl)-L-threonine, isolated in 1969 from the total tRNA of yeast, has been determined[144] for its relevance to the conformations of anticodon loops of tRNA, and the base pairing and base stacking interactions of modified nucleosides.

Less-common amino-acids and their derivatives studied include: bis copper(II) D-penicillamine disulphide nonahydrate,[145] L-mimosine sulphate hydrate,[146] meso-3,3′-dithiobisvaline dihydrate,[147] meso-lanthionine dihydrochloride,[148] L-thioproline,[149] *N*-acetyl-L-norvaline,[150] N^{α}-acetyl-L-glutamine,[151] and *N*-benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester.[152]

3,4-Dehydro-DL-proline readily dimerizes;[153] *X*-ray crystal analysis shows that dimerization leads to only D,D- and L,L-stereoisomers.

N.M.R. Spectroscopy.—^{1}H N.m.r. studies of amino-acids and their derivatives continue to provide information on fundamental structural features, such as protonation equilibria for L-cysteine as a function of pH,[154] and ligand sites in mercury(II) complexes of cysteine, cysteine methyl ester, and *S*-methyl-cysteine.[155] Conformational studies of a familiar type, employing both ^{1}H and ^{13}C n.m.r., deal with *N*-formyl alanine amide and *N*-methylamide,[156] *N*-acetyl-*N*-methyl

[136] T. J. Kistenmacher, G. A. Rand, and R. E. Marsh, *Acta Cryst.*, 1974, **B30**, 2573.
[137] H. Einspahr and C. E. Bugg, *Acta Cryst.*, 1974, **B30**, 1037.
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[139] V. Cody, *J. Amer. Chem. Soc.*, 1974, **96**, 6720.
[140] A. Camerman and N. Camerman, *Canad. J. Chem.*, 1974, **52**, 3042.
[141] A. N. Chekhlov, Y. T. Struchkov, and A. I. Kitaigorodskii, *Kristallografiya*, 1974, **19**, 981.
[142] Y. S. Wong, N. J. Taylor, P. C. Chieh, and A. J. Carty, *J.C.S. Chem. Comm.*, 1974, 625.
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[146] A. Mostad, E. Rosenqvist, and C. Romming, *Acta Chem. Scand.* (*B*), 1974, **28**, 249.
[147] L. G. Warner, T. Ottersen, and K. Seff, *Acta Cryst.*, 1974, **B30**, 1077.
[148] R. E. Rosenfield and R. Parthasarathy, *J. Amer. Chem. Soc.*, 1974, **96**, 1925.
[149] K. K. Chacko, *Cryst. Struct. Comm.*, 1974, **3**, 561.
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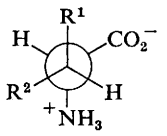
[153] I. L. Karle, H. C. J. Ottenheym, and B. Witkop, *J. Amer. Chem. Soc.*, 1974, **96**, 539.
[154] D. B. Walters and D. E. Leyden, *Analyt. Chim. Acta*, 1974, **72**, 275.
[155] G. A. Neville and T. Drakenberg, *Canad. J. Chem.*, 1974, **52**, 616.
[156] V. N. Solkan and V. F. Bystrov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1308.

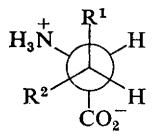
alanine methyl ester,[157] and Boc-glycine.[158] A study of the pH dependence of vicinal coupling constants for histidine and its N^{im}-benzyl, N^{α}-acyl, and *O*-methyl derivatives indicates a *gauche* conformation for histidine in basic solution[159] due to electrostatic interaction between the carboxy and imidazole groups, but in acidic or isoelectric solutions 'the ions show equally populated conformations' (*sic*). ^{1}H N.m.r. data for proline[160, 161] and for hydroxy-L-proline and the *allo*-diastereoisomer[162] have been interpreted in terms of conformational mobility of the pyrrolidine ring[160, 161] and in terms of the torsion angle of the plane of the carboxy-group (around the C_{α}–C^{O} axis),[162] respectively. A study of amino-acids in the solid state has been published.[163]

An extensive literature is accumulating on ^{13}C n.m.r. characteristics of the common amino-acids. Data for eight amino-acids are available,[164] and exploration of some of the factors determining chemical shifts has yielded ^{13}C n.m.r. titration curves of individual carbon atoms of representative amino-acids, with interpretation of the observed shifts.[165] ^{13}C Spin lattice relaxation times T_1 of several amino-acids as a function of pD and of concentration,[166] including data for glycine and lysine,[167] reveal a strong dependence of carboxy carbon T_1 on these parameters, accounted for[166] by intermolecular association.

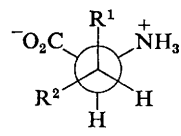
^{13}C–C–C–^{1}H Coupling constants for the carboxy carbon and β-hydrogen atoms in amino-acids give information on side-chain conformation which is not available from ^{1}H–^{1}H coupling constants alone.[168] Data for 1M solutions of amino-acids (^{13}C in natural abundance) are interpreted[168] to show that aspartic acid exists at pH 11 in conformations (24), (25), and (26) ($R^1 = CO_2H$, R^2 = Me)



(24)



(25)



(26)

in proportions 15 : 62 : 23, and that for valine at pH 5.7, the respective proportions ($R^1 = R^2$ = Me) are 17 : *ca.* 60 : *ca.* 20. ^{13}C–^{13}C Coupling constants for ^{13}C-enriched amino-acids[169] (including 85% ^{13}C-enriched alanine, valine, leucine, and isoleucine[170]) have been reported, as has their dependence on pH,[170] though

