1
Amino-acids

BY G. C. BARRETT

1 Introduction

The layout for this chapter follows that used in previous volumes. Coverage is intended to be thorough as far as the chemistry of the amino-acids is concerned, but excludes most of the biological literature. The patent literature is also excluded, but this is felt to be not a serious omission in a continuous series; the Sections 16 (Fermentations) and 34 (Amino-acids, Peptides, and Proteins) of *Chemical Abstracts* provide easy access to this aspect of the literature.

Nomenclature.—IUPAC–IUB Recommendations (1974) on the nomenclature of amino-acids [1] (reproduced in Chapter 6) include no drastic revision of current usage, but some opportunities have been taken to decide between rival systems. In particular, the imidazole nitrogen atoms of histidine should be distinguished as π and τ (*pros* and *tele*; the N atom closest to the side-chain CH_2 group, and that farthest from the side-chain CH_2 group, respectively).

Textbooks and Reviews.—Synthetic methods [2] and other specific topics [2, 3] are covered in recent textbooks. Reviews are cited elsewhere in this chapter.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—Implications of the occurrence of non-protein amino-acids in plants have been discussed.[4]

Sensitive g.l.c. assay techniques permit the identification of amino-acids in marine sediments [5] and in meteorites;[6] the NASA Viking programme leading to exploration on Mars will involve the use of these techniques for assessing the

[1] *Pure Appl. Chem.*, 1974, **40**, 315; *European J. Biochem.*, 1975, **53**, 1; *Biochem. J.*, 1975, **149**, 1; *Biochemistry*, 1975, **14**, 449; *Rev. Soc. Quim. Mexico*, 1975, **19**, 33.

[2] (*a*) 'Methodicum Chimicum', ed. F. Korte, Vol. 6, Thieme Verlag, Stuttgart and Academic Press, New York, 1975; (*b*) 'Houben-Weyl: Methoden der organischen Chemie', 4th edn., ed. E. Müller, Vol. XV, ed. E. Wunsch, Thieme Verlag, Stuttgart, 1974.

[3] 'Amino-acid Metabolism', ed. D. A. Bender, Wiley, Chichester, 1975; 'Critical Stability Constants', ed. A. E. Martell and R. M. Smith, Volume 1: 'Amino-acids', Plenum, New York, 1974.

[4] L. Fowden, *Recent Adv. Phytochem.*, 1974, **8**, 95; A. Kjaer and P. O. Larsen, in 'Biosynthesis', ed. T. Geissman (Specialist Periodical Reports), The Chemical Society, London, 1973, Vol. 2, p. 71.
1973, **2**, 71; E. A. Bell, in 'Chemotaxonomy of the *Leguminosae*', ed. J. B. Harborne, Academic Press, London, 1971, p. 179.

[5] J. K. Whelan, *J. Chromatog.*, 1975, **111**, 337.

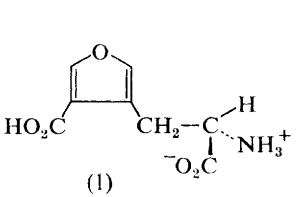
[6] (*a*) J. G. Lawless and M. P. Romiez, *Adv. Mass Spectrometry*, 1974, **6**, 143; (*b*) J. G. Lawless and E. Peterson, *Origins Life*, 1975, **6**, 3; (*c*) G. E. Pollock, C. N. Cheng, S. E. Cronin, and K. A. Kvenvolden, *Geochim. Cosmochim. Acta*, 1975, **39**, 1571; see also ref. 81*c*.

existence of life in former times, in case no evidence for surviving life forms is found.[7] At least 23 amino-acids are present in the Murchison meteorite, as shown using g.l.c.-m.s. techniques,[6b] and are thought to arise through extra-terrestrial abiotic synthesis[6b, 6c] since they are racemic; a clinching argument for their abiotic origin is the demonstration[6c] that isovaline from this source is also racemic.

Seeds of *Combretum zeyheri* contain *N*-methyl-L-tyrosine,[8] while branches of *Limonium vulgare* contain 2-trimethylaminopropionic acid[9] and 2-trimethylamino-6-oxoheptanoic acid (as the choline ester).[10] L-Dopa found in *Hygrocybe conica* and *H. ovina* is responsible for the formation of red and black colours in these toadstools after bruising.[11]

Unusual amino-acids from microbial sources include 3-cyclohexenylglycine from *Streptomyces tendae*,[12] and δ-aminovaleric acid from rumen ciliate protozoa.[13] Elastatinal, a microbial elastase inhibitor, releases (2*RS*),(3*S*)-α-[2-iminohexahydro-4-pyrimidyl]glycine on acid hydrolysis, and is similar in this respect to the chymostatins (Vol. 6, p. 7).[14] The literature on microbial synthesis and production of amino-acids can only be represented generally here; recent reviews are available[15] and papers describing the fermentative production of L-proline or L-tryptophan[16] by auxotrophs of *Corynebacterium glutamicum* are typical of a substantial amount of current literature in this area.

New Natural Free Amino-acids.—L-3-(3-Carboxyfuran-4-yl)alanine (1) exists in the free state in *Phyllotopsis nidulans*[17] and in *Tricholomopsis rutilans* fruiting bodies.[18] Simultaneous independent investigation of the same species has occurred with *Pentaclethra macrophylla*,[19, 20] seeds of which contain penmacric



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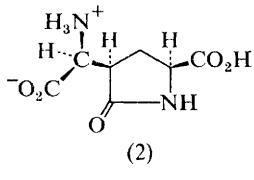

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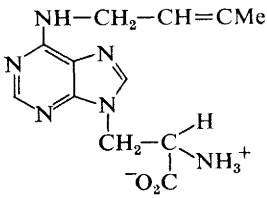
[7] P. H. S. Tsang, *Chemistry*, 1975, **48**, 15.
[8] K. Mwauluka, E. A. Bell, B. V. Charlwood, and J. M. Briggs, *Phytochemistry*, 1975, **14**, 1657.
[9] F. Larher and J. Hamelin, *Phytochemistry*, 1975, **14**, 205.
[10] F. Larher and J. Hamelin, *Phytochemistry*, 1975, **14**, 1789.
[11] W. Steglich and R. Preuss, *Phytochemistry*, 1975, **14**, 1119.
[12] W. Koenig, H. Hagenmaier, and U. Daehn, *Z. Naturforsch.*, 1975, **30b**, 626.
[13] W. Tsutsumi, R. Onodera, and M. Kandatsu, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 711.
[14] A. Okura, H. Morishima, T. Takita, T. Aoyagi, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1975, **28**, 337.
[15] W. M. Weigert, H. Offermanns, and P. Scherbereich, *Angew. Chem.*, 1975, **87**, 372; *Angew. Chem. Internat. Edn.*, 1975, **14**, 330; O. Tajima, *Hakko Kogaku Zasshi*, 1975, **53**, 482; R. Naumski, *Kem. Ind.*, 1975, **24**, 165.
[16] H. Hagino and K. Nakayama, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 343; K. Araki, Y. Takasawa, and J. Nakajima, *ibid.*, p. 1193.
[17] R. R. Doyle and B. Levenberg, *Phytochemistry*, 1974, **13**, 2813.
[18] S. Hatanaka and Y. Niimura, *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, 1975, **25**, 35 (*Chem. Abs.*, 1975, **84**, 40 800); *Phytochemistry*, 1975, **14**, 1436.
[19] A. Welter, M. Marlier, and G. Dardenne, *Bull. Soc. chim. belges*, 1975, **84**, 243; A. Welter, J. Jadot, G. Dardenne, M. Marlier, and J. Casimir, *Phytochemistry*, 1975, **14**, 1347.
[20] E. I. Mbadiwe, *Phytochemistry*, 1975, **14**, 1351.

acid [20] whose structure has been elucidated in full detail [19] as 3(*R*)-[1′(*S*)-amino-carboxymethyl]-2-pyrrolidone-5(*S*)-carboxylic acid (2). *X*-Ray and n.m.r. studies indicate a C_s-envelope conformation for (2) both in crystal and solution states.[19]

Of three unusual amino-acids found in *Mycena pura*,[21] *viz.* γ-methylene-, γ-ethylidene-, and γ-propylidene-L-glutamic acids, the third has not been found previously in Nature. A similar situation occurs for *Combretum zeyheri*,[22] seeds of which contain L-3-(3-aminomethylphenyl)alanine in addition to the 3-hydroxymethylphenyl and 3-carboxyphenyl analogues previously reported. New amino-acids have been isolated from fruit bodies of *Lactarius quietus* (L-2-amino-4-methylpimelic acid),[23] and from seeds of *Aleurites fordii* (L-3-carboxy-1,2,3,4-tetrahydro-β-carboline).[24] In addition to 13 known non-protein amino-acids, marine red algae contain pyrrolidine-2,5-dicarboxylic acid and *N*-methylmethionine sulphoxide.[25]

Lupinic acid (3), β-[6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purin-9-yl]-alanine, is the first reported example of a naturally occurring purine derivative



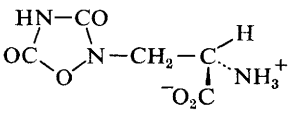
(3)

linked through one of its ring nitrogen atoms to an amino-acid moiety (although compounds of this type have been synthesized).[26] It is a novel zeatin metabolite, isolated from *Lupinus angustifolius* seedlings; the available 40 μg was insufficient to allow determination of its absolute configuration.

The structure β-(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)-L-alanine (4) assigned [27] to quisqualic acid (from *Quisqualis fructus*) has been confirmed by synthesis;[27] hydrolysis by alkali gives the novel 2-amino-3-(1-hydroxyureido)propionic acid (5),[27] which might be expected to accompany quisqualic acid in the natural source.

New microbial metabolites include the anti-tumour agent (6) from *Streptomyces sviceus*;[28] the homologue (6; H in place of OH) was recently found in the same

[21] S. Hatanaka and H. Katayama, *Phytochemistry*, 1975, **14**, 1434.
[22] K. Mwauluka, B. V. Charlwood, J. M. Briggs, and E. A. Bell, *Biochem. Physiol. Pflanz.*, 1975, **168**, 15.
[23] S. Hatanaka, H. Iizumi, A. Tsuji, and R. Gmelin, *Phytochemistry*, 1975, **14**, 1559.
[24] T. Okuda, T. Yoshida, N. Shiola, and J. Nobuhara, *Phytochemistry*, 1975, **14**, 2304.
[25] G. Impellizzeri, S. Mangiafico, G. Oriente, M. Piattelli, S. Sciuto, E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Phytochemistry*, 1975, **14**, 1549.
[26] J. K. MacLeod, R. E. Summons, C. W. Parker, and D. S. Letham, *J.C.S. Chem. Comm.*, 1975, 809.
[27] T. Takemoto, K. Koike, T. Nakajima, and S. Arihara, *Yakugaku Zasshi*, 1975, **95**, 448 (*Chem. Abs.*, 1975, **83**, 97 875); *ibid.*, p. 326 (*Chem. Abs.*, 1975, **83**, 43 706).
[28] D. G. Martin, C. G. Chidester, S. A. Mizsak, D. J. Duchamp, L. Baczynskyj, W. C. Krueger, R. J. Wnuk, and P. A. Meulman, *J. Antibiotics*, 1975, **28**, 91; L. J. Hanka, S. A. Gerpheide, P. R. Spieles, D. G. Martin, P. A. Belter, T. A. Coleman, and H. F. Meyer, *Antimicrobial Agents and Chemotherapy*, 1975, **7**, 807.



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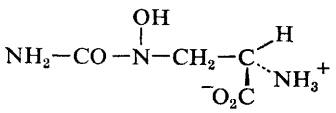

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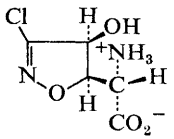

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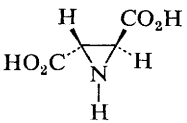

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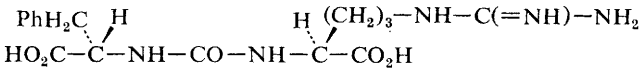

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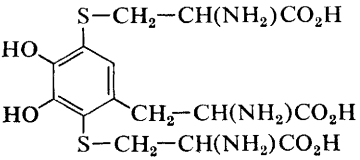
culture (Vol. 6, p. 6). L-*threo*-β-Hydroxyaspartic acid,[29] L-β-(5-hydroxy-2-pyridyl)alanine and L-β-(3-hydroxyureido)alanine,[30] L-*trans*-2,3-dicarboxyaziridine (7),[31] and the urea (8) derived from L-phenylalanine and L-arginine[32] have also been isolated from *Streptomyces* cultures. Cultures of *Claviceps fusiformis* deprived of oxygen accumulate N^{α}-methyl-4-dimethylallyl-L-tryptophan;[33] the non-methylated amino-acid was itself isolated from the same source previously.

Novel amino-acids isolated from higher organisms are 2,5-*S*,*S*-dicysteinyldopa (9) from the eye of the alligator *Lepisosteus spatula*,[34] and cystathionine sulphoxide and perhydro-1,4-thiazepine-3,5-dicarboxylic acid from the urine of a cystathioninuric patient.[35]



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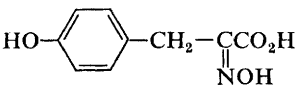

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Although formally outside the scope of this chapter, the report of the isolation[36] of the tyrosine analogue (10) from the marine sponge *Hymeniacidon sanguinea* deserves mention.

[29] T. Ishiyama, T. Furuta, M. Takai, Y. Okimoto, S. Aizawa, A. Shimazu, and H. Yonehara, *J. Antibiotics*, 1975, **28**, 821.

[30] S. Inouye, T. Shomura, T. Tsuruoka, Y. Ogawa, H. Watanabe, J. Yoshida, and T. Niida, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2669.

[31] H. Naganawa, N. Usui, T. Takita, M. Hamada, and H. Umezawa, *J. Antibiotics*, 1975, **28**, 828.

[32] K. Fujimoto, K. Tatsuta, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 685.

[33] K. D. Barrow and F. R. Quigley, *Tetrahedron Letters*, 1975, 4269.

[34] S. Ito and J. A. C. Nicol, *Tetrahedron Letters*, 1975, 3287.

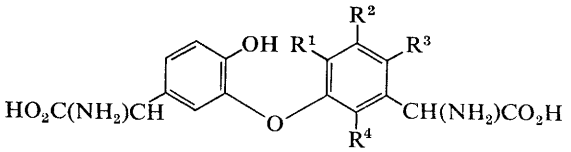
[35] H. Kodama, Y. Ishimoto, M. Shimomura, T. Hirota, and S. Ohmori, *Physiol. Chem. Phys.*, 1975, **7**, 147 (*Chem. Abs.*, 1975, **83**, 56 395).

[36] G. Cimino, S. De Stefano, and L. Minale, *Experientia*, 1975, **31**, 756.

New Amino-acids from Hydrolysates.—The outstanding new example under this heading is the discovery[37] of γ-carboxy-L-glutamic acid in several locations in Vitamin K-dependent prothrombin[37–40] and in mineralized tissue proteins.[38] It survives alkaline hydrolysis, but is quantitatively decarboxylated in 0.05M-HCl at 100 °C.[38]

The presence of an α-aminoadipic acid δ-semialdehyde residue in myelin basic protein from bovine brain has been established[41] through reduction with NaB^3H_4 and isolation of ^3H-labelled ε-hydroxynorleucine from alkaline hydrolysates. Further fascinating work on collagen cross-links has been reported,[42] leading to structure assignment to a new hydroxy-aldolhistidine trifunctional cross-link from cow skin collagen.

Peptide antibiotics and related compounds providing new derivatives of the protein amino-acids on acid hydrolysis are cerexins A and B (first appearance of L-*threo*-γ-hydroxylysine)[43] and actinomycin Z_1 (3-hydroxy-5-methylproline).[44] More complicated phenylglycine derivatives are released from ristocetin A,[45, 46] actinoidin, ristomycin, and vancomycin;[46] the derivative (11; R^1 = Me, R^2 = OH,



(11)

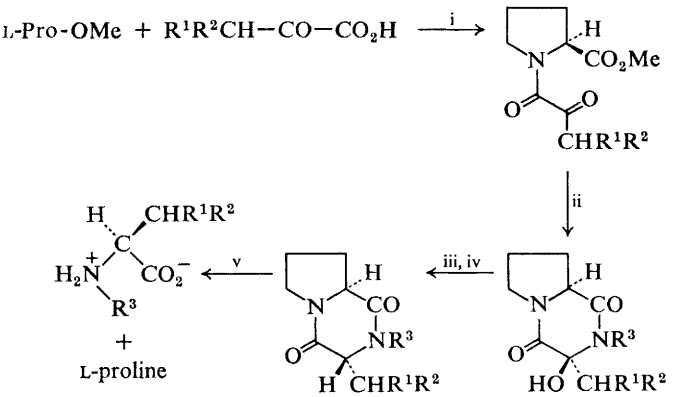
R^3 = R^4 = H) is present in hydrolysates of ristocetin A,[45] and structure (11; R^1 = R^2 = H, R^3 = R^4 = OH) is established[46] for actinoidinic acid, present in hydrolysates of the other antibiotics. Substantial progress towards elucidation of structure of vancomycin has been made;[46] it is thought to include three oxygenated phenylglycine units and two chloro-β-hydroxytyrosine units.

3 Chemical Synthesis and Resolution of Amino-acids

Asymmetric Synthesis.[47]—Further development of asymmetric hydrogenation using ruthenium(II) chiral phosphine catalysts has been reported, leading to moderate optical yields for synthesis of 2-aminoalkanoic acids from α-acylamidoacrylic acids.[48, 49]

[37] H. R. Morris, *Biochem. Soc. Trans.*, 1975, **3**, 465.
[38] P. V. Hauschka, J. B. Liau, and P. M. Gallop, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3925.
[39] H. R. Morris, M. R. Thompson, and A. Dell, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 856.
[40] P. Fernlund, J. Stenflo, P. Roepstorff, and J. Thomsen, *J. Biol. Chem.*, 1975, **250**, 6125; J. B. Howard, M. O. Fausch, and G. L. Nelsestuen, *ibid.*, p. 6178.
[41] E. Wada and T. Tsumita, *Jap. J. Exp. Med.*, 1975, **45**, 313.
[42] T. Housley, M. L. Tanzer, E. Henson, and P. M. Gallop, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 824.
[43] J. Shoji and H. Hinoo, *J. Antibiotics*, 1975, **28**, 60.
[44] E. Katz, K. T. Mason, and A. B. Mauger, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 502.
[45] T. M. Harris, J. R. Fehlner, A. B. Raabe, and D. S. Tarbell, *Tetrahedron Letters*, 1975, 2655.
[46] G. A. Smith, K. A. Smith, and D. H. Williams, *J.C.S. Perkin I*, 1975, 2108.
[47] For a review see J. W. Scott and D. Valentine, *Science*, 1974, **184**, 943.
[48] B. R. James, D. K. W. Wang, and R. F. Voigt, *J.C.S. Chem. Comm.*, 1975, 574.
[49] W. S. Knowles, M. J. Sabacky, B. D. Vineyard, and D. J. Weinkauff, *J. Amer. Chem. Soc.*, 1975, **97**, 2567.

While enzymic synthesis of L-amino-acids from DL-α-hydroxy-acids[50] and synthesis of L-aspartic acid derivatives from addition of (*S*)-PhCHMeNH$_2$ to dimethyl fumarate or maleate[51] illustrate familiar principles, asymmetric induction in the synthesis of *N*-benzoyl-*N*[(*R*)-α-ferrocenylisobutyl]-L- and -D-valine t-butylamide *via* a four-component condensation involving benzoic acid, (*R*)-α-ferrocenylamine, Me_2CHCHO, and Bu^tNC is particularly interesting because of its extremely high stereoselectivity.[52] Another highly selective example is the general synthesis of L-amino-acids and their *N*-methyl derivatives from corresponding α-keto-acids using L-proline as chiral agent (Scheme 1).[53]



Reagents: i, DCCI; ii, NH_3–$MeOCH_2CH_2OMe$; iii, TFA; iv, H_2 with Adams' catalyst; v, H_3O^+

Scheme 1

Asymmetric synthesis of threonine and allo-threonine from optically active *N*-salicylideneglycine cobalt(III) complexes has been reported.[102]

General Methods of Synthesis.—Isocyanides continue to appeal as starting materials in general syntheses of α-amino-acids. α-Isocyano-alkanoate esters $CNCHR^1CO_2R^2$ yield *N*-formylamino-acid esters on hydrolysis; they may be prepared by alkylation of alkyl isocyanoacetates (R^1 = H)[54–57] after metallation, though the nature of R^2 influences ratios of mono- and di-alkylated products.[54] Alkylation with a ketone gives β-branched amino-acids when reaction conditions causing dehydration of the β-hydroxyalkanoate are employed,[55] or threonine analogues when aldehydes are used.[57] t-Alkylglycines are obtained by addition of a Grignard reagent to an α-isocyanoacrylate [$R^1R^2C{=}C(NC)CO_2Et$ + $R^3MgBr \rightarrow R^1R^2R^3CCH(NHCHO)CO_2Et$].[58] Good yields of phenylglycines

[50] H. Matsushima, K. Murata, and Y. Mase, *Hakko Kogaku Zasshi*, 1975, **53**, 443 (*Chem. Abs.*, 1975, **83**, 129 947); *ibid.*, p. 450.
[51] Y. Nakajima, J. Oda, and Y. Inouye, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2065.
[52] R. Urban and I. Ugi, *Angew. Chem.*, 1975, **87**, 67.
[53] B. W. Bycroft and G. R. Lee, *J.C.S. Chem. Comm.*, 1975, 988.
[54] U. Schollkopf, D. Hoppe, and R. Jentsch, *Chem. Ber.*, 1975, **108**, 1580.
[55] H. J. Praetorius, J. Flossdorf, and M. R. Kula, *Chem. Ber.*, 1975, **108**, 3079.
[56] K. Bischofberger, R. H. Hall, and A. Jordaan, *J.C.S. Chem. Comm.*, 1975, 806.
[57] K. Matsumoto, Y. Urabe, Y. Ozaki, T. Iwasaki, and M. Miyoshi, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1869.
[58] U. Schollkopf and R. Meyer, *Angew. Chem.*, 1975, **87**, 624.

are obtained[59] by successive lithiation, carboxylation, and hydrolysis of $PhCH_2NC$.

Ogura and Tsuchihashi's extraordinary new synthesis (Vol. 7, p. 6) has been exemplified further[60] in a synthesis of *N*-lauroylvaline methyl ester from $MeSOCH_2SMe$ and Bu^tCN.

Methods employing Schiff bases as starting materials involve either metallation followed by alkylation[59, 61] [use of dithioacetals $(RS)_2C{=}NCH_2CO_2Et$[61] is noteworthy], or more novel procedures, *e.g.* $Cl_3CCH{=}NCO_2Et + RMgX \rightarrow Cl_3CCHRNHCO_2Et \rightarrow {}^-O_2CCHRNH_3{}^+$,[62] and $MeCH{=}NCHMePh + Me_3SiCN \rightarrow MeCH(CN)N(SiMe_3)CHMePh \rightarrow$ DL-alanine in 37% yield.[63]

α-Hydroxy-,[64, 65, 119] α-methoxy-,[66, 116] or α-alkanethio-hippuric acids[67, 116] may be employed in new α-amino-acid syntheses since they are amidoalkylating agents towards aromatic compounds (*e.g.* $PhCONHCH(OH)CO_2H + ArH \rightarrow PhCONHCHArCO_2H$),[64, 67] active methylene compounds,[65] and alkenes.[66]

Classical procedures of amino-acid synthesis continue to be methods of first choice in many areas. The hydantoin synthesis,[68, 69] Strecker synthesis,[215] acetamidomalonate synthesis,[70, 71] use of ethyl α-nitroacetate,[72] and extension of a side-chain through functionalized amino-acids (*e.g.* β-chloro-L-alanine[73] and *trans*-4-bromoproline[74]) and through αβ-dehydro-α-amino-acids,[26, 75] are representative examples of methods used for synthetic objectives described in later sections of this chapter. Use of 2-phenylimidazol-5-ones in synthesis of *N*-benzoylamino-acids (a relative of the azlactone synthesis) has been described.[76]

A new synthesis of amino-acids[77] is general in the sense that an excellent reagent (RuO_2) is available for oxidizing the aryl moiety of an aralkylamine to a carboxy-group, *e.g.* $ArCHR(CH_2)_nNH_2 \rightarrow {}^-O_2CCHR(CH_2)_nNH_3{}^+$; tyrosine can be oxidized to aspartic acid by this method.[77] A classical method for converting an α-amino-acid into its β-homologue is illustrated for the conversion of *Z*-L-Pro-OH into 'α-L-homoproline' by treatment with diazomethane (Wolff rearrangement) followed by de-protection.[78]

[59] T. Oguri, T. Shioiri, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 173.
[60] K. Ogura, I. Yoshimura, N. Katoh, and G. Tsuchihashi, *Chem. Letters*, 1975, 803.
[61] D. Hoppe, *Angew. Chem.*, 1975, **87**, 450.
[62] C. Kashima, Y. Aoki, and Y. Omote, *J.C.S. Perkin I*, 1975, 2511.
[63] Y. Nakajima, T. Makino, J. Oda, and Y. Inouye, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 571.
[64] D. Ben-Ishai, I. Satati, and Z. Berler, *J.C.S. Chem. Comm.*, 1975, 349.
[65] D. Ben-Ishai, Z. Berler, and J. Altman, *J.C.S. Chem. Comm.*, 1975, 905.
[66] J. Altman, R. Moshberg, and D. Ben-Ishai, *Tetrahedron Letters*, 1975, 3737.
[67] U. Zoller and D. Ben-Ishai, *Tetrahedron*, 1975, **31**, 863.
[68] M. Winn, R. Rasmussen, F. Minard, J. Kyncl, and N. Plotnikoff, *J. Medicin. Chem.*, 1975, **18**, 434.
[69] A. H. El Masry, S. E. El Masry, L. E. Hare, and R. E. Counsell, *J. Medicin. Chem.*, 1975, **18**, 16.
[70] T. Shiba, Y. Mukunoki, and H. Akiyama, *Bull. Chem. Soc. Japan*, 1975, **48**, 1902.
[71] J. W. Thanassi, *Bio-org. Chem.*, 1975, **4**, 132.
[72] A. Rosenthal and B. Cliff, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1975, **2**, 263.
[73] H. Nishimura, A. Mizuguchi, and J. Mizutani, *Tetrahedron Letters*, 1975, 3201.
[74] F. M. Kaspersen and U. K. Pandit, *J.C.S. Perkin I*, 1975, 1617, 1798.
[75] W. Maerki and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 1471.
[76] G. M. Devasia and C. R. Pillai, *Tetrahedron Letters*, 1975, 4051.
[77] D. C. Ayres, *J.C.S. Chem. Comm.*, 1975, 440.
[78] L. Balaspiri, B. Penke, G. Papp, G. Dombi, and K. Kovacs, *Helv. Chim. Acta*, 1975, **58**, 969.

Prebiotic Synthesis; Model Reactions.—Reports of the synthesis of amino-acids from simple molecules under simulated prebiotic conditions now usually promote a feeling of indifference, but a report that no amino-acids are formed by low-pressure Hg lamp irradiation of a CH_4–N_2–H_2O mixture (1 : 1 : 1)[79] catches us in mid-yawn. Reviews of more productive experiments of this type are available,[80–83] and further results on direct carboxylation of aliphatic amines by formic acid under glow discharge electrolysis conditions,[81b, 84] and on the accumulation of urea, amino-acids, and u.v.-absorbing substances on alumino-silicates saturated with Ca^{2+}, NH_4^+, and Fe^{3+} salts when heated in an atmosphere of CO + NH_3,[85] have been published. Specific factors (metal ion catalysis) favouring formation of cystine in irradiated mixtures of NH_4SCN, HCHO, KH_2PO_4, and $Ca(OAc)_2$ have been studied (other amino-acids are also formed under such conditions).[86]

More evidence that α-aminonitriles are the primary condensation products in these processes, *e.g.* their formation behind shock waves in CH_4–C_2H_6–NH_3–H_2O mixtures,[87] is provided indirectly by the link between the difficulty of hydrolysis of α-alkyl-α-aminonitriles and the fact that α-alkyl-α-amino-acids are not formed in simulated prebiotic reaction mixtures.[88] Experiments with poly(α-cyanoglycine) and HCN suggest that the presence of α-amino-acids was not necessarily a prerequisite for the chance synthesis of the first proteins.[89]

Protein and Other Naturally Occurring Amino-acids.—Although general synthetic methods outlined in the preceding section are represented here in reports of new syntheses of natural amino-acids, another common approach, the use of the protein amino-acids as starting materials for the synthesis of related compounds, is also illustrated.