[157] M. Goodman, F. Chen, and C. Y. Lee, *J. Amer. Chem. Soc.*, 1974, **96**, 1479.
[158] M. Branik and H. Kessler, *Tetrahedron*, 1974, **30**, 781.
[159] R. C. Weinkam and E. C. Jorgensen, *J. Amer. Chem. Soc.*, 1974, **96**, 6084.
[160] M. Ellenberger and L. Pogliani, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 613.
[161] M. Ellenberger, L. Pogliani, K. Haeuser, and J. Valat, *Chem. Phys. Letters*, 1974, **27**, 419.
[162] L. Pogliani and M. Ellenberger, *J. Amer. Chem. Soc.*, 1974, **96**, 1621.
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[164] W. Voelter, S. Fuchs, R. H. Seuffer, and K. Zech, *Monatsh.*, 1974, **105**, 1110.
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[169] J. A. Sogn, L. C. Craig, and W. A. Gibbons, *J. Amer. Chem. Soc.*, 1974, **96**, 4694.
[170] T. D. Son, S. Fermandjian, E. Sala, R. Mermet-Bouvier, M. Cohen, and P. Fromageot, *J. Amer. Chem. Soc.*, 1974, **96**, 1484.

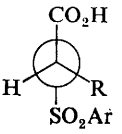
their interpretation in terms of electronic structure and conformation cannot yet be attempted.[169]

Magnetic resonance studies of nitrogen nuclei are still in their pioneering phase, and recent papers deal with ^{14}N n.m.r. of amino-acids, peptides, and derivatives in 0.2M-aqueous solution,[171] ^{14}N,^{2}H pure nuclear quadrupole resonance of deuteriated amino-acids at 77 K,[172] and natural-abundance ^{15}N n.m.r. of eight amino-acids, including L-arginine,[173] for which the pH-dependence of ^{15}N chemical shifts may be related to various ionized species.

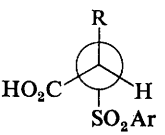
O.R.D. and C.D. Spectra.—Studies of the chiroptical properties of amino-acids are being entered into with more scope for interpretation of spectra in terms of conformational isomerism; excepting a study of transitions in the far-u.v., all the more superficial collecting of data has already been carried out for amino-acids. Recent studies of aromatic amino-acids (D-phenylglycine,[174] α-methyl-L-tyrosine,[175] and *p*-hydroxy-D-phenylglycine [176]) include a thorough analysis of the conformations in solution of *p*-hydroxyphenylglycine and its amide; judged by o.r.d. and c.d. data, torsion angles ψ near $-5°$ and χ near 75° are assumed by these compounds.[176]

A good deal of data collected for L-cystine can be interpreted in terms of the chirality of the disulphide chromophore, and the near-u.v. c.d. of solutions of this amino-acid has been analysed in terms of conformer populations.[177] Effects of temperature and salt concentration on the c.d.[178, 179] and o.r.d.[179] of *N*-acetyl-L-alanine *N'*-methylamide [178, 179] and other alanine derivatives [178] have been studied. The *N*-acetyl *N'*-methylamide in 1,2-dichloroethane adopts an intramolecularly hydrogen-bonded conformation at lower temperatures and an increasing amount of a non-hydrogen-bonded form appears as the temperature of the solution is raised,[179] ΔH^0 for the transition between the two forms being 2570 ± 5 cal mol^{-1} and $\Delta S^0 = 6.56 \pm 0.1$ e.u.[179]

Dansyl-L-amino-acids show a positive Cotton effect centred near 260 nm in MeOH, in most cases studied.[180] The assumption [180] that conformation (27) is adopted by dansyl-L-amino-acids showing a positive Cotton effect, but that an alternative staggered conformation (28) accounts for a negative Cotton effect, appears to need more justification than has been provided.



(27)



(28)

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[179] G. M. Crippen and J. T. Yang, *J. Phys. Chem.*, 1974, **78**, 1127.
[180] T. Polonski, A. Chimiak, and M. Kochman, *Tetrahedron*, 1974, **30**, 641.

Mass Spectrometry.—The two categories into which mass spectrometric studies of amino-acids might be divided, one covering interpretation of fragmentation modes, the other dealing with analytical applications, are both represented this year. The mass spectra of dimethyl glutamate and its deuteriated derivatives [181] indicate sequential loss of MeOCO and MeOH giving a prominent *m/e* 116 peak, and base peak *m/e* 84, respectively, the latter being formed by hydrogen transfer from C-3 with the formation of a protonated pyrrol-5(4*H*)-one. The mass spectrum of arginine obtained using fast heating rates at the probe shows an $M + 1$ peak (*m/e* 175), while slower heating rates give spectra with highest-mass fragments shown to be *m/e* 158 and 157 (loss of NH_3 or H_2O, respectively, from parent ion);[182] an abundant *m/e* 115 peak may be useful in interpretation of the mass spectra of underivatized arginine peptides. High-pressure chemical ionization mass spectrometric studies continue [183] with studies of association occurring between valine and proline with their protonated ions under CIMS conditions; it is suggested [183] that such studies will contribute knowledge of the energetics of ions of biologically important molecules in their biological environments.