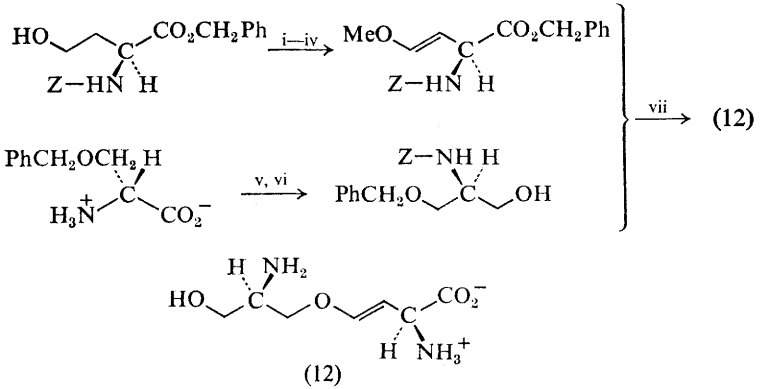
Several syntheses have been described for γ-carboxyglutamic acid,[39, 40, 75, 90] some[75, 90] yielding the γγ′-di-t-butyl ester α-methyl ester suitable for use in peptide synthesis. The pyroglutamic acid homologue 3,5-di(methoxycarbonyl)-pyrrolid-2-one has also been prepared.[75]

Longicatenamycin constituents *threo*-β-hydroxy-L-glutamic acid, L-2-amino-5-methylhexanoic acid, L-2-amino-6-methylheptanoic acid, L-2-amino-7-methyl-octanoic acid, and DL-5-chlorotryptophan,[70] DL-furanomycin,[91] an antibiotic α-amino-acid containing a 2,5-dihydrofuran moiety, have been synthesized.

Examples of the use of protein amino-acids in the synthesis of other natural amino-acids involve β-chloro-L-alanine in syntheses of *S-trans*-propenyl-L-

[79] J. P. Ferris and C. T. Chen, *J. Amer. Chem. Soc.*, 1975, **97**, 2962.
[80] K. Harada, in 'Chemistry and Biochemistry of Amino-acids, Peptides and Proteins', ed. B. Weinstein, Vol. 2, Dekker, New York, 1974.
[81] 'Origin of Life and Evolutionary Biochemistry', ed. K. Dose, S. W. Fox, and G. A. Deborin, Plenum, New York, 1974: (*a*) K. Dose, p. 69; (*b*) K. Harada, p. 183; (*c*) K. A. Kvenvolden, p. 301.
[83] K. Dose, *BioSystems*, 1975, **6**, 224.
[83] W. Groth, *BioSystems*, 1975, **6**, 229.
[84] K. Harada and T. Iwasaki, *Chem. Letters*, 1975, 185.
[85] G. Poncelet, A. T. van Assche, and J. J. Fripiat, *Origins Life*, 1975, **6**, 401.
[86] K. Bahadur and P. Sen, *Z. Mikrobiol.*, 1975, **15**, 143.
[87] A. Bar-Nun, *Origins Life*, 1975, **6**, 109.
[88] M. Béjaud, L. Mion, J. Taillades, and A. Commeyras, *Tetrahedron*, 1975, **31**, 403.
[89] R. Minard, W. Yang, P. Varma, J. Nelson, and C. Matthews, *Science*, 1975, **190**, 387.
[90] N. T. Boggs, R. E. Gawley, K. A. Koehler, and R. G. Hiskey, *J. Org. Chem.*, 1975, **40**, 2850.
[91] T. Masamune and M. Ono, *Chem. Letters*, 1975, 625.

cysteine [73] and of quisqualic acid (4),[27] use of *O*-tosyl-L-serine methyl ester in the synthesis of γ-carboxy-L-glutamic acid,[90] and use of D-serine and L-homoserine in the synthesis of rhizobitoxine [(12) in Scheme 2].[92] L-Histidine is used for the

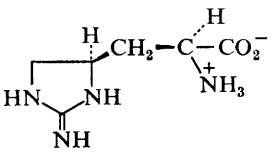


(12)

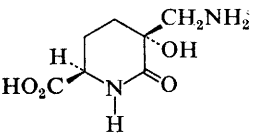
Reagents: i, CrO_3–py–CH_2Cl_2; ii, MeOH–$CH(OMe)_3$; iii, Ac_2O/cation exchange resin; iv, 180 °C; v, $LiAlH_4$; vi, Z-Cl; vii, dichlorobis(benzonitrile)palladium(II)

Scheme 2

synthesis [93] of enduracididine (13), a component amino-acid of enduracidin; Bamberger cleavage of L-histidine methyl ester, followed by catalytic hydrogenation, gives a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-2,4,5-triaminopentanoic acid methyl esters, from which both natural (2*S*,4*R*) and allo-enduracididines were prepared by de-protection and guanidination.[93] 2,5-*S*,*S*-Dicysteinyldopa (9) is synthesized from L-cysteine, L-dopa, and O_2 using mushroom tyrosinase.[34]



(13)



(14)

Synthesis of higher homologous amino-acids of natural origin is represented by (−)-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, present in pepstatins,[94] and for tabtoxinine-δ-lactam (14).[95]

α-Alkyl- and αα-Dialkyl-amino-acids.—Further details of routes reported last year (Vol. 7, p. 10) leading to α-alkyl-substituted ornithines [96] and to 2-amino-4-chloroalkanoic acids [97] have been published. Chlorinolysis of methionine

[92] D. D. Keith, J. A. Tortora, K. Ineichen, and W. Leimgruber, *Tetrahedron*, 1975, **31**, 2633.
[93] S. Tsuji, S. Kusumoto, and T. Shiba, *Chem. Letters*, 1975, 1281.
[94] M. Kinoshita, A. Hagiwara, and S. Aburaki, *Bull. Chem. Soc. Japan*, 1975, **48**, 570.
[95] D. L. Lee and H. Rapoport, *J. Org. Chem.*, 1975, **40**, 3491.
[96] M. M. Abdel-Monem, N. E. Newton, B. C. Ho, and C. E. Weeks, *J. Medicin. Chem.*, 1975, **18**, 600.
[97] Y. Urabe, M. Miyoshi, and K. Matsumoto, *Agric. and Biol. Chem. (Japan)*,, 1975, **39**, 1085.

derivatives yields 2-amino-3,4,4,4-tetrachlorobutanoic acid derivatives in addition to the 4,4,4-trichloro analogues previously reported;[98] *N*-aryl substituents are not cleaved.[97] Electrochemical reduction of 2-amino-4,4,4-trichlorobutanoic acid[99] gives the 4,4-dichloro-analogue, *alias* armentomycin, a powerful antibacterial agent isolated in 1967 from *Streptomyces armentosus*. Electrochemical reduction of *N*-benzyloxycarbonyl 2-amino-3,4,4,4-tetrachlorobutanoic acid methyl ester gives the armentomycin analogue $Cl_2C{=}CHCH(NH_3^+)CO_2^-$ after removal of protecting groups.[99]

N-Protected esters of 3,4-dehydro-DL-proline give the corresponding stereoisomeric 3,4-epoxy-proline derivatives with peroxytrifluoroacetic acid.[100]

α-Hydroxy-α-amino-acids and α-Amino-acids with Hydroxy-groups in the Side-chain.—Novel applications of α-hydroxyhippuric acid and analogues in amino-acid synthesis[64–67] are particularly promising because of the ready availability of these starting materials using standard methods of synthesis. Oxygenation at pH 11 of optically active *N*-salicylidenealanine cobalt(III) complexes gives the corresponding diastereoisomeric α-hydroxyalanine complexes.[101] Corresponding *N*-salicylideneglycine complexes react with acetaldehyde at pH 11 to give a threonine–allothreonine mixture after electrochemical decomposition and removal of Co^{2+} ions, constituting a new asymmetric synthesis when the starting material is one of the two possible enantiomers.[102] More conventional syntheses of *threo*-4,4,4-trichlorothreonine,[57] and DL-*threo*- and DL-*erythro*-3-(*p*-fluorophenyl)-,[103] -3-(*o*-tolyl)-,[104] -3-(3,4-dihydroxyphenyl)-,[105] and -3-(3′,4′-methylenedioxyphenyl)serines[106] using corresponding aldehydes as starting materials have been reported.

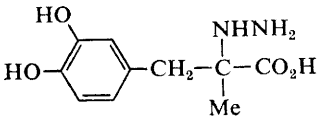
Aromatic and Heterocyclic Amino-acids.—Continuing improvements in the methods available for the synthesis of L-dopa, *e.g.* from 3-aminotyrosine[107] or from *N*-benzoyl-3-(4-hydroxy-3-methoxyphenyl)alanine,[108] have been described. 2,3,4-Trihydroxy-L-phenylalanine has been synthesized from *S*-methyl-L-cysteine and pyrogallol in the presence of L-tyrosine phenol-lyase.[109] Several combinations of carboxy-, hydroxy-, and amino-methylated derivatives are obtained by reaction of a suspension of L-dopa in MeOH–Et_2O containing diazomethane during 24 h.[110] 6-Chloro-D-tryptophan (a non-nutritive sweetening agent) can be prepared by nitration, reduction, diazotization, and Sandmeyer reactions applied to D-tryptophan.[111]

[98] Y. Urabe, T. Okawara, K. Okukura, M. Miyoshi, and K. Matsumoto, *Synthesis*, 1974, 440.
[99] Y. Urabe, T. Iwasaki, K. Matsumoto, and M. Miyoshi, *Tetrahedron Letters*, 1975, 997.
[100] C. B. Hudson, A. V. Robertson, and W. R. J. Simpson, *Austral. J. Chem.*, 1975, **28**, 2479.
[101] N. G. Faleev, Y. N. Belokon, V. M. Belikov, and L. M. Melnikova, *J.C.S. Chem. Comm.*, 1975, 85.
[102] Y. N. Belokon, V. M. Belikov, S. V. Vitt, M. M. Dolgaya, and T. F. Saveleva, *J.C.S. Chem. Comm.*, 1975, 86.
[103] N. Blazevic and F. Zymalkowski, *Arch. Pharm.*, 1975, **308**, 541.
[104] A. Hajos, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 471.
[105] B. Hegedues, A. F. Krasso, K. Noack, and P. Zeller, *Helv. Chim. Acta*, 1975, **58**, 147.
[106] K. Eisele, *Z. Naturforsch.*, 1975, **30c**, 538.
[107] B. Rzeszotarska and K. Pawelczak, *Farm. Pol.*, 1975, **31**, 137 (*Chem. Abs.*, 1975, **83**, 79 563).
[108] E. O. Renth, *Angew. Chem.*, 1975, **87**, 379; *Angew. Chem. Internat. Edn.*, 1975, **14**, 361.
[109] P. Rapp, H. Kumagai, H. Yamada, T. Ueno, and H. Fukami, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 241.
[110] J. F. Suida, *J. Org. Chem.*, 1975, **40**, 3611.
[111] T. Moriya, K. Hagio, and N. Yoneda, *Bull. Chem. Soc. Japan*, 1975, **48**, 2217.

Nucleotide analogues of α-amino-acids with variously substituted purinyl and pyrimidinyl side-chains are attracting increasing interest (see also List, p. 12). Standard methods yield *cis*-4-adeninyl-, guaninyl-, hypoxanthinyl-, uracilyl-, and thyminyl-L-prolines [74] and DL-β-[6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purin-9-yl]alanine.[26]

Monosaccharide-based α-amino-acids are also important synthetic objectives (sugars linked to amino-acid moieties *via* a C^1—C^α bond are represented in the polyoxins), and recent syntheses include L-2-(β-D-mannofuranosyl)- and -(β-D-lyxofuranosyl)glycines [56] and L- and D-2-(1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)glycines [72] (see also List, p. 12).

N-Substituted Amino-acids.—Excluding N-protected amino-acids prepared for use in peptide synthesis, and work relevant to the coverage of this section mentioned elsewhere in this chapter,[229] recent papers have described the conversion of BOC-dehydroamino-acid methyl esters into *N*-methyl homologues using MeI–K_2CO_3 in DMF or Me_2SO_4–K_2CO_3 in MeCN;[112] also, the synthesis of N^β-oxalyl-L-αβ-diaminopropionic acid (a neurotoxin),[113] and the synthesis of 'Carbidopa' (15), a hydrazine analogue of α-methyldopa which is a potent



(15)

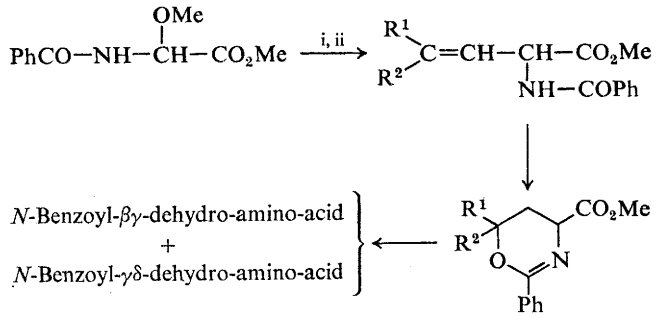
inhibitor of extra-cerebral aromatic amino-acid decarboxylase and enhances the efficiency of dopa *in vivo*.[114]

α-Aza-amino-acids.—In anticipation of an increasing volume of work under this heading, this new section is launched with one representative citation, describing the synthesis of *N*-benzoyl-α-aza-ornithine phenyl ester, a potent trypsin inhibitor.[115]

Amino-acids with Unsaturated Functional Groups in Side-chains.—Novel procedures have been devised, or have arisen from work with other objectives, for the synthesis of unsaturated amino-acids. α-Methoxy-α-amino-acids, prepared from amino-acid esters or amides by *N*-chlorination (Bu^tOCl) and dehydrochlorination followed by base-catalysed addition of MeOH to the resulting α-iminocarboxylic acid, give αβ-dehydroamino-acids on treatment with DABCO.[116] Synthesis of dehydrophenylalanine from phenylalanine *via* 2-trifluoromethyl-4-benzyloxazolone exemplifies an alternative route for the conversion of an α-amino-acid into its αβ-dehydro-analogue.[117] Conversion of 2-amino-3,4,4,4-tetrachlorobutanoic acid into 2-amino-4,4-dichlorobut-3-enoic acid by electrochemical reduction [99] cannot be developed into a general synthetic method for

[112] D. H. Rich, J. Tam, P. Mathiaparanam, and J. Grant, *Synthesis*, 1975, 402.
[113] S. L. N. Rao, *Biochemistry*, 1975, **14**, 5218.
[114] S. Vickers, E. K. Stuart, H. B. Hucker, and W. J. A. Vanden Heuvel, *J. Medicin. Chem.*, 1975, **18**, 134.
[115] C. J. Gray and R. C. Parker, *Tetrahedron*, 1975, **31**, 2940.
[116] H. Poisel and U. Schmidt, *Chem. Ber.*, 1975, **108**, 2547, 2917.
[117] E. G. Breitholle and C. H. Stammer, *Tetrahedron Letters*, 1975, 2381.

αβ-dehydroamino-acids (and requires protection of both NH_2 and CO_2H groups), but alkylation of alkenes with methyl α-methoxyhippurate [66] gives 2-phenyloxazines (Scheme 3) which give mixtures of βγ- and γδ-dehydroamino-acids, as their *N*-benzoyl derivatives, on hydrolysis.[66]



Reagents: i, $R^1R^2C{=}CH_2$; ii, BF_3

Scheme 3

Amino-acids containing Sulphur.—Straightforward syntheses of cysteine derivatives [34, 73, 118] have been reported. Protein amino-acids modified by substitution of the α-hydrogen atom by a sulphur grouping are of interest because the trifunctional moiety —NH—CR(S—)—CO— exists in gliotoxins and aranotins, and amino-acids may be modified in this way by N-protection, conversion into an α-imino-acid derivative, and addition of thioacetic acid,[116, 119] or from an α-hydroxy-α-benzamido-acid,[116, 120] prepared similarly from the α-imino-acid intermediate [116] or by the addition of an amide to an α-keto-acid.[120]

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

Compound [a]	*Ref.*
β-Fluoro-L-alanine	121
β-Fluoro-DL-phenylalanine	121
D- and L-Homohistidine	122
N^{β}-[*p*-(Fluorosulphonyl)benzyl]-L-asparagine	123
N^{β}-[*p*-(Fluorosulphonyl)benzyl]-L-glutamine	123
4′-Azido-2′-nitro-L-phenylalanine	124
2-Amino-4-(3,4-dihydroxyphenyl)butyric acid ('homo-dopa')	68
2-Amino-4-(3,4,5-trihydroxyphenyl)butyric acid	68
2-Amino-2-methyl-4-(3,4-dihydroxyphenyl)butyric acid ('α-methyl-homo-dopa')	68
α-Methyl-(3-allyl-4-hydroxyphenyl)alanine	69
α-Methyl-(3-methallyl-4-hydroxyphenyl)alanine	69
α-Methyl-(3-propenyl-4-hydroxyphenyl)alanine	69
α-Methyl-(3-propyl-4-hydroxyphenyl)alanine	69

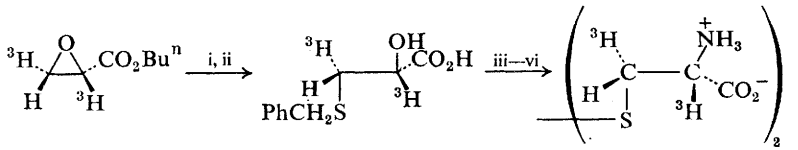
[118] R. T. Borchardt and Y. S. Wu, *J. Medicin. Chem.*, 1975, **18**, 300.
[119] R. K. Olsen and A. J. Kolar, *Tetrahedron Letters*, 1975, 3579.
[120] H. C. J. Ottenheijm, A. D. Potman, and T. Van Vroonhoven, *Rec. Trav. chim.*, 1975, **94**, 135.
[121] J. Kollonitsch, S. Marburg, and L. M. Perkins, *J. Org. Chem.*, 1975, **40**, 3808.
[122] W. Bloemhoff and K. E. T. Kerling, *Rec. Trav. chim.*, 1975, **94**, 182.
[123] M. Mokotoff, S. Brynes, and J. F. Bagaglio, *J. Medicin. Chem.*, 1975, **18**, 888.
[124] F. Fahrenholz and G. Schimmack, *Z. physiol. Chem.*, 1975, **356**, 469.

Compound [a]	*Ref.*
α-Methyl-(3-isobutyl-4-hydroxyphenyl)alanine	69
α-Methyl-[3-(2′,3′-epoxypropyl)-4-hydroxyphenyl]alanine	69
α-Methyl-[3-(2′-substituted)-5′-dihydrobenzofuryl]alanines	69
o- and *p*-Substituted phenylglycines	64
L-Canavaninosuccinic acid	125
S-Adenosyl-L-homocysteines with modified ribose moiety	118
N^{ϵ}-(4,6-Dimethyl-2-pyrimidinyl)lysine	126
N^{ϵ}-(4-Methyl-6-oxo-1,6-dihydro-2-pyrimidinyl)lysine	126
N^{ϵ}-(5-Methyl-6-oxo-1,6-dihydro-2-pyrimidinyl)lysine	126
N^{δ}-(5-Methyl-2-pyrimidinyl)ornithine	126
N^{δ}-(5-Propyl-2-pyrimidinyl)ornithine	126
N^{δ}-(5-Butyl-2-pyrimidinyl)ornithine	126
N^{δ}-(5-Pentyl-2-pyrimidinyl)ornithine	126
N^{δ}-(4,6-Dimethyl-2-pyrimidinyl)ornithine	126
O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-threonine *N*-methylamide	127
O-[2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl] L-serine *N*-methylamide	127
O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetyl-DL-serine *N*-methylamide	128
O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetyl-DL-threonine *N*-methylamide	128

[a] Other new amino-acids, and labelled analogues of known amino-acids, mentioned elsewhere in this chapter, are not repeated in this list.

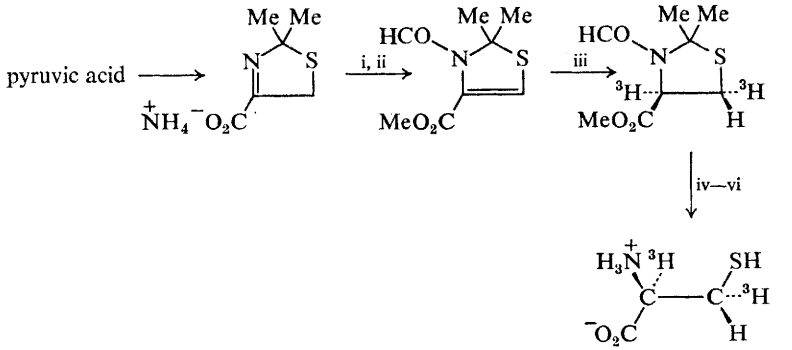
Labelled Amino-acids.—Considerable ingenuity is involved in devising routes to strategically labelled compounds, and the routes in themselves are often of outstanding interest in the general context of organic synthesis. ^{3}H-Labelled cysteine,[129] cystine,[130, 131] and valine,[132] as well as ^{15}N,^{2}H-labelled valine,[133] have been synthesized to assess aspects of penicillin biosynthesis. The routes to L-[α-^{3}H]cystine [131] and D-[α-^{3}H]valine [132] involve conventional α-tritiation (Ac_2O–$MeCO_2{}^3H$) of the isotopically natural amino-acids, while the synthesis [133] of (2*S*,3*S*)-[4,4,4-$^{2}H_3$]valine-^{15}N involving treatment of mesaconic acid-[*methyl*-$^{2}H_3$] with $^{15}NH_3$ and β-methylaspartase follows the route established earlier (Vol. 6, p. 22) for the synthesis of the [4-^{13}C]-analogue. The synthesis of (3*R*,3′*R*)-[3,3′-$^{3}H_2$]cystine and its (3*S*,3′*S*)-isomer outlined in Scheme 4 starts with labelled n-butyl glycidate,[130] and is related to a route established earlier (Vol. 6, p. 21) for the synthesis of labelled valines. The α-^{3}H in the resulting amino-acid was replaced by ^{1}H by equilibration. An alternative route (Scheme 5) starting with pyruvic acid [129] has been employed in the synthesis of (2*R*,3*R*)-[2,3-$^{3}H_2$]cysteine and its (2*R*,3*S*)-[3-^{3}H] analogue.[129]

[125] G. A. Rosenthal, *Analyt. Biochem.*, 1975, **65**, 60.
[126] F.-S. Tjoeng, E. Kraas, E. Stark, E. Breitmaier, and G. Jung, *Chem. Ber.*, 1975, **108**, 862.
[127] V. A. Derevitskaya and O. S. Novikova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1436.
[128] M. N. Mirzayanova, I. V. Medvedeva, and A. Y. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 697.
[129] D. J. Morecombe and D. W. Young, *J.C.S. Chem. Comm.*, 1975, 198.
[130] D. J. Aberhardt, L. J. Lin, and J. Y.-R. Chu, *J.C.S. Perkin I*, 1975, 2517.
[131] B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 123.
[132] B. W. Bycroft, C. M. Wels, K. Corbett, A. P. Maloney, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 923.
[133] F. C. Huang, J. A. Chan, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1975, **97**, 3858.



Reagents: i, $PhCH_2S^-Na^+$; ii, H_3O^+; iii, resolve; iv, substitution of OH by NH_2 *via* Br; v, $Na-NH_3$; vi, O_2

Scheme 4



Reagents: i, Me_2SO_4; ii, HCO_2COMe; iii, $^3H_2$/10% Pd–C; iv, NaOH; v, resolve; vi, N-HCl

Scheme 5

[4′-^{3}H]-L-Phenylalanine has been prepared from *p*-chloro-L-phenylalanine,[134] and the [2,3-$^3H_2$] analogue from 2-(acetamido)cinnamoyl-L-glutamic acid by $^3H_2$–Pd reduction;[135] [2,3-$^3H_2$]-L-tyrosine has been synthesized in a similar way.[135] The distribution of the ^{3}H label in L-[2,3-$^3H_2$]-phenylalanine and -tyrosine is approximately equal in the two positions, with the C-3 label predominantly in the *pro-S* position but of lower configurational purity than expected from the presumed stereospecificity of the method of synthesis.[135] Related degradative studies for establishing ^{3}H-distribution have been reported for [2,3-$^3H_2$]-L-valine [136] and for DL-[G-^{3}H]phenylalanine.[137] [5-^{3}H]-L-Ornithine and [6-^{3}H]-L-lysine may be prepared from γ-cyano-L-α-aminobutyric acid and from δ-cyano-L-norvaline, respectively, using NaB^3H_4 [138] as an alternative to catalytic tritiation (Vol. 6, p. 21).

^{14}C-Labelled amino-acid synthesis is illustrated for N^4-[*ethyl*-^{14}C]-L-asparagine [139] and L-4-azaleucine-[*dimethyl*-^{14}C].[140] While ^{3}H- and ^{14}C-labelling offers advantages in biosynthetic and physiological studies, ^{13}N-labelling (glutamic acid

[134] J. Kovacs, I. Teplan, and I. Mezo, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 109.
[135] G. W. Kirby, S. Narayanaswami, and P. S. Rao, *J.C.S. Perkin I*, 1975, 645.
[136] P. Adriaens, B. Meesschaert, and H. Vanderhaeghe, *Analyt. Biochem.*, 1975, **69**, 297.
[137] M. C. Clifford, A. E. Evans, A. E. Kilner, and D. C. Warrell, *J. Labelled Compounds*, 1975, **11**, 435.
[138] I. Mezo, M. Havranek, I. Teplan, J. Benes, and B. Tanacs, *Acta Chim. Acad. Sci. Hung.*, 1975, **85**, 201.
[139] R. W. Dineen and D. O. Gray, *Radiochem. Radioanalyt. Letters*, 1975, **22**, 205.
[140] L. I. Harrison, H. N. Christensen, M. E. Handlogten, A. L. Oxender, and S. C. Quay, *J. Bacteriol.*, 1975, **122**, 957.

and glutamine),[141] ^{15}N-labelling (glutamic acid,[142] pyroglutamic acid,[142] ornithine,[143] and valine [133]), and ^{18}F-labelling (DL-[4-^{18}F]-phenylalanine) [144] continue to be practised. Selective α-deuteriation of amino-acids $NH_2(CH_2)_n$-CO_2H (n = 2, 3, or 5) may be accomplished [145] by treatment with 5N-^{2}HCl at 117 °C during 20—60 h, and Pt-catalysed ^{2}H exchange of the aromatic protons of L-phenylalanine has been studied.[146] Amino-acids 85% enriched with ^{13}C have been prepared for ^{13}C–^{13}C coupling constant studies.[147, 148]

Resolution of Amino-acids.—Work continues on the applicability of chiral macroheterocycles in resolution of hexafluorophosphate salts of DL-amino-acid esters through differential complexation (Vol. 6, p. 21; Vol. 7, p. 15);[149–151] current studies deal with the testing of several macrocyclic ethers containing 2,2′-substituted-1,1′-binaphthyl units connected through various aliphatic ether chains,[150] and chromatographic resolution using the macrocycle covalently bonded to silica gel.[151]