Analytical studies by g.c.–m.s. of volatile derivatives of amino-acids deal with β-aminoisobutyric acid in urine at 1 ng levels,[184] and 12 amino-acids in biological samples [185] (quadrupole m.s. of *N*-trifluoroacetyl n-butyl esters); also of *N*-methylamino-acids present in hydrolysates of actomyosin from heart-cell cultures.[186] Trimethylsilyl derivatives have been employed in similar studies, for the analysis of thyroxine at 100 pg levels,[187] and common amino-acids at 10—100 femtomole levels.[188]

Other Physical and Theoretical Studies.—Non-routine physical studies (and their possible value in a biological context) include surface tension of solutions in 0.1M-NaCl (comparison of hydrophobicity of side-chains),[189] dielectric increments of homologous series of αω-amino-acids (adoption of extended conformations),[190] depolarized Rayleigh scattering (ψ for glycine is $170 \pm 10°$),[191] polarized i.r. of N-deuteriated L-alanine,[192] and studies of the temperature dependence of u.v. spectra of tyrosine and tryptophan [193] and of *N*-acetyltryptophan ethyl ester and tyrosine and phenylalanine analogues (in comparison with temperature effects on protein spectra).[194]

[181] E. Lerch and M. Hesse, *Helv. Chim. Acta*, 1974, **57**, 1584.
[182] R. J. Beuhler, E. Flanigan, L. J. Greene, and L. Friedman, *J. Amer. Chem. Soc.*, 1974, **96**, 3990.
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[188] F. P. Abramson, M. W. McCaman, and R. E. McCaman, *Analyt. Biochem.*, 1974, **57**, 482.
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[191] C. Destrade and C. Clement, *Compt. rend.*, 1974, **278**, *C*, 733.
[192] R. F. Adamowicz and M. L. Sage, *Spectrochim. Acta*, 1974, **30A**, 1007.
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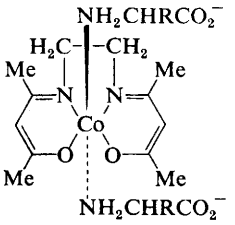
[194] A. Cooper, *F.E.B.S. Letters*, 1974, **48**, 101.

MO calculations of conformations adopted by γ-aminobutyric acid [195, 196] and alanine [196] present different conclusions from those published earlier; notably,[195] γ-aminobutyric acid adopts a highly folded structure.

Determination of Absolute Configuration and Optical Purity of Amino-acids.—A modification of Horeau's procedure has been described,[197] so that it may be applied to amines and amino-acids. 1-(2-Phenylbutyroyl)imidazole is advocated as reagent for the new procedure, and its validity was established using esters of alanine, leucine, and tryptophan.[197]

The optical purity of samples of amino-acids available only in 50–100 mg quantities can be determined by exploiting the greatly enhanced rotation resulting from the equilibration of the sample with $K_2[Co(acac_2en)(Gly)_2]$ to give an equilibrium concentration of the complex (29) after *ca.* 10 h at pH 10.[198] Comparison of observed specific rotations with those for standards prepared from



(29)

optically pure samples under identical conditions gives a measure of the optical purity of the test sample. An enzymic method for the assessment of the optical purity of radioactively labelled L-amino-acids employs t.l.c. estimation of the α-keto-acid formed by D-amino-acid oxidase.[199]

5 Chemical Studies of Amino-acids

Racemization.—An extensive review has appeared dealing with amino-acid racemases.[200] The mechanism of the racemization of L-cystine (the only protein amino-acid to suffer racemization during the hydrolysis of proteins with 6N hydrochloric acid) is considered to involve the formation of an acid enol stabilized by the inductive effect of the partially or fully charged β-heteroatom;[201] some support for this hypothesis is given by the fact that *S*-(2,4-dinitrophenyl)-L-cysteine and 2,3-diaminopropionic acid both racemize at rates similar to that of L-cystine, in acid solutions.[201]

Continuation of studies described last year (Volume 6, p. 28) on the use of aspartic acid racemization as an index for the age of fossil bones leads to an estimate of ⩽50 000 years for the age of several Californian Paleo-Indian

[195] B. Pullman and H. Berthod, *Compt. rend.*, 1974, **278**, *D*, 1433.
[196] S. Parthasarathy, M. M. Dhingra, and G. Govil, *Indian J. Chem.*, 1974, **12**, 805.
[197] H. Brockmann and N. Risch, *Angew. Chem. Internat. Edn.*, 1974, **13**, 664.
[198] Y. Fujii and H. Yoneda, *Chem. Letters*, 1974, 43; Y. Fujii, *Bull. Chem. Soc. Japan*, 1974, **47**, 2856.
[199] M. J. Hardy, *Analyt. Biochem.*, 1974, **57**, 529.
[200] K. Soda, *Seikagaku*, 1974, **46**, 203.
[201] S. J. Jacobson, C. G. Wilson, and H. Rapoport, *J. Org. Chem.*, 1974, **39**, 1075.

skeletons.[202] The technique is useful for dating bones which are either too old or too small for radiocarbon dating.[203] Misgivings about the possible influence of the molecular environment of the amino-acid on the timekeeping of this novel chemical clock are largely overcome by the way in which the method is applied;[203] a bone which can be accurately dated by the radiocarbon technique is used to estimate the first-order racemization rate constant for the aspartic acid it contains, for a particular site, so that other bones at the same site can be dated on the basis of their D- and L-aspartic acid content.[203]

General Reactions.—Mutarotation of solutions of L-amino-acids in aqueous potassium carbonate is ascribed to carbamate formation.[204] The general trend towards more positive rotations with time may be the basis of a method of assigning absolute configuration to acid-sensitive amino-acids (*i.e.* amino-acids which would not survive the conditions required for the application of the Clough–Lutz–Jirgensons rule).[204]

General reactions which will enhance the value of amino-acids as intermediates in organic synthesis include conversion of amino-acid esters into α-alkoxycarbonyl diazoalkanes under mild conditions with *p*-nitro- or 2,4-dinitrobenzenediazonium tetrafluoroborate,[205] synthesis of α-fluoro-acids by diazotization of amino-acids in 70% HF in pyridine,[206] reduction with diborane to 2-aminoalkanols (reductive dehalogenation of ring-chloro- or ring-bromo-phenylalanines is avoided),[207] and dianion formation from *NN*-dimethylglycine followed by nucleophilic addition to benzophenone.[208]