More conventional procedures are illustrated in papers describing papain-catalysed acylation of arylamines [152] and carboxyhydrazides [153] by Z-DL-Ala-OH, and the use of carboxypeptidase A for resolution of α-methylphenylalanine and α-methylvaline as their *N*-TFA derivatives, based on the same enantiomeric selectivity principle.[154] *N*-Acetyl derivatives of L-dopa and α-methyldopa may be resolved as di-n-butylamine and hydrazine salts, respectively, through preferential crystallization of one enantiomer.[155]

N-Thiobenzoyl-α-amino-acids may be resolved in the conventional way, using a chiral amine,[156] their particular merit being their long-wavelength absorption (*ca.* 370 nm in aqueous media) which generates easily measured circular dichroism in partially resolved salts, from which the degree of resolution achieved by their crystallization may be assessed. 2-Phenylthiazol-5-ones obtained by cyclization of *N*-thiobenzoyl-DL-arginine and -lysine are substrates for trypsin, giving corresponding *N*-thiobenzoyl-L-amino-acids as hydrolysis products.[157] This provides a novel method for converting a D- or DL-α-amino-acid quantitatively

[141] A. S. Gelbard, L. P. Clarke, J. M. McDonald, W. G. Monahan, R. S. Tilbury, T. Y. T. Kuo, and J. S. Laughlin, *Radiology*, 1975, **116**, 127.
[142] W. Greenaway and F. R. Whatley, *J. Labelled Compounds*, 1975, **11**, 395.
[143] K. W. Hindmarsh, N. W. Hamon, and W. J. Ostapovich, *Canad. J. Pharm. Sci.*, 1975, **10**, 73.
[144] R. W. Goulding and S. W. Gunasekera, *Internat. J. Appl. Radiat. Isotopes*, 1975, **26**, 561.
[145] V. P. Kumarev and G. A. Almanov, *Zhur. fiz. Khim.*, 1975, **49**, 1361.
[146] R. S. Norton and J. H. Bradbury, *J. Catalysis*, 1975, **39**, 53.
[147] S. Fermandjian, D. S. Tran, J. Savrda, E. Sala, R. Mermet-Bouvier, E. Bricas, and P. Fromageot, *Biochem. Biophys. Acta*, 1975, **399**, 313.
[148] D. S. Tran, S. Fermandjian, E. Sala, R. Mermet-Bouvier, and P. Fromageot, *J. Amer. Chem. Soc.*, 1975, **97**, 1267.
[149] D. J. Cram, R. S. Helgeson, L. R. Sousa, J. M. Timko, M. Newcomb, P. Moreau, F. De Long, G. W. Gokel, and D. H. Hoffman, *Khim. geterotsikl. Soedinenii*, 1975, 1299.
[150] G. W. Gokel, J. M. Timko, and D. J. Cram, *J.C.S. Chem. Comm.*, 1975, 394, 444.
[151] G. Dotsevi, Y. Sogah, and D. J. Cram, *J. Amer. Chem. Soc.*, 1975, **97**, 1259.
[152] J. L. Abernethy, F. G. Howell, A. Ledesma, D. Doose, and R. Everett, *Tetrahedron*, 1975, **31**, 2659.
[153] J. L. Abernethy, D. Srulevitch, and M. J. Ordway, *J. Org. Chem.*, 1975, **40**, 3445.
[154] J. Turk, G. T. Panse, and G. R. Marshall, *J. Org. Chem.*, 1975, **40**, 953.
[155] S. Yamada, M. Yamamoto, and I. Chibata, *J. Org. Chem.*, 1975, **40**, 3360.
[156] G. C. Barrett and P. R. Cousins, *J.C.S. Perkin I*, 1975, 2313.
[157] M. A. Coletti-Previero and C. Axelrud-Cavadore, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 844.

into its L-enantiomer; an *in vivo* equivalent of this process is the conversion of D-lysine into L-lysine *via* L-pipecolic acid in *Neurospora crassa*.[158]

'Resolution' of a DL-amino-acid by destruction of one enantiomer is a well-known application of enzyme specificity, but the equivalent process, involving the more rapid destruction of one enantiomer of a DL-amino-acid by chiral longitudinally polarized electrons produced by β-decay processes, still appears to be unsubstantiated. The notion that L-amino-acids predominated in prebiotic times through secondary effects of chiral radiation[159] has been tested[160–163] through subjecting DL-leucine as a film to irradiation during 1.34 years with left-circularly polarized electrons; after this time, the residual amino-acid comprised 50.8% L- and 49.2% D-leucine.[160–162] However, this is still within experimental error,[162] and similar studies indicated no difference in absorption of right- or left-circularly polarized γ-radiation by D- or L-tryptophan.[163] A reasoned assessment of stereospecific discrimination expected in such high-energy processes as the interaction of elementary particles with pure crystalline chiral media suggests that effects will be very small,[164] though the expectation that higher discrimination could be found in solutions involving inherently chiral solvated electrons could not be confirmed in studies with simple chiral organic compounds.[164] An alternative effect of chiral electrons, that of favouring the crystallization of one enantiomer from a racemic melt, was demonstrated for sodium ammonium tartrate.[165]

Analytical resolution of DL-amino-acids by g.l.c. and by high-pressure liquid chromatography is discussed in Section 6.

4 Physical and Stereochemical Studies of Amino-acids

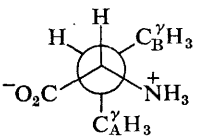
Crystal Structures of Amino-acids.—Neutron diffraction analysis of L-cysteine[166] and of glycine hydrochloride[167] provides definitive hydrogen locations and conformational features.

X-Ray crystal analysis of naturally occurring amino-acids and related compounds has been reported for L-cystine dihydrobromide dihydrate,[168] *S*-methyl-lanthionine dihydrate,[169] DL-tyrosine hydrochloride,[170] 3,5,3′-tri-iodo-L-thyronine methyl ester,[171] and *N*-(5-*O*-phosphopyridoxyl)-L-tyrosine hepta-hydrate.[172]

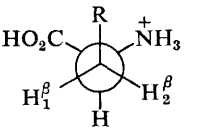
[158] W. U. Mueller and E. Leistner, *Z. Naturforsch.*, 1975, **30c**, 253.
[159] T. L. V. Ulbricht, *Nature*, 1975, **258**, 383; *Origins Life*, 1975, **6**, 303; *New Scientist*, 1975, **67**, 479.
[160] W. A. Bonner, *J. Mol. Evol.*, 1974, **4**, 23.
[161] W. A. Bonner, M. A. van Dort, and M. R. Yearian, *Nature*, 1975, **258**, 419.
[162] W. A. Bonner and J. J. Flores, *Origins Life*, 1975, **6**, 187.
[163] L. Keszthelyi and I. Vincze, *Radiat. Environ. Biophys.*, 1975, **12**, 181.
[164] M. M. Ulrich and D. C. Walker, *Nature*, 1975, **258**, 418.
[165] K. L. Kovacs and A. S. Garay, *Nature*, 1975, **254**, 538.
[166] K. A. Kerr, J. P. Ashmore, and T. F. Koetzle, *Acta Cryst.*, 1975, **B31**, 2022.
[167] A. R. Al-Karaghouli, F. E. Cole, M. S. Lehmann, C. F. Miskell, J. J. Verbist, and T. F. Koetzle, *J. Chem. Phys.*, 1975, **63**, 1360.
[168] R. E. Rosenfield and R. Parthasarathy, *Acta Cryst.*, 1975, **B31**, 816.
[169] J. R. Knox and P. C. Keck, *Acta Cryst.*, 1975, **B31**, 2698.
[170] B. Khawas, *Indian J. Phys.*, 1975, **49**, 403.
[171] V. Cody, *J. Medicin. Chem.*, 1975, **18**, 126.
[172] A. Mangia, M. Nardelli, G. Pelizzi, C. B. Volrattorni, A. Orlacchio, and C. Turano, *J.C.S. Perkin II*, 1975, 60.

Absolute configuration assigned to 3(*R*)-[1′(*S*)-aminocarboxymethyl]-2-pyrrolidone-5(*S*)-carboxylic acid (2) on the basis of o.r.d. and c.d. data has been confirmed by X-ray crystal analysis.[19]

N.M.R. Spectroscopy.—The major applications of n.m.r. spectroscopy of amino-acids continue to be the establishment of solution conformations and of protonation equilibria. Detailed interpretation of n.m.r. data for β-functional α-amino-acids in terms of rotamer populations in solution has been reported from several laboratories,[173–178] including further details of work [174] reported in preliminary communication form last year (Vol. 7, p. 18). Valine, isoleucine, and allo-isoleucine were shown, on the basis of ^1H–^1H and ^1H–^{13}C coupling constant data, to adopt similar conformations around their C^α—C^β bond, *e.g.* (16) for L-valine, and comparisons of data for these three amino-acids indicate that the upfield ^{13}C-resonance for valine can be assigned to C^γ_A.[175] L-Aspartic acid is common to the other studies of conformational preferences involving the



(16)



(17)

C^α—C^β bond in β-functional amino-acids,[174, 176–178] and the situation for serine,[173, 176] cysteine,[177] phenylalanine,[173, 176] histidine,[173] and tyrosine [176] has also been considered. The aliphatic amino-acids of this family adopt the rotamer (17) in which the three bulky groups are preferentially gauche in acidic solutions,[176, 177] primarily as a result of the structure of the surrounding solvent,[176] but the aromatic analogues show smaller preference for this rotamer indicating that steric effects exert a dominant influence.[176] These results have been obtained through 220 MHz ^1H n.m.r. studies [177] and the use of selectively deuteriated amino-acids to exploit the vicinal coupling constant between the carbonyl carbon atom and a β-proton.[176] N.m.r. data have been reported for diastereoisomers of β-hydroxyaspartic acid.[178]

Conformational studies of a more conventional type have been reported for Boc-amino-acids (using ^{13}C n.m.r. and i.r. data),[179] and for a variety of proline derivatives.[180–185] Rotameric equilibria in *N*-acyl-prolines,[181, 182] and ring conformations for L-proline [184] and derivatives [183] and their correlation with

[173] S. Fujiwara, H. Ishizuka, and S. Fudano, *Chem. Letters*, 1974, 1281.
[174] P. E. Hansen, J. Feeney, and G. C. K. Roberts, *J. Magn. Resonance*, 1975, **17**, 249.
[175] J. G. Batchelor and J. Feeney, *J.C.S. Chem. Comm.*, 1975, 503.
[176] M. Kainosho and K. Ajisaka, *J. Amer. Chem. Soc.*, 1975, **97**, 5630.
[177] B. J. Dale and D. W. Jones, *Spectrochim. Acta*, 1975, **31A**, 83.
[178] E. Luboch and J. F. Biernat, *Roczniki Chem.*, 1974, **48**, 2181.
[179] M. Branik and H. Kessler, *Chem. Ber.*, 1975, **108**, 2176, 2722.
[180] I. Z. Siemion, T. Wieland, and K. H. Pook, *Angew. Chem. Internat. Edn.*, 1975, **14**, 702.
[181] H. Nishihara, K. Nishihara, T. Uefuji, and N. Sakota, *Bull. Chem. Soc. Japan*, 1975, **48**, 553.
[182] E. W. B. de Leer and J. M. van der Toorn, *Rec. Trav. chim.*, 1975, **94**, 119.
[183] L. Pogliani, *Spectroscopy Letters*, 1975, **8**, 37.
[184] L. Pogliani, M. Ellenberger, and J. Valat, *Org. Magn. Resonance*, 1975, **7**, 61.
[185] G. V. Fazakerly and G. E. Jackson, *J.C.S. Perkin II*, 1975, 567.

long-range coupling constant data,[183] are described. Related work covers *N*-acetyl-*N*-methyl-L-alanine-[*dimethylamide*-$^2H_6$],[186] and β- and γ-amino-acids.[187]

Geminal ^2J(C,C) and vicinal ^3J(C,C) ^{13}C–^{13}C coupling constants are small (0—5 Hz) and only observable in ^{13}C-enriched amino-acids if the enrichment factor is substantially greater than 50%, and if Fourier transform acquisition times are greater than about 0.8 s.[147, 148] Vicinal coupling between CO and C^γ is shown by amino-acids with a four-carbon sequence (Asp, Thr, and Val), while C^α–C^δ coupling is shown by higher homologues.[148] Information on torsion angles should be contained in ^{13}C–^{13}C coupling constant data,[148] and further studies are under way to see what possibilities exist for conformational studies of amino-acids using this approach.[148]

N.m.r. studies of histidine deal with pH-dependence of ^{13}C chemical shift data and ^1H–^{13}C coupling constants for the imidazole moiety.[188] A splendid example of the merits of ^{15}N n.m.r. describes the use of pH-shift data to show that the τ-H tautomer of L-histidine predominates in alkaline solution.[189] ^{15}N n.m.r. data of a more fundamental type (relaxation times and chemical shifts) as a function of pH have been reported for glycine in aqueous solutions,[190] and ^{13}C n.m.r. data are available for the same system.[191] ^1H and ^{19}F n.m.r. titration curves (in ^2H_2O and H_2O, respectively), have been measured for 2- and 4-fluoro-histidines.[192]

^{13}C-Resonances for the ε- and *N*-methyl carbon atoms are shifted progressively downfield through the series lysine, N^ε-methyl-lysine, and NN^ε-dimethyl-lysine.[193]

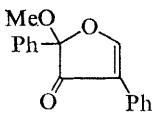
Binding of metal ions to lysine and histidine,[194] or to thiaproline,[185] has been reported, representing an application of n.m.r. of increasing importance. A novel application, dipolar splitting in ^1H n.m.r. shown for the nematic lyotropic phase formed between optically active sodium dodecyl sulphate and D- and L-alanine, respectively, reveals that the D-alanine complex is some 6% less ordered than that formed with L-alanine.[195]

O.R.D. and C.D. Spectra.—Aliphatic L-α-amino-acids show a weak Cotton effect in the 240—250 nm wavelength range in addition to the diagnostic 215 nm Cotton effect. The longer-wavelength features are best studied by c.d., which shows the presence of the long-wavelength Cotton effect in spectra of L-leucine and its esters, as well as corresponding *N*-methanesulphonyl derivatives and *N*-hydroxy-analogues.[196] Previous assignments of the long-wavelength c.d. of aliphatic amino-acids, in terms of a charge-transfer transition of a non-bonding

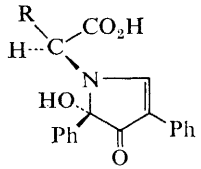
[186] Y. Imanishi, K. Kugimiya, and T. Higashimura, *Polymer*, 1975, **16**, 345.
[187] N. S. Ham, '7th Jerusalem Symposium on Quantum Chemistry and Biochemistry', 1974, 261 (*Chem. Abs.*, 1975, **83**, 39 073).
[188] R. E. Wasylishen and G. Tomlinson, *Biochem. J.*, 1975, **147**, 605.
[189] K. Kawano and Y. Kyogoku, *Chem. Letters*, 1975, 1305.
[190] T. K. Leipert and J. H. Noggle, *J. Amer. Chem. Soc.*, 1975, **97**, 269.
[191] H. Pearson, D. Gust, I. M. Armitage, H. Huber, J. D. Roberts, R. E. Stark, R. R. Vold, and R. L. Vold, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1599.
[192] H. J. C. Yeh, K. L. Kirk, L. A. Cohen, and J. S. Cohen, *J.C.S. Perkin II*, 1975, 928.
[193] C. S. Baxter and P. Byvoet, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 514.
[194] W. Voelter, G. Sokolowski, U. Weber, and U. Weser, *European J. Biochem.*, 1975, **58**, 159.
[195] A. S. Tracey and P. Diehl, *F.E.B.S. Letters*, 1975, **59**, 131.
[196] T. Polonski, *Tetrahedron*, 1975, **31**, 347.

electron on nitrogen to a π^*-antibonding orbital of the carboxy chromophore, are supported.[196]

Further examples of the use of c.d. spectra in assignments of absolute configuration to amino-acids after introducing a suitable chromophore have been reported for [Cu(succinimidato)$_2$(amino-acidato)$_2$] complexes,[197] ketimines,[198] *N*-thiobenzoyl [156] and *N*-(2,4-dinitrophenyl) derivatives,[199] and pyrrolinones (19)



(18)



(19)

obtained through condensation of α-amino-acids with 2-methoxy-2,4-diphenylfuran-3(2*H*)-one (18).[200] Although (19) is formed together with its diastereoisomer by reaction of (18) with an L-α-amino-acid, this is apparently not a drawback to the use of the pyrrolinones (19) for the chirospectral assignment of absolute configuration to amino-acids since the longest-wavelength Cotton effect (centred at *ca.* 380 nm) is positive in every case for reaction products (19) from 24 L-α-amino-acids.[200] On this basis, these are more reliable than most of the many other chromophoric derivatives proposed for the same purpose in recent years; assignment of absolute configuration to rhizobitoxine [(12) in Scheme 2] using this method required careful use of analogues of known absolute configuration,[201] and the chirospectral assignment was proved to be correct through stereochemically unambiguous total synthesis.[92] Like any other empirical method, however, the underlying reasons for the relationship between sign of longest-wavelength Cotton effect and absolute configuration need to be established so that this method may be used with full confidence.

An important observation, relevant to the interpretation of the c.d. behaviour of *N*-acyl-L-amino-acids in different solvents, has emerged from n.m.r. and c.d. study of *trans*- and *cis*-isomers of *N*-acyl-L-prolines;[181] the $n \rightarrow \pi^*$ c.d. developed in the *cis*-amide chromophore in these compounds is opposite in sign to that of the *trans*-isomer.

Further data on factors determining the c.d. developed in the chiral disulphide chromophore are provided through study of *S*-alkylthio-L-cysteines and glutathione.[202]

Absolute configurational assignments to penmacric acid (2) made with the help of c.d. data have been confirmed by *X*-ray crystal analysis.[19]

Mass Spectrometry.—The mass spectrum of methionine can be interpreted to indicate the formation of EtS^+ by successive elimination of CO_2H and $CH_2{=}CHNH_2$.[203]

[197] F. Kerek and G. Snatzke, *Angew. Chem.*, 1974, **87**, 133.
[198] G. Bettoni, V. Tortorella, A. Hope, and B. Halpern, *Tetrahedron*, 1975, **31**, 2383.
[199] M. Kawai, U. Nagai, and M. Katsumi, *Tetrahedron Letters*, 1975, 2845.
[200] V. Toome, S. de Bernardo, and M. Weigele, *Tetrahedron*, 1975, **31**, 2625; V. Toome and G. Reymond, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 75.
[201] D. D. Keith, S. de Bernardo, and M. Weigele, *Tetrahedron*, 1975, **31**, 2629.
[202] M. Ottnad, C. Ottnad, P. Hartter, and G. Jung, *Tetrahedron*, 1975, **31**, 1155.
[203] C. G. Van den Heuvel and N. M. M. Nibbering, *Org. Mass Spectrometry*, 1975, **10**, 250.

This section does not attempt a comprehensive coverage of mass spectrometry of amino-acid derivatives since most papers describing this topic are linked with peptide sequence methodology. A representative paper[204] describes the identification of amino-acids and peptides after reaction with fluorescamine or with 2-methoxy-2,4-diphenylfuran-3(2*H*)-one (18).

Other Physical and Theoretical Studies.—MO calculations leading to predicted conformations for amino-acid derivatives have been reported for *N*-acetyl *N*-methylamides of L-lysine,[205] L-leucine,[206] and glycine.[207] Laser-Raman spectra and depolarized Rayleigh scattering data for the *N*-methylamide of pyroglutamic acid reveal a change in torsion angle ψ_1 from $+169°$ to $-20°$ from crystal to aqueous solution states.[208]

I.r. spectroscopic studies of ^{14}C-labelled amino-acids[209] and of L- and DL-alanine[210] have been reported; complementary n.m.r. and i.r. studies of amino-acid derivatives are cited in the n.m.r. section.[178,179] Molar magnetic susceptibilities of alkali-metal salts of amino-acids have been determined.[211]

Physical properties at surfaces and interfaces, with particular reference to prebiotic events concerning amino-acids, have been studied for simple systems. Partitioning of amino-acids between water and aqueous micellar sodium dodecanoate and between hexane and water trapped in dodecylammonium propionate has been investigated.[212] Although asymmetric adsorption of phenylalanine enantiomers and asymmetric polymerization of DL-aspartic acid on kaolin has been ruled out,[162] (+)-quartz in powdered form is claimed to be capable of preferential adsorption of the D-enantiomer from anhydrous DMF solutions of DL-alanine,[213] leading to solutions with D : L ratio 49.5 : 50.5.

Determination of Absolute Configuration of Amino-acids.—Chemical correlation allows allotment of (*S*)-configuration to (+)-α-ethylphenylglycine,[214] and use of amino-acid oxidases leads to allotment of the L-configuration to the alternamic acid residue in alternariolide, a novel toxin reported last year (Vol. 7, p. 2).[215]

5 Chemical Studies of Amino-acids

Racemization.—Amino-acids present in fossils found in temperate regions (average temperature 25 °C) are completely racemized after *ca.* 100 000 years, but L-aspartic acid (racemization rate constant 8.29×10^{-4} year^{-1}) undergoes

[204] D. G. Pritchard, W. C. Schnute, and C. W. Todd, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 312.
[205] B. S. Zhorov, E. M. Popov, and V. A. Govyrin, *Mol. Biol.* (*Moscow*), 1975, **9**, 415 (*Chem. Abs.*, 1975, **83**, 73 844).
[206] I. S. Maksumov, S. F. Arkhipova, G. M. Lipkind, and E. M. Popov, *Khim. prirod. Soedineniya*, 1975, **11**, 211.
[207] R. Rein, V. Renugopalakrishnan, S. Niv, and T. J. Swissler, *Internat. J. Quantum Chem., Quantum Biol. Symp.*, 1975, **2**, 99.
[208] M. Allard, M. Avignon, and A. M. Bellocq, *Biopolymers*, 1975, **14**, 1565.
[209] L. Zilka, *Radioisotopy*, 1975, **16**, 215.
[210] N. Kaneko, H. Takahashi, and K. Higasi, *Bull. Chem. Soc. Japan*, 1975, **48**, 1961.
[211] M. A. Bernard, N. Bois, and M. Daireaux, *Canad. J. Chem.*, 1975, **53**, 3167.
[212] J. H. Fendler, F. Nome, and J. Nagyvary, *J. Mol. Evol.*, 1975, **6**, 215.
[213] W. A. Bonner, P. R. Kavasmaneck, F. S. Martin, and J. J. Flores, *Origins Life*, 1975, **6**, 367.
[214] H. Mizuno and S. Yamada, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 527.
[215] T. Okuno, Y. Ishita, A. Sugawara, Y. Mori, K. Sawai, and T. Matsumoto, *Tetrahedron Letters*, 1975, 335.

relatively rapid racemization in fossils and in biological cul-de-sacs such as tooth enamel. An interesting consequence is that *ca.* 8% of the aspartic acid content of tooth enamel of a warm-blooded animal (*e.g.* man) would be the D-enantiomer after 60 years; furthermore, aspartic acid residues in proteins with a long *in vivo* lifetime might undergo racemization sufficient to form 'mutant' proteins with some involvement in the ageing process.[216] An immediate application is suggested,[216] the checking of controversial claims to longevity by certain Georgians (if still equipped with their own teeth).

Among several reports of pyridoxal-mediated reactions of amino-acids (see also p. 23) a study of the racemization of L-glutamic acid by various pyridoxal–copper(II) systems [217] is eligible for citation in this section. An interesting example of enantiomeric differentiation is provided by the faster racemization of L-alanine by the (*S*)-2′-nitro-5-nitroso-6,6′-dimethylbiphenyl-2-ol–copper(II) system compared with that of D-alanine.[218] A thorough mechanistic study of base-catalysed racemization of *NN*-di(carboxymethyl)-D-phenylglycine, in the presence of metal ions, indicates removal of the α-proton by HO^- in all systems studied.[219]

The α-epimerization step in the conversion of isoleucine into allo-isoleucine may be effected using isobutyric anhydride.[220]

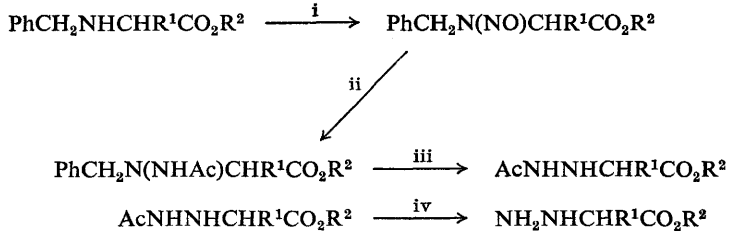
General Reactions.—α-Amino-acids are converted into corresponding carbonyl derivatives on treatment with *N*-sulphinylaniline, analogous to the Strecker degradation;[221] glycine is exceptional in being oxidized to formic acid in this reaction.[221] Oxidation of amino-acids with H_2O_2 or H_2O_2–$CuSO_4$ has been studied, leading to a number of unexpected observations (ornithine gives β-alanine *via* 4-aminobutyric acid; proline gives 3-hydroxyproline).[222] Direct addition of α-amino-acids to alkenes in Ac_2O gives *N*-acetyl-2-pyrrolines or isomeric acetamido-cyclobutanones.[223] A limited study of thermal condensation reactions occurring in mixtures of six amino-acids (including glutamic acid in excess) has been reported; three peptides were isolated from the glass-like melt.[224] Mannich reactions involving amino-acids have been surveyed.[225]

ATP-activated amino-acids become paramagnetic in a solution containing an acyl-tRNA-synthetase, the unpaired electron being located on the carboxy-group as a rule (but on the thiol group in the exceptional case of cysteine).[226] Further details of free-radical formation during reactions of amino-acids with sugar derivatives have been published.[227]

Full details are now available [228] of synthesis of α-substituted α-diazo-esters from amino-acid esters (Vol. 6, p. 30). Yamada's group [229] has also established

[216] P. M. Helfman and J. L. Bada, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2891.
[217] M. Ando and S. Emoto, *Bull. Chem. Soc. Japan*, 1975, **48**, 1655.
[218] K. Hirota, *Bull. Chem. Soc. Japan*, 1975, **48**, 2509.
[219] L. G. Stadtherr and R. J. Angelici, *Inorg. Chem.*, 1975, **14**, 925.
[220] G. Flouret and S. H. Nakagawa, *J. Org. Chem.*, 1975, **40**, 2635.
[221] T. Taguchi, S. Morita, and Y. Kawazoe, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 2654.
[222] H. A. Gruber and E. F. Mellon, *Analyt. Biochem.*, 1975, **66**, 78.
[223] F. Texier and O. Yebdri, *Tetrahedron Letters*, 1975, 855.
[224] P. Melius and J. Y. P. Sheng, *Bio-org. Chem.*, 1975, **4**, 385.
[225] J. H. Short and C. W. Ours, *J. Heterocyclic Chem.*, 1975, **12**, 869.
[226] C. Courty, *Compt. rend.*, 1975, **281**, *D*, 583.
[227] M. Namiki and T. Hayashi, *J. Agric. Food Chem.*, 1975, **23**, 487.
[228] N. Takamura, T. Mizoguchi, K. Koga, and S. Yamada, *Tetrahedron*, 1975, **31**, 227.
[229] K. Achiwa and S. Yamada, *Tetrahedron Letters*, 1975, 2701.

a convenient route to L-hydrazino-acids (Scheme 6) for use in their newly introduced asymmetric synthesis of α-amino-acids (Vol. 7, p. 6).