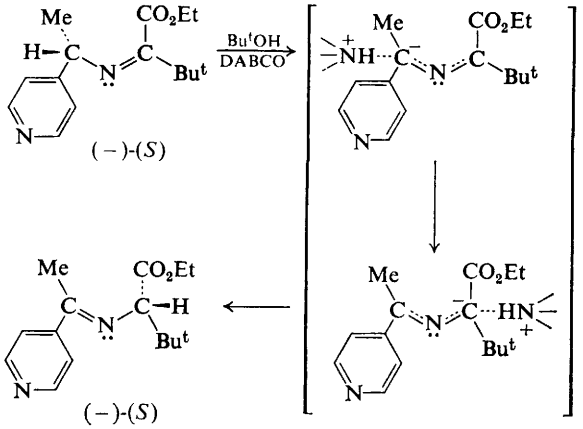
Five mechanistic schemes can be considered for the Dakin–West synthesis of α-acetamidoalkyl methyl ketones from amino-acids with acetic anhydride and pyridine, and rate studies support [209] the mechanism [210] involving a 4-(pyrid-4-yl)oxazolone intermediate.

Interaction of amino-acids with *p*-benzoquinones provides a basis for colorimetric quantitation, previously assumed to involve charge-transfer complex formation but now shown [211] to lead to 2,5-disubstituted quinones (30).

Stereospecific transamination and isotopic exchange of the α-proton of an amino-acid (Scheme 6),[212] involving suprafacial proton transfer, depends on the ability of the bulky pyridyl and t-butyl groups to maintain conformation through the transition state.

Nitrosation studies of imines under mildly acidic conditions have been reported for proline,[213, 214] hydroxyproline,[214] sarcosine,[214] and *N*-acyl tryptophans,[215] a

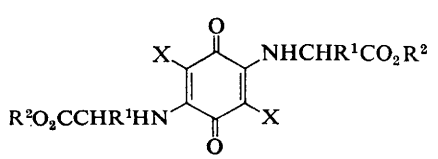
[202] J. L. Bada, R. A. Schroeder, and G. F. Carter, *Science*, 1974, **184**, 791.
[203] J. L. Bada, R. A. Schroeder, R. Protsch, and R. Berger, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 914.
[204] W. L. F. Armarego and B. A. Milloy, *J.C.S. Perkin I*, 1974, 1905.
[205] J. F. McGarrity, *J.C.S. Chem. Comm.*, 1974, 558.
[206] G. A. Olah and J. Welch, *Synthesis*, 1974, 652.
[207] M. L. Anhoury, M. Arickx, P. Crooy, R. De Neys, and J. Eliaers, *J.C.S. Perkin I*, 1974, 191.
[208] B. Angelo, *Compt. rend.*, 1974, **278**, *C*, 383.
[209] N. L. Allinger, G. L. Wang, and B. R. Dewhurst, *J. Org. Chem.*, 1974, **39**, 1730.
[210] W. Steglich and G. Hofle, *Tetrahedron Letters*, 1968, 1619.
[211] R. Foster, N. Kulevsky, and D. S. Wanigasekera, *J.C.S. Perkin I*, 1974, 1318.
[212] M. D. Broadhurst and D. J. Cram, *J. Amer. Chem. Soc.*, 1974, **96**, 581.
[213] A. Okany, T. F. Massiah, L. J. Rubin, and K. Yates, *Canad. J. Chem.*, 1974, **52**, 1050.
[214] S. S. Mirvish, J. Sams, T. Y. Fan, and S. R. Tannenbaum, *J. Nat. Cancer Inst.*, 1973, **51**, 1833 (*Chem. Abs.*, 1974, **80**, 142 053).
[215] R. Bonnett and R. Holleyhead, *J.C.S. Perkin I*, 1974, 962; see also K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.*, (*C*), 1969, 954.



Scheme 6

prime concern being the assessment of maximum amounts of *N*-nitroso-compounds which could be formed in the stomach or in stored food (less than 0.9 p.p.b. in canned meat [213]).

Reactions of amino-acids with carbohydrates include a preparation of 3-phenylfuran from phenylalanine and glucose,[216] an unusual Ugi reaction involving arabinose, glycine, and cyclohexyl isocyanide to give (31) and open-chain



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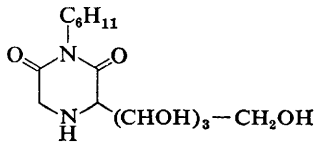

(31)

analogues,[217] and formation of a free radical derived from L-scorbamic acid through reaction of an amino-acid with dehydro-L-ascorbic acid.[218] Pyrolysis of alkyl amino-acids at 500 °C gives decarboxylation and condensation products including amines and pyridines.[219] Valine treated similarly, in the presence of tricaproin, gives caproamide, *N*-isobutylcaproamide, and capronitrile.[220]

Papers dealing with the synthesis of amino-acid derivatives for purposes other than peptide synthesis include vinyl ester synthesis by ester interchange,[221] *N*-methylation of Z- and Boc-amino-acids with MeI–Ag_2O–DMF,[222] synthesis of

[216] K. Misselhorn and H. Bruckner, *Chem., Mikrobiol., Technol. Lebensmitteln*, 1974, **3**, 25.
[217] A. I. Polyakov, N. N. Aseeva, and V. G. Bezrukova, *Izvest. Akad. Nauk S.S.S.R.*, 1974, 1589.
[218] T. Kurata and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1981; M. Namiki, M. Yano, and T. Hayashi, *Chem. Letters*, 1974, 125.
[219] Y. C. Lien and W. W. Nawar, *J. Food Sci.*, 1974, **39**, 914; M. A. Ratcliff, E. E. Medley, and P. G. Simmonds, *J. Org. Chem.*, 1974, **39**, 1481.
[220] Y. C. Lien and W. W. Nawar, *J. Food Sci.*, 1974, **39**, 917.
[221] K. Geckeler and E. Bayer, *Chem. Ber.*, 1974, **107**, 1271.
[222] K. Okamoto, H. Abe, K. Kuromizu, and N. Izumiya, *Mem. Fac. Sci., Kyushu Univ., Ser. C*, 1974, **9**, 131.