Reagents: i, $NaNO_2$–aq. HCl; ii, Zn–AcOH–Ac_2O; iii, H_2–Pd–C; iv, 6N-HCl

Scheme 6

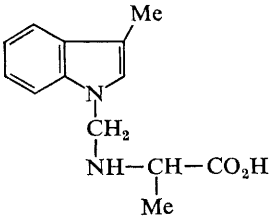
Amino-acids can be recovered from their hydrochlorides by treatment in DMF with $(Me_3Si)_2NH$ and hydrolysis of the resulting *NO*-bis(trimethylsilyl) derivatives.[230] *NO*-Bis(dimethylphenylsilyl)amino-acids are conveniently prepared in THF using dimethylphenylsilane and $NiCl_2$.[231] A useful procedure for synthesis of esters from N-protected amino-acids employs a hydrazone and peracetic acid at 0—10 °C.[232] De-protection procedures are augmented by two recent studies, one providing delicate selectivity in removal of Boc-groups in the presence of t-butyl esters [233] and the other providing means (FSO_3H or $MeSO_3H$) for stripping most of the commonly used amino-acid protecting groups.[234]

Specific Reactions.—The uses of readily available amino-acids as starting materials for synthesis of terpenes, carbohydrates, and alkaloids have been reviewed.[235]

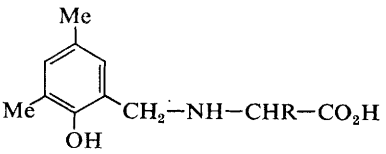
Oxidation studies (see also preceding section) based on aliphatic amino-acids [manganese(III) as oxidant] [236] and products from tryptophan (peroxyacetic acid as oxidant) [237] have been reported. *N*-Acetyltyrosine ethyl ester and N^{α}-acetyllysine react with formaldehyde in EtOH at 37 °C during several days to give complex mixtures, but identifiable products (20) and (21) are formed when the amino-acid derivatives are replaced by 2,4-dimethylphenol or 3-methylindole together with glycine, alanine, or valine.[238]

Nitrosation at the indole nitrogen atom of *N*-acetyltryptophan has been confirmed [239] by the appearance of new signals in the 500—550 p.p.m. region of the ^{15}N n.m.r. spectrum of the product obtained using $Na^{15}NO_2$, and independent

[230] A. I. Yurtanov, Y. A. Davidovich, and S. V. Rogozhin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1420; *Synthesis*, 1975, 113.
[231] M. Abe, K. Adachi, T. Takiguchi, Y. Iwakura, and K. Uno, *Tetrahedron Letters*, 1975, 3207.
[232] R. Bywood, G. Gallagher, G. K. Sharma, and D. Walker, *J.C.S. Perkin I*, 1975, 2019.
[233] J. Goodacre, R. J. Ponsford, and I. Stirling, *Tetrahedron Letters*, 1975, 3609.
[234] H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1164.
[235] S. Yamada and K. Koga, *Yuki Gosei Kagaku Kyokai Shi*, 1975, **33**, 535.
[236] A. M. Beg and Kamaluddin, *Acta Chim. Acad. Sci. Hung.*, 1975, **86**, 65.
[237] W. E. Savage, *Austral. J. Chem.*, 1975, **28**, 2275.
[238] M. K. Dewar, R. B. Johns, D. P. Kelly, and J. F. Yates, *Austral. J. Chem.*, 1975, **28**, 917.
[239] R. Bonnett, R. Holleyhead, B. L. Johnson, and E. W. Randall, *J.C.S. Perkin I*, 1975, 2261.



(20)

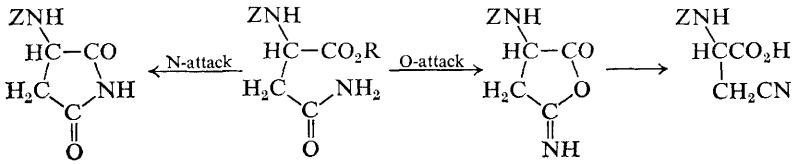


(21) R = H, Me, or Pr^i

evidence for the same site of nitrosation in *N*-acetyltryptophan esters is provided by the susceptibility of the products to chymotrypsin-catalysed hydrolysis.[240]

Studies with basic amino-acids cover synthesis of *N*-DNP-ornithine and lysine,[241] identification of the unprotonated ε-amino-group as the site of reaction between lysine and *p*-nitrophenyl acetate at pH 8.1—10.1,[242] periodate oxidation of δ-hydroxylysine to Δ^1-pyrroline-5-carboxylic acid *via* α-aminoglutaric acid γ-semi-aldehyde,[243] and synthesis of τ- and π-carboxymethyl-histidines.[244]

Asparagine active esters, *e.g.* (22; R = C_6F_5), provide the first[245] demonstration of competitive O- and N-attack at a carbonyl group under neutral conditions (Scheme 7); however, neither intramolecular process is fast enough to



Scheme 7

compete with the intermolecular aminolysis reactions applied to (22) when used in peptide synthesis.[245] High-yield syntheses of β-aspartate and γ-glutamate monoesters by copper(II)-catalysed hydrolysis of corresponding diesters represent improvements of a well-known procedure.[246]

Specific Reactions of Amino-acids Related to Biochemical Processes.—Several reports of pyridoxal-mediated processes have appeared, dealing with β-elimination from *S*-(*p*-substituted phenyl)cysteines and *O*-phosphothreonine,[247] β-decarboxylation of L-aspartic acid,[248] retro-aldol cleavage of threonine and β-hydroxyvaline,[249] monodecarboxylation of α-amino-α-alkylmalonate esters,[71] and racemization of L-glutamic acid.[217]

[240] T. B. Brown and M. F. G. Stevens, *J.C.S. Perkin I*, 1975, 2357.
[241] J. L. Brooks, *Analyt. Biochem.*, 1975, **66**, 290.
[242] D. S. Kristol, P. Krautheim, S. Stanley, and R. C. Parker, *Bio-org. Chem.*, 1975, **4**, 299.
[243] G. Y. Wu and S. Seifter, *Analyt. Biochem.*, 1975, **67**, 413.
[244] T. Weighardt and H. J. Gorne, *Bio-org. Chem.*, 1975, **4**, 30.
[245] L. Kisfaludy, I. Schon, M. Renyei, and S. Gorog, *J. Amer. Chem. Soc.*, 1975, **97**, 5588.
[246] R. L. Prestidge, D. R. K. Harding, J. E. Battersby, and W. S. Hancock, *J. Org. Chem.*, 1975, **40**, 3287.
[247] Y. Murakami and H. Kondo, *Bull. Chem. Soc. Japan*, 1975, **48**, 125, 541.
[248] N. Y. Sakkab and A. E. Martell, *Bio-inorg. Chem.*, 1975, **5**, 67.
[249] J. A. Marcello, A. E. Martell, and E. H. Abbott, *J.C.S. Chem. Comm.*, 1975, 16.

Further confirmation of specific interactions between inosine and L-lysine [250a] or D- or L-tryptophan [250b] (Vol. 6, p. 31) has been provided. Cationic micelles (cetyltrimethylammonium bromide) catalyse nucleophilic aromatic substitution of 2,6-dinitro-4-trifluoromethylbenzenesulphonate by amino-acids with hydrophobic side-chains.[251]

A recent structural assignment to the *p*-benzoquinone-cysteine ethyl ester condensation product has been corrected so as to conform with earlier conclusions.[252]

Effects of Electromagnetic Radiation on Amino-acids.—U.v. photochemistry of amino-acids has been reviewed for the areas of photosensitized reactions [253] and their quenching of singlet oxygen.[254] Photo-oxidation of methionine [255] and reactions of photolytically produced hydrogen atoms with cysteine and penicillamine have been studied.[256] Luminescence properties of aromatic amino-acids,[257, 258] products of laser flash photolysis [259] of tryptophan in aqueous solution, and radical-anion formation from u.v.-irradiated tryptophan amides [260] have been reported.

Ultrasound (800 kHz) irradiation of representative amino-acids in dilute aqueous solutions at isoelectric points (histidine also at pH 2 and 10) during 6 h gives NH_3, primary amines, HCHO, and other carbonyl compounds, in simple cases.[261] Conversion into other α-amino-acids is also observed (*e.g.* cysteine give cystine and serine) but between 39 and 92% of the starting material survives the irradiation.[261]

Radiolysis studies of cysteine,[262, 264] *N*-acetylcysteine,[263] and *N*-acetylmethionine,[264] *N*-acetylalanine,[265] tryptophan,[266] proline,[267] and histidine,[268] and broader studies of the same type,[269] have been reported.

[250] (*a*) Y. Suzuki, K. Hara, and T. Hirahara, *Bull. Chem. Soc. Japan*, 1975, **48**, 2149; (*b*) Y. Suzuki, K. Hara, and T. Hirahara, *ibid.*, p. 2342.
[251] C. A. Bunton and J. L. Wright, *Tetrahedron*, 1975, **31**, 3013.
[252] F. McCapra and Z. Razari, *J.C.S. Chem. Comm.*, 1975, 42; *corrigendum, ibid.*, p. 492; G. Prota and E. Ponsiglione, *Tetrahedron Letters*, 1972, 1327.
[253] G. Jori, *Photochem. and Photobiol.*, 1975, **21**, 463.
[254] I. B. C. Matheson, R. D. Etheridge, N. R. Kratowich, and J. Lee, *Photochem. and Photobiol.*, 1975, **21**, 165.
[255] S. G. Cohen and S. Ojanpera, *J. Amer. Chem. Soc.*, 1975, **97**, 5633.
[256] J. T. Wu and R. R. Kuntz, *Radiation Res.*, 1975, **64**, 662.
[257] D. V. Bent and E. Hayon, *J. Amer. Chem. Soc.*, 1975, **97**, 2599, 2606, 2612.
[258] C. Helene, T. Montenay-Garestier, and M. Charlier, *An. Acad. Bras. Cienc.*, 1975, **45** (Supplement), 59.
[259] F. D. Bryant, R. Santus, and L. I. Grossweiner, *J. Phys. Chem.*, 1975, **79**, 2711.
[260] V. B. Ilyasova, C. A. Azizova, and L. P. Kayushin, *Stud. Biophys.*, 1975, **49**, 143.
[261] W. H. Staas and L. A. Spurlock, *J.C.S. Perkin I*, 1975, 1675.
[262] G. C. Goyal and D. A. Armstrong, *Canad. J. Chem.*, 1975, **53**, 1475.
[263] G. Saxebol and O. Herskedal, *Radiation Res.*, 1975, **62**, 395.
[264] J. H. Hadley and W. Gordy, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3486.
[265] G. Saxebol, *Internat. J. Radiation Biol. Related Studies in Phys., Chem., Med.*, 1975, **27**, 293.
[266] J. L. Redpath, R. Santus, J. Ovadia, and L. I. Grossweiner, *Internat. J. Radiation Biol. Related Studies in Phys., Chem., Med.*, 1975, **27**, 201.
[267] J. Kopoldova and M. Voracek-Hubsch, *Z. Naturforsch.*, 1975, **30c**, 474.
[268] E. Westhof, W. Flossman, and A. Mueller, *Internat. J. Radiation Biol. Related Studies in Phys., Chem., Med.*, 1975, **27**, 51.
[269] T. Gejvall and G. Lofroth, *Radiation Effects*, 1975, **25**, 187; Y. Tal and M. Farragi, *Radiation Res.*, 1975, **62**, 337, 347; J. E. Aldrich, K. Y. Lam, P. C. Shragge, and J. W. Hunt, *ibid.*, 1975, **63**, 42.

6 Analytical Methods

This section is sub-divided as in previous volumes; there is some overlap in sub-divisions dealing with colorimetric procedures and specific amino-acid assays, and with the preceding section dealing with chemical reactions of amino-acids.

Gas–Liquid Chromatography.—G.l.c. techniques for amino-acid analysis are now widely used; general techniques have been reviewed [270] and the use of g.l.c.–m.s. techniques for trace amino-acid analysis is well illustrated for studies of meteorites [6, 7] and of bacterial cultures.[12]

Volatile derivatives used for the g.l.c. identification of amino-acids, as reported in the 1975 literature, are *N*-trifluoroacetyl butyl esters,[271, 272] (of guanidino-acids [272]), 1,1,1,3,3,3-hexafluoropropyl esters,[273] *N*-pentafluoropropionyl 2,2,3,3,3-pentafluoropropyl esters,[274] *N*-heptafluorobutyryl n-propyl esters,[275] isobutyl esters,[276] and isoamyl esters.[277] Alternative approaches described in recent years are further illustrated for acetylated methylthiohydantoins [278] and 2-trifluoromethyloxazol-3-in-5-ones,[279] these heterocyclic compounds being readily prepared from amino-acids and in the latter case providing a method for the quantitative analysis of phenylalanine in blood serum.[279] L-Tryptophan condensed with formaldehyde gives 2,3,4,5-tetrahydro-β-carboline-4-carboxylic acid, whose trimethylsilyl derivative is suitable for g.l.c.–m.s. analysis.[280]

The g.l.c. technique lends itself particularly well to the determination of optical purity of amino-acids (for reviews see refs. 281, 282; for applications in meteorite studies and in aspartic acid analysis of fossils and tooth enamel see refs. 6*c* and 216, respectively). This is achieved either by conversion of the amino-acid into a pair of volatile diastereoisomers (*N*-trifluoroacetyl 2-butyl esters,[5] and *l*-menthyl esters,[283, 284] or *N*-trifluoroacetyl-L-prolyl methyl [285] and butyl esters [286] have been employed; the optical purity of aspartic acid has been determined by reaction with L-leucine-*N*-carboxyanhydride followed by conversion of the diastereoisomeric dipeptides into volatile derivatives [216]), or by resolving a volatile derivative of the amino-acid on an optically active stationary phase [161, 283, 287–289] (*N*-trifluoroacetyl-L-methionyl-L-methionine cyclo-

[270] B. Kolb, in ref. 2*b*, Vol. 1B, p. 1020.
[271] P. Morard, M. Garcia, and J. P. Cabassy, *Analusis*, 1975, **3**, 451.
[272] H. Patel and B. D. Cohen, *Clin. Chem.*, 1975, **21**, 838.
[273] J. D. M. Pearson and D. F. Sharman, *J. Neurochem.*, 1975, **24**, 1225.
[274] S. Wilk and M. Orlowski, *Analyt. Biochem.*, 1975, **69**, 100.
[275] J. F. March, *Analyt. Biochem.*, 1975, **69**, 420.
[276] S. L. MacKenzie and D. Tenaschuk, *J. Chromatog.*, 1975, **104**, 176; **111**, 413.
[277] P. Felker and R. Bandurski, *Analyt. Biochem.*, 1975, **67**, 245.
[278] J. C. Cavadore and J. Derancourt, *Biochimie*, 1974, **56**, 1151.
[279] O. Grahl-Nielsen and B. Moerik, *Biochem. Med.*, 1975, **12**, 143.
[280] B. S. Middleditch, *Analyt. Letters*, 1975, **8**, 397.
[281] E. Gil-Av, *J. Mol. Evol.*, 1975, **6**, 131.
[282] K. D. Haegele, P. Y. Howard, and W. Parr, *Origins Life*, 1975, **6**, 195.
[283] H. Iwase, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1604, 1608.
[284] M. Hasegawa and I. Matsubara, *Analyt. Biochem.*, 1975, **63**, 308.
[285] J. D. Kemp, *Physiol. Plan.*, 1975, **35**, 53.
[286] H. Iwase, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 217.
[287] F. Andrawes, R. Brazell, W. Parr, and A. Zlatkis, *J. Chromatog.*, 1975, **112**, 197.
[288] W. A. Koenig and G. J. Nicholson, *Analyt. Chem.*, 1975, **47**, 951.
[289] R. Charles, U. Beitler, B. Feibush, and E. Gil-Av, *J. Chromatog.*, 1975, **112**, 121.

hexyl ester[287] and related dipeptide derivatives,[288] and *N*-lauroyl-L-valine t-butylamide[161] or 2-methyl-2-heptadecylamide,[289] or *N*-docosanoyl-L-valine t-butylamide,[289] have been used successfully as stationary phases).