N-TFA amino-acid trimethylsilyl esters by successive treatment with Me_3SiCl and TFA-anhydride,[223] and *O*- and *N*-diethylborylation of amino-acids with Et_3B.[224] Comparison has been made[225] of the various reagents which may be used for the synthesis of trimethylsilyl derivatives of amino-acids. Most of the common *N*- and side-chain-protecting groups may be stripped from amino-acids with boron tribromide[226] or with trifluoromethanesulphonic acid,[227] without additional complications with methionine, tryptophan, or tyrosine.

Specific Reactions.—Syntheses of natural products starting from glutamic acid have been recorded for both enantiomers of disparlure (*cis*-7,8-epoxy-2-methyloctadecane),[228] for D-ribose,[229] and proline[85] and hydroxyproline.[71] A synthesis of trichotomine, the blue pigment from *Clerodendron trichotomum*, starts with L-tryptophan;[230] particularly interesting microbiological syntheses, the conversion of amino-acids into higher alcohols by auxotrophic mutants of *Saccharomyces cerevisiae*,[231] and the biosynthesis of linalool from leucine in *Cinnamomum camphora* var. *linalooliferium* (the first demonstration of isoprenoid biosynthesis in a higher plant),[232] have been reported.

Decarboxylation of tryptophan is achieved in 45% yield by heating the chelates obtained with cupric or zinc acetates;[233] decarboxylation kinetics have been established for *N*-salicylidene-DL-valine,[234] and $Cu(dpa)^{2+}$-catalysed hydrolysis of glycine methyl esters [dpa = bis-(2-pyridylmethyl)amine] has been studied.[235]

Aziridines react with methionine in dilute aqueous solutions at pH 7.4 to give sulphonium salts,[236] indicating a likely site for attack by biological alkylating agents. Under defined conditions, L-methionine-*S*- (or -*R*-) sulphoximine is converted into the sulphoxide with retention of configuration at sulphur, without affecting the α-amino-group.[237] Phosgene reacts with methionine at nitrogen, to give carbonylbis(L-methionine), which in the form of its bis(*p*-nitrophenyl ester) is advocated[238] as a cross-linking agent for insulin, removable by CNBr.

Specific Reactions of Amino-acids Related to Biochemical Processes.—Interactions between amino-acids and nucleotides which can be surmised to have occurred in the prebiotic milieu have been discussed.[239]

Oxidative transformations of aromatic amino-acids have received careful quantitative study, including the cyclization of dopa to dopachrome (32) *en*

[223] H. R. Kricheldorf and M. Fehrle, *Synthesis*, 1974, 420.
[224] R. Koster and E. Rothgery, *Annalen*, 1974, 112.
[225] S. V. Rogozhin, Y. A. Davidovich, S. M. Andreev, N. V. Mironova, and A. I. Yurtanov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1868.
[226] A. M. Felix, *J. Org. Chem.*, 1974, **39**, 1427.
[227] H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J.C.S. Chem. Comm.*, 1974, 107.
[228] S. Iwaki, S. Marumo, T. Saito, M. Yamada, and K. Katagiri, *J. Amer. Chem. Soc.*, 1974, **96**, 7842.
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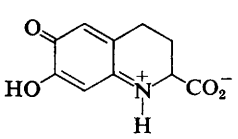
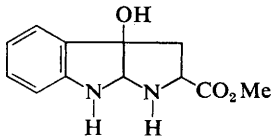
[232] T. Suga, T. Hirata, T. Shishibori, and K. Tange, *Chem. Letters*, 1974, 189.
[233] T. Kametani, T. Suzuki, K. Takahashi, and K. Fukumoto, *Synthesis*, 1974, 131.
[234] M. Malherbe and G. Chatelus, *Compt. rend.*, 1974, **278**, *C*, 1205.
[235] R. Nakon, P. R. Rechani, and R. J. Angelici, *J. Amer. Chem. Soc.*, 1974, **96**, 2117.
[236] P. A. Capps and A. R. Jones, *J.C.S. Chem. Comm.*, 1974, 320.
[237] R. A. Stephani and A. Meister, *Tetrahedron Letters*, 1974, 2307.
[238] W. D. Busse and F. H. Carpenter, *J. Amer. Chem. Soc.*, 1974, **96**, 5947.
[239] C. Saxinger and C. Ponnamperuma, *Origins Life*, 1974, **5**, 189.

route to melanin,[240] autoxidation of tryptophan at 100 °C (maximum rate at pH 5.3 through the acid pH range),[241] and photosensitized oxygenation of tryptophan methyl ester to give (33).[242] Evidence for the formation of a 1 : 1



(32)



(33)

molecular complex between ATP and tryptophan, or *N*-acetyltryptophanamide, has been derived [243] from difference u.v. spectrometry.

Binding of Ca^{2+} ions by amino-acids in aqueous solutions has been studied.[244]

Effects of Electromagnetic Radiation on Amino-acids.—Reversible effects dealing with concentration dependence of fluorescence quenching of tyrosine,[245] and luminescence [246] and phosphorescence [247] studies of tryptophan, the latter in relation to the phosphorescence of a Trp residue at different protein locations, have been reported. Photoelectron quantum yields of representative amino-acids in the 180—240 nm wavelength range exhibit wavelength dependences similar to those of corresponding poly(amino-acids).[248]

Pulse radiolysis of selenomethionine,[249] and a study of radicals formed by γ-irradiation of solid threonine [250] and by photolysis of amino-acids in solution in the presence of ferricyanide ions or tris-(1,10-phenanthroline)iron,[251] are continuations of earlier studies of a conventional type, while an extraordinary implication has emerged from positron annihilation studies of amino-acids.[252] Since triplet intensities of positron annihilation time spectra of a D-amino-acid differ from those of its enantiomer, subsequent β-decay can lead to a minute initial asymmetry of otherwise racemic amino-acids, from which the natural state of affairs favouring one enantiomer could have developed.[252]

6 Analytical Methods

General.—An extensive survey of modern assay procedures, including several protein amino-acids, is available.[253] Microdetermination of amino-acids using

[240] T. E. Young, J. R. Griswold, and M. H. Hulbert, *J. Org. Chem.*, 1974, **39**, 1980.
[241] M. Stewart and C. H. Nicholls, *Austral. J. Chem.*, 1974, **27**, 205.
[242] M. Nakagawa, T. Kaneko, K. Yoshikawa, and T. Hino, *J. Amer. Chem. Soc.*, 1974, **96**, 624.
[243] F. Morita, *Biochim. Biophys. Acta*, 1974, **343**, 674.
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[246] J. Moan, *J. Chem. Phys.*, 1974, **60**, 3859; D. Muller, M. Ewald, and G. Durocher, *Canad. J. Chem.*, 1974, **52**, 407.
[247] J. U. von Schutz, J. Zuclich, and A. H. Maki, *J. Amer. Chem. Soc.*, 1974, **96**, 714.
[248] R. J. Dam, C. A. Burke, and O. H. Griffith, *Biophys. J.*, 1974, **14**, 467.
[249] M. Tamba, S. Bonora, and R. Badiello, *Z. Naturforsch.*, 1974, **29b**, 571.
[250] D. M. Close and R. S. Anderson, *J. Chem. Phys.*, 1974, **60**, 2828.
[251] A. L. Poznyak, S. I. Arzhankov, and G. A. Shagisultanova, *Biofizika*, 1974, **19**, 233.
[252] S. A. Garay, L. Keszthelyi, I. Demeter, and P. Hrasko, *Nature*, 1974, **250**, 332.
[253] 'Methods of Enzymatic Analysis', second English edition, ed. H. U. Bergmeyer, Vol. 4, Academic Press, New York, 1974.

dansyl chloride,[254] and analysis of amino-acids in micro-quantities in nerve tissue and individual neurons,[255] are subjects of recent reviews.

Gas–Liquid Chromatography.—The derivatives of amino-acids which are suitable for g.l.c. analysis have been reviewed,[256] with particular emphasis on silyl derivatives.[256a] There is the customary expression of preference for one or another of the perfluoroacyl N-substituents and alkyl ester groups as a means of converting amino-acids in mixtures into volatile derivatives for g.l.c. analysis, and *N*-trifluoroacetyl n-butyl esters,[257–261] *N*-heptafluorobutyryl n-propyl[262, 263] and isobutyl[264] esters, and *N*-acetyl n-propyl esters[265, 266] are represented in this year's literature. Reaction of amino-acids with 1,3-dichloro-1,1,3,3-tetrafluoropropanone gives 2,2-bis(chlorodifluoromethyl)oxazolidinones which are advocated[267] as volatile derivatives for g.l.c. analysis. The identification of histidine by standard methods, *e.g.* as the N^{α},N^{im}-bis(trifluoroacetyl) derivative n-butyl ester, involves a derivative with a tendency to decompose during handling, and the more stable N^{im}-ethoxycarbonyl-N^{α}-trifluoroacetyl n-butyl ester gives more reliable results.[261]

Sample preparation techniques during these derivative-forming procedures are crucial if the identification of nanomole quantities of amino-acids is to be achieved, and the experience of some of the leading innovators in the field has been summarized.[268] An improved procedure for the preparation of *N*-trifluoroacetyl amino-acid n-butyl esters of nanomole amounts of amino-acids has been described.[257]

Methylthiohydantoins obtained by Edman degradation may be identified by g.l.c. of their trimethylsilyl derivatives,[269] and all protein amino-acids give satisfactory derivatives for g.l.c., except arginine, hydroxyproline, and hydroxylysine. If a flame photometric sulphur detector is used with the gas chromatograph, in place of the flame ionization detector, a number of extraneous peaks accompanying the silylated methylthiohydantoins may be avoided.[270]

Two main approaches to the estimation of the enantiomeric composition of partly racemic amino-acids have been further illustrated this year. *N*-Trifluoroacetyl cyclohexyl esters of L,L-dipeptides may be used as stationary phases for separation of enantiomeric amino-acids in the form of a volatile derivative, and isoleucyl-isoleucine, norvalyl-norvaline, and butyryl-butyrine are superior as