Ion Exchange and Partition Column Chromatography.—Improvements to amino-acid analyser techniques[290, 291] including a simplified buffer system for gradient elution analysis of the protein amino-acids[292, 293] have been described. A system for single-column amino-acid analysis which also copes with amino-sugars has been developed,[294] and progress towards sub-nanomole automated amino-acid analysis has been discussed.[295] The high-pressure partition chromatography technique has been advocated for ion-exchange chromatographic analysis of amino-acids,[296] allowing 16 protein amino-acids to be separated in 45 min, and analysed in the picomole range using fluorescamine as colour reagent.

Analysis of basic amino-acids by ion-exchange methods calls for modified amino-acid analyser techniques; recent papers[297–304] describe resolutions of lysine, thialysine, and selenalysine,[298] techniques for hydroxylysine[299] and 3-methylhistidine,[300] and the separation of the various basic amino-acids and their *N*-methyl derivatives.[301–304]

Chromatographic analysis of cysteine and related sulphur-containing amino-acids has been reviewed,[305] and determination of *S*-adenosyl-methionine and -homocysteine in tissue has been described.[306] Separation of tri-iodothyronine, thyroxine, and various iodinated tyrosines and histidines can be achieved on Biogel P-2.[307]

The possibility of interaction between an amino-acid and components of buffer systems must be borne in mind, and the formation of double or asymmetric peaks for L-*trans*-3-hydroxyproline has been traced to reversible complex formation with hydroxy-acids.[308]

Analysis of β-aminoisobutyric acid[309] and γ-aminobutyric acid[310] in physiological fluids using the autoanalyser has been described.

High-pressure liquid chromatography has already proved its value in analytical chemistry, and recent papers in the amino-acid field describe the separation of

[290] S. J. Potashner and J. D. Knowles, *Analyt. Biochem.*, 1975, **65**, 435.
[291] R. W. Giese and J. F. Riordan, *Analyt. Biochem.*, 1975, **64**, 588.
[292] D. G. Redman, *J. Chromatog.*, 1975, **104**, 178.
[293] Y. Houpert, P. Tarallo, and G. Siest, *J. Chromatog.*, 1975, **115**, 33.
[294] A. G. Georgiadis, J. W. Coffey, J. G. Hamilton, and O. N. Miller, *Analyt. Biochem.*, 1975, **67**, 453.
[295] R. L. Niece, *J. Chromatog.*, 1975, **103**, 25.
[296] W. Voelter and K. Zech, *J. Chromatog.*, 1975, **112**, 643; *Chromatographia*, 1975, **8**, 350.
[297] R. S. Ersser, *J. Chromatog.*, 1975, **115**, 612.
[298] C. De Marco, A. Rinaldi, S. Dernini, P. Cossu, and D. Cavallini, *J. Chromatog.*, 1975, **114**, 291.
[299] N. Blumenkrantz and G. Asboe-Hansen, *Clin. Biochem.*, 1975, **8**, 177.
[300] C. C. Long and J. W. Geiger, *Biochem. Med.*, 1975, **12**, 267.
[301] H. W. Lange and K. Hempel, *J. Chromatog.*, 1975, **107**, 389.
[302] A. Beckerton, P. J. Buttery, F. J. Bailey, and N. Bolton, *J. Chromatog.*, 1975, **104**, 170.
[303] R. T. Markiw, *Biochem. Med.*, 1975, **13**, 23.
[304] C. G. Zarkadas, *Canad. J. Biochem.*, 1975, **53**, 96.
[305] M. Friedman and A. T. Noma, *Nutr. Clin. Nutr.*, 1975, **1**, 521.
[306] J. Hoffmann, *Analyt. Biochem.*, 1975, **68**, 522.
[307] P. Thomopoulos, *Analyt. Biochem.*, 1975, **65**, 600.
[308] M. Man, R. M. Gryder, and E. Adams, *Analyt. Biochem.*, 1975, **63**, 513.
[309] E. Solem, D. P. Agarwal, and H. W. Goedde, *Clin. Chim. Acta*, 1975, **59**, 203.
[310] B. S. Glaeser and T. A. Hare, *Biochem. Med.*, 1975, **12**, 274.

phenylthiohydantoins[311] and the estimation of optical purity of amino-acids after conversion into diastereoisomeric *N*-[(+)-10]camphorsulphonyl *p*-nitrobenzyl esters.[312]

Thin-layer Chromatography.—Suitable conditions have been found[313] for the separation of protein amino-acids in hydrolysates, using electrophoresis followed by t.l.c. on cellulose. A novel technique, t.l.c. on strong acid cation exchange resin with part of the layer in the Na^+ form, the remainder in the Li^+ form, shows its worth in the separation of asparagine from glutamine.[314] Double spots for L-3,3′,5-tri-iodothyronine in certain eluents are due to the use of 50% aqueous propyleneglycol as solvent for placing the sample on the chromatogram.[315]

The already extensive literature on quantitative t.l.c. of phenylthiohydantoins has been augmented further;[316–319] fluorescein-thiohydantoins can be detected on micro-polyamide layers at picomole levels.[320]

Dansylamino-acids can be detected on thin layers at extremely low concentrations, quantitative estimation of leucine at $\leqslant 10^{-11}$ mol l^{-1} being typical;[321] cyclohepta-amylose increases the fluorescence intensity of these derivatives 10-fold, and stabilizes the fluorescence[322] (see also Vol. 7, p. 29). No improvement is obtained using 4-dimethylaminoazobenzene-4′-sulphonyl chloride for chromophoric labelling of amino-acids since 10^{-10}—10^{-11} mol l^{-1} are lower limits.[323] Extraordinary sensitivity is provided by use of radioactively labelled materials;[324] use of ^{3}H-labelled dansyl chloride leads to the possibility of detection of femtomolar amounts of amino-acids, and further development of the double isotope dansylation technique (^{3}H-dansyl chloride and ^{14}C-labelled amino-acids as internal standards; Vol. 6, p. 37) permits the analysis of putative amino-acid transmitters glutamic acid and glutamine, aspartic acid, glycine, alanine, serine, taurine, and γ-aminobutyric acid, in *ca.* 50 μg of pigeon optic nerve.[325]

Colorimetric Procedures.—Mention has been made elsewhere in this chapter of colorimetric analysis procedures, and the papers collected together here describe fundamental or comparative studies. Although quantitative colorimetric assays of amino-acids, using the fluorescent pyrrolinones obtained on reaction with fluorescamine,[326] have much merit, modified conditions for the *o*-phthalaldehyde

[311] J. X. De Vries, R. Frank, and C. Birr, *F.E.B.S. Letters*, 1975, **55**, 65.
[312] H. Furukawa, E. Sakikibara, A. Kamei, and K. Ito, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1625.
[313] R. L. Munier and B. Longchambon-Faivre, *Compt. rend.*, 1975, **280**, *D*, 919.
[314] A. Varadi, *J. Chromatog.*, 1975, **110**, 166.
[315] F. G. Stanford and M. L. Golder, *J. Chromatog.*, 1975, **104**, 474.
[316] D. A. Walz and J. Reuterby, *J. Chromatog.*, 1975, **104**, 180.
[317] G. K. Zwolinski and L. R. Treiber, *J. Chromatog.*, 1975, **107**, 311.
[318] M. Kubota, N. Takahashi, K. Goto, and T. Murachi, *Analyt. Biochem.*, 1975, **64**, 494.
[319] K. D. Kulbe and Y. M. Nogueira-Hattesohl, *Analyt. Biochem.*, 1975, **63**, 624.
[320] K. Muramoto, H. Kawauchi, and K. Tuzimura, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2241.
[321] B. Loessner, R. Jork, and H. Matthies, *Acta Biol. Med. Ger.*, 1975, **34**, 1.
[322] T. Kinoshita, F. Iinuma, K. Atsumi, Y. Kanada, and A. Tsuji, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1166.
[323] J.-K. Lin and J.-Y. Chang, *Analyt. Chem.*, 1975, **47**, 1634.
[324] S. R. Burzynski, *Analyt. Biochem.*, 1975, **65**, 93.
[325] P. M. Beart and S. R. Snodgrass, *J. Neurochem.*, 1975, **24**, 821.
[326] A. M. Felix, V. Toome, S. De Bernardo, and M. Weigele, *Arch. Biochem. Biophys.*, 1975, **168**, 601.

procedure show that it can be made some 5—10 times more sensitive than the fluorescamine or ninhydrin procedures, permitting quantitative analysis of picomole amounts.[327] Similar sensitivity is claimed for the fluorimetric assay of pyridoxal derivatives of amino-acids.[328]

Successive treatment of tryptophan or dopa with formaldehyde and glyoxylic acid gives fluorescent products permitting the identification of these amino-acids in tissue by microspectrofluorimetry.[329]

Other Analytical Methods.—An electrophoretic method is suitable for the determination of *S*-methylmethionine from plant sources;[330] the technique is represented in publications cited elsewhere in this chapter.[313]

Determination of Specific Amino-acids.—Several papers in other sections deal with techniques for the determination of specific amino-acids, but the papers collected here use well-established methods or tailor-made modifications.

Determination of specific amino-acids present in physiological fluids has been reviewed.[331] Most of the literature cited here deals with clinical assays, with methods for proline and hydroxyproline,[332–336] and methods for aromatic amino-acids,[337–346] being particularly numerous. Among the latter group of papers are methods for determination of tyrosine,[337, 338, 342] thyroxine and triiodothyronine,[339–341] tryptophan,[342–344] 5-hydroxytryptophan,[345] and histidine.[346] Enzymic assays are presented for arginine[347, 348] and for asparagine[348] as well as for some of the aromatic amino-acids.[337, 344, 346] A method has been developed for the analysis of diaminopimelic acid in urine,[349] and a new colorimetric procedure for the determination of cystine has been announced.[350]

[327] J. R. Benson and P. E. Hare, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 619.
[328] K. Hempel, H. W. Lange, and N. Lustenberger, *Instrum. Forsch.*, 1974, **2**, 13.
[329] O. Lindvall, A. Bjorklund, B. Falck, and L. A. Svensson, *Histochemistry*, 1975, **46**, 27.
[330] G. N. Khuchna and A. B. Stasyak, *Priklady Biokhim. Mikrobiol.*, 1975, **11**, 914.
[331] F. A. Ibbott, in 'Clinical Chemistry: Principles and Techniques', ed. R. J. Henry, D. C. Cannon, and J. W. Winkelman, Harper and Row, New York, 1974, 2nd edn., p. 565.
[332] N. Blumenkrantz and G. Asboe-Hansen, *Analyt. Biochem.*, 1975, **63**, 331.
[333] R. Nagel and M. Keysser, *Deutsch. Gesundheitswes.*, 1975, **30**, 2289.
[334] B. T. Nobbs, A. W. Walker, and T. J. Davies, *Clin. Chim. Acta*, 1975, **64**, 219.
[335] P. Vratny, M. Stefl, and I. Trcka, *Chem. Listy*, 1975, **69**, 379.
[336] T. Tepper and L. De Vos, *Clin. Chim. Acta*, 1975, **59**, 373.
[337] A. Kumar and G. D. Christian, *Clin. Chem.*, 1975, **21**, 325.
[338] J. Chrastil, *Analyt. Chem.*, 1975, **47**, 2293.
[339] P. S. Zavadskii, A. V. Negovskaya, and A. S. Ametov, *Patol. Fiziol. Eksp. Ter.*, 1975, 83.
[340] M. I. Surks and J. H. Oppenheimer, *Methods Enzymol.*, 1975, **36A**, 537.
[341] A. Burger, C. Sakaloff, V. Staeheli, M. B. Vallotton, and S. H. Ingbar, *Acta Endocrinol.*, 1975, **80**, 58; V. Chan, T. Merrett, J. Landon, A. M. Linden, and M. Joustra, *Ann. Clin. Biochem.*, 1975, **12** (Part 4), 173.
[342] S. S. Hassan, *Analyt. Chem.*, 1975, **47**, 1429.
[343] A. Dalby and C.-Y. Tsai, *Analyt. Biochem.*, 1975, **63**, 283.
[344] K. Shikata, H. Obata, and T. Tokuyama, *Technol. Rep. Kansai Univ.*, 1975, **16**, 103 (*Chem. Abs.*, 1975, **83**, 189 981).
[345] K. Shimomura, T. Fukushima, and T. Danno, *J. Pharm. Pharmacol.*, 1975, **27**, 197.
[346] T. T. Ngo, *Internat. J. Biochem.*, 1975, **6**, 371.
[347] G. Gaede and M. Grieshaber, *Analyt. Biochem.*, 1975, **66**, 393.
[348] T. T. Ngo, *Internat. J. Biochem.*, 1975, **6**, 663.
[349] J. Krysciak, *Chem. Analysis*, 1975, **20**, 549, 635.
[350] M. Horiuchi and T. Chiba, *J. Pharm. Soc. Japan*, 1975, **95**, 1263.