[254] V. Neuhoff, *Mol. Biol., Biochem. Biophys.*, 1973, **14**, 85.
[255] N. N. Osborne, *Progr. Neurobiol.*, 1973, **1**, Part IV, 299.
[256] (*a*) V. Marek, *Chem. Listy*, 1974, **68**, 250; (*b*) A. Darbre, *Biochem. Soc. Trans.*, 1974, **2**, 70.
[257] P. Cancalon and J. D. Klingman, *J. Chromatog. Sci.*, 1974, **12**, 349.
[258] B. Thom and J. W. Parsons, *J. Chromatog.*, 1974, **90**, 370.
[259] J. P. Ussary, *Food Prod. Develop.*, 1973, **7**, 84, 86, 88.
[260] M. Sakamoto, K. I. Kajiyama, and H. Tonami, *J. Chromatog.*, 1974, **94**, 189.
[261] I. M. Moodie, *J. Chromatog.*, 1974, **99**, 495.
[262] M. A. Kirkman, *J. Chromatog.*, 1974, **97**, 175.
[263] C. W. Moss and M. A. Lambert, *Analyt. Biochem.*, 1974, **59**, 259.
[264] S. L. Mackenzie and D. Tenaschuk, *J. Chromatog.*, 1974, **97**, 19.
[265] R. F. Adams, *J. Chromatog.*, 1974, **95**, 189.
[266] B. W. Hanny and C. D. Elmore, *J. Agric. Food Chem.*, 1974, **22**, 476.
[267] P. Husek, *J. Chromatog.*, 1974, **91**, 475, 483.
[268] F. E. Kaiser, C. W. Gehrke, R. W. Zumwalt, and K. C. Kuo, *J. Chromatog.*, 1974, **94**, 113.
[269] W. M. Lamkin, J. W. Weatherford, N. S. Jones, T. Pan, and D. N. Ward, *Analyt. Biochem.*, 1974, **58**, 422.
[270] W. M. Lamkin, N. S. Jones, T. Pan, and D. N. Ward, *Analyt. Biochem.*, 1974, **58**, 549.

bases for the stationary phase in comparison with dipeptide derivatives reported earlier (Volume 6, p. 34).[271] The alternative approach, using an achiral stationary phase, involves conversion of the sample into a mixture of diastereoisomeric amides or esters, for example with (+)-isoketopinyl, (−)-dihydroteresantalinyl, or (−)-teresantalinyl chlorides,[272] or with perfluoroacyl-L-prolyl chlorides;[273, 274] extensions of these studies[274, 275] have employed *N*-trifluoroacetyl-L-prolyl,[274] -L-hydroxyprolyl,[275] and -L-thiazolidin-4-carbonyl[275] n-butyl esters of the amino-acid.

Ion-exchange and Partition Column Chromatography.—Most of the papers eligible for inclusion in this Section describe improvements to amino-acid analyser technique, including aspects of sample preparation and colorimetric estimation. Older generation analysers can be up-dated to accommodate wider ranges of amino-acids,[276] though with correct use of buffers a single-column analysis of 44 compounds can be performed in 5½ h.[277]

The operating conditions of the amino-acid analyser for threonine and serine,[278] lysine,[279, 280] ornithine, $\alpha\gamma$-diaminobutyric acid, and $\alpha\beta$-diaminopropionic acid,[280] and several less-common dibasic amino-acids[281] have been discussed, as have the effects of glucosamine on routine amino-acid assay.[282] Components of a mixture of acidic and neutral amino-acids emerge from a column of SE-Sephadex C-25 in order of their pK_a values.[283]

6-Acetimidyl-lysine residues in proteins subjected to routine hydrolysis prior to amino-acid analysis are only slowly hydrolysed to lysine (6M-hydrochloric acid at 110 °C), and partial hydrolysis to estimate the protein content of this residue has been investigated.[284] Normal conditions of hydrolysis may be used for synthetic peptides containing β-(uracil-1-yl)alanine, β-(thymin-1-yl)alanine, and β-(cytosin-1-yl)alanine, but β-(adenin-9-yl)alanine residues are converted into glycine;[285] however, treatment with 60% perchloric acid at 130 °C during 15 min was satisfactory for peptides containing these residues.[285]

The importance of pre-treatment of materials is illustrated[286] for Dowex-50 cation-exchange resins, which when used straight from the bottle contribute a contaminant equivalent in ninhydrin colour intensity to 27 μmol glycine.

Examples of more recent techniques are separation of phenylthiohydantoins by high-pressure liquid chromatography,[287] and separations of amino-acids as

[271] P. Y. Howard and W. Parr, *Chromatographia*, 1974, **7**, 283.
[272] T. Nambara, J. Goto, K. Taguchi, and T. Iwata, *J. Chromatog.*, 1974, **100**, 180.
[273] H. Iwase and A. Murai, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 8, 1455.
[274] H. Iwase, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2075.
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[276] H. E. Eick, P. H. Ward, and B. Kassell, *Analyt. Biochem.*, 1974, **59**, 482.
[277] P. L. Y. Lee, *Biochem. Med.*, 1974, **10**, 107.
[278] J. Oulevey and R. Heitefuss, *J. Chromatog.*, 1974, **94**, 283.
[279] C. Dennison, *J. Chromatog.*, 1974, **89**, 84.
[280] R. W. Longton, V. J. Berzinskas, A. Y. Balekjian, and S. B. Needleman, *Analyt. Biochem.*, 1974, **57**, 343.
[281] M. M. Herbst, B. G. Baltimore, G. Bozler, and H. A. Barker, *Analyt. Biochem.*, 1974, **58**, 322.
[282] J. F. Bouhours, *J. Chromatog.*, 1974, **94**, 307.
[283] D. P. Thornhill, *J. Chromatog.*, 1974, **90**, 354.
[284] B. V. Plapp and J. C. Kim, *Analyt. Biochem.*, 1974, **62**, 291.
[285] M. T. Doel, A. S. Jones, and R. T. Walker, *Tetrahedron*, 1974, **30**, 2755.
[286] R. D. Greenland, W. Roth, T. Gieske, and I. A. Michaelson, *Analyt. Biochem.*, 1974, **62**, 305.
[287] A. Haag and K. Langer, *Chromatographia*, 1974, **7**, 659.

perchlorate salts in organic solvents on porous silica by the ion-pair partition principle.[288]

Thin-layer Chromatography.—Techniques for minimizing sources of error in the quantitative analysis of amino-acids by t.l.c. have been reviewed,[289] and a review of non-destructive detection methods in t.l.c.[290] has appeared.

T.l.c. analysis of amino-acids in blood [291] and in protein hydrolysates [292] has been described, including an example of non-destructive location of separated amino-acids.[292] Conditions for the separation of hydroxyproline from allo-hydroxyproline have been established.[293] Further illustration of the sensitivity of the fluorescamine method for the detection of primary amines and amino-acids on t.l.c. plates is provided by separations at the 10 ng [294] and <100 pmol [295] levels. A complex multi-step reaction mechanism is involved in the fluorescamine procedure.[296]

Two-dimensional t.l.c. separation of 24 amino-acid phenylthiohydantoins [297] employs routine detection methods, but the use of the calcein fluorophore procedure permits the detection of phenylthiohydantoins at less than nanomole amounts.[298] Dansyl derivatives [266, 299] and related 5-dibutylaminonaphthalene-1-sulphonyl derivatives [300] of amino-acids still offer increased sensitivity even with the improvements contributed by the fluorescamine procedure to the analysis of un-derivatized amino-acids; picomole amounts can be detected,[300] and the analysis of the amino-acids present in *ca.* 1 mg of brain tissue is feasible using dansyl-^{14}C derivatives.[299]

Colorimetric Procedures for the Analysis of Amino-acids.—Such drawbacks as may still be associated with the major procedures for amino-acid colorimetry and fluorimetry are either readily overcome or are of a minor nature. The ninhydrin reaction is not quantitative with cysteine and lysine, and possible reasons for the non-ideal stoicheiometry have been discussed.[301] The isatin procedure is superior to the ninhydrin method for proline in amino-acid mixtures.[302] Further examples are provided of the conversion of imines into fluorescamine-sensitive primary amines;[303] N^{α}-methylamino-acids give methylamine with *N*-chlorosuccinimide, by which *N*-methylalanine may be detected at 100 picomole levels.[303]

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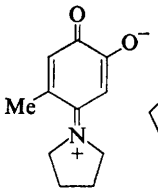
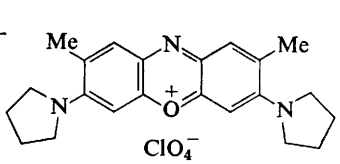
The use of *o*-phthalaldehyde is advocated for the detection of histidine and related primary amines at 4–5 ng ml^{-1} levels by fluorimetry,[304] and the enhancement of the fluorescence of dansyl amino-acids by the addition of *cyclo*-heptaamylose permits their detection at 50 picomol ml^{-1} levels.[305] Fluorophores formed from dopa and from dopamine by successive treatment with formaldehyde and glyoxylic acid show markedly different excitation and emission spectra, so that these amines should be identifiable in tissue.[306]

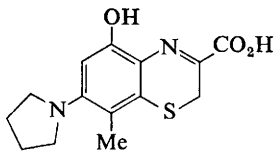
Oxidative deamination of amino-acids by the model aminochrome (34) gives the greenish-blue phenoxazine dye (35);[307] although proline and hydroxyproline



(34)



(35)



(36)

do not react, and cysteine reacts atypically in giving (36). The reaction is suggested to have some diagnostic value. The related *p*-benzoquinone reaction (λ_{max} 490 nm for amino-acids, λ_{max} 525 nm for prolines) is highly sensitive;[308] incidentally, the interpretation of this process as charge-transfer complex formation [308] needs revision in view of the isolation of 2,5-disubstituted quinones (30) in preparative scale studies.[211]

Other Analytical Methods.—A paper surveying rapid automated microbioassay of amino-acids [309] provides an entry to the extensive literature covering the technique. Further illustration of a method based on the addition of a known quantity of labelled amino-acid to an 'unknown' mixture, followed by addition to specific tRNA, has been provided,[310] with an indication of the lower limit in the nanomole range, and an accuracy ±15%. Low voltage electrophoresis on thin layers of microcrystalline cellulose offers an alternative means of separating amino-acids in biological samples.[311]

Determination of Specific Amino-acids.—Relatively well-established methods are applied to the assay of cysteine,[312] ergothioneine,[313] and hydroxyproline.[314, 315] The nitroprusside–methionine colour reaction provides the basis for an automated analysis of the amino-acids in biological samples,[316] and a sensitive pro-

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cedure for the analysis of *N*-methylalanine uses nitroprusside and acetaldehyde.[317]

The cystine-binding protein isolated from *E. coli* has been exploited in an assay for this amino-acid, using the uptake of added [^{14}C]cystine as the basis for quantitation.[318] Other biologically oriented assays include immobilized amino-acid decarboxylases as electrodes in a simple cell, with electrode response in the presence of the appropriate amino-acid being proportional to concentration with a precision in the order of 2%;[319] immobilized tryptophanase or tryptophanase–lactate dehydrogenase has been used in an L-tryptophan assay.[320] Fluorimetric methods are particularly suitable for tryptophan[321–323] and 5-hydroxytryptophan,[324] and for tyrosine.[325, 326] Representative papers[327–331] describe thyroxine assay procedures, involving the ^{125}I-labelled amino-acid,[327] a Sephadex binding method,[328, 329] and the Thyopac-4 kit for routine assay.[330, 331]

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