1

Amino-acids

BY G. C. BARRETT

1 Introduction

As in the preceding volumes of this Specialist Periodical Report, the coverage of the chemical and biochemical literature on the amino-acids is intended to be thorough, but the biological aspects, such as metabolism, biosynthesis, and distribution in the bio- and geo-spheres of the common amino-acids, are only covered through representative citations.

Textbooks and Reviews.—A textbook [1] and symposium proceedings,[2] covering general aspects and analysis, respectively, have been published in 1981. The analytical theme also features as a component of a review of the analysis of amino-acids and other constituents of single cells.[3] Physical and chemical properties of amino-acids [4] and theoretical analysis of hydration of amino-acids and peptides [5] have also been reviewed; other reviews appearing in the recent literature are cited in the appropriate sections of this chapter.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—The biosynthesis, occurrence, and properties of γ-carboxyglutamic acid have been reviewed,[6] and, on an extra-terrestrial level, the results for amino-acid content of moon soil and meteorites have been brought together.[7]

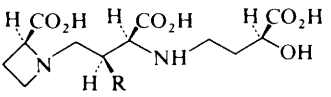
In selecting papers from the 1981 literature for inclusion in this section, the broad routine literature on the distribution of the common amino-acids is largely excluded. As in earlier volumes, however, items of exceptional interest, usually relating to geological or extra-terrestrial sources, are cited. The distribution of common and uncommon amino-acids in the Isua rocks of south-western Greenland of age *ca.* 3800 My has been determined [8] to counter a claim, based on laser-Raman spectroscopic analysis, that amino-acids in microfossil crystal regions (and therefore insulated from leaching and infusion processes) may be of ancient derivation. Since amino-acids that are unstable to geological processes were found

[1] 'Aminosäuren', J. Breuer and S. Kowalewsi, Thieme Verlag, Stuttgart, 1981.
[2] 'Proceedings of a Symposium on Amino-acid Analysis', ed. J. M. Rattenbury, Horwood, Chichester, 1981.
[3] N. N. Osborne in 'Methods in Neurobiology', ed. R. LaRue, Plenum Press, New York, 1981, Vol. 1, p. 135.
[4] P. O. Larsen in 'Biochemistry of Plants', ed. B. J. Miflin, 1980, Vol. 5, p. 225.
[5] Y. Paterson, G. Nemethy, and H. A. Scheraga, *Ann. N.Y. Acad. Sci.*, 1981, **367**, 132.
[6] J. P. Bumier, M. Borowski, B. C. Furie, and B. Furie, *Mol. Cell. Biochem.*, 1981, **39**, 191.
[7] S. W. Fox, K. Harada, and P. E. Hare, *Subcell. Biochem.*, 1981, **8**, 357.
[8] B. Nagy, M. H. Engel, J. E. Zumberge, H. Ogino, and S. Y. Chang, *Nature (London)*, 1981, **289**, 53.

in the crushed rock samples,[8] it was contended that these were laid down since the last of the Isua metamorphic episodes; the extent of racemization is by no means complete, suggesting a date of a few ten thousand years and also implying that the amino-acid content is the result of continuous seepage into the rocks from lichen since the end of the last ice age.[8] The structures of a group of amino-acids found in the Murchison meteorite have also been discussed in relation to inferences that might be drawn – are they synthesized by the condensation of simple precursors under the influence of energy input and catalysts, or are they the remnants of living organisms, racemized and degraded or rearranged through the trauma undergone by the meteorite preceding its arrival on Earth? The presence of six of the seven acyclic six-carbon amino-acids in the Murchison meteorite has been confirmed,[9] although their relative proportions differ from those for amino-acid mixtures formed through electric discharge and Fischer–Tropsch-type syntheses. The major structural type represented in the amino-acid content of this meteorite is the α-branched α-amino-acid; the positive identification of 2-amino-2-ethylbutyric acid, 2-amino-2,3-dimethylbutyric acid, pseudo-leucine (*C*-t-butylglycine), and 2-methylnorvaline represents the first report of the 'natural' occurrence of these amino-acids.[9] Leucine and isoleucine (also *allo*-isoleucine), reported earlier, were also confirmed to be present, and the presence of norleucine is described as 'probable'.[9]

Thirteen common amino-acids have been identified in the 6M hydrochloric acid hydrolysate of humic acids extracted from Spanish lignite.[10]

Some other 'first occurrences' of amino-acids in plant sources have been reported. N^{δ}-Methylornithine has been found in *Atropa belladonna* and shown to be the biosynthetic precursor of atropine.[11] *N*-Carboxymethyl-L-serine is present



(1) R = OH, mugineic acid
(2) R = H, 2′-deoxymugineic acid

in asparagus shoots.[12] Mugineic acid[13] (1) (see Vol. 12, p. 3) and 2′-deoxymugineic acid[14] (2) are iron(II)-chelating constituents of wheat and barley; the 2′-deoxy compound was isolated from root washings of water-cultured wheat (*Triticum aestivum*) under iron-deficient media.[14] *N*-Malonyl-1-aminocyclopropanecarboxylic acid has been recognized as a metabolite of 1-aminocyclopropanecarboxylic acid in buckwheat,[15] while α-(methylenecyclopropyl)glycine, formed from threonine and a one-carbon unit from methionine, is an intermediate in the biosynthesis of β-(methylenecyclopropyl)alanine (the toxic amino-acid,

[9] J. R. Cronin, W. E. Gandy, and S. Pizzarella, *J. Mol. Evol.*, 1981, **17**, 265.
[10] R. Moliner and J. M. Gavilan, *Fuel*, 1981, 64 (*Chem. Abstr.*, 1981, **94**, 124358).
[11] S. H. Hedges and R. B. Herbert, *Phytochemistry*, 1981, **20**, 2064.
[12] T. Kasai and S. Sakamura, *Agric. Biol. Chem.*, 1981, **45**, 1483.
[13] Y. Sugiura, H. Tanaka, Y. Mino, T. Ishida, N. Ota, M. Inoue, K. Nomoto, H. Yoshioka, and T. Takemoto, *J. Am. Chem. Soc.*, 1981, **103**, 6979.
[14] K. Nomoto, H. Yoshioka, M. Arima, S. Fushiya, S. Takagi, and T. Takemoto, *Chimia*, 1981, **35**, 249.
[15] N. Amrhein, D. Schneebeck, H. Skorupka, S. Tophof, and J. Stoeckigt, *Naturwissenschaften*, 1981, **68**, 619.

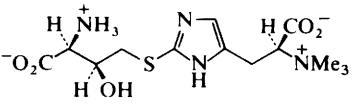
hypoglycin, present in *Blighia sapida*).[16] Briefer mention can be made of other amino-acids in new locations: L-4-oxalysine in *Streptomyces roseoviridofuscus*,[17] 2,4-*trans*-4,5-dihydroxypipecolic acid and *cis*-5-hydroxypipecolic acid from leaves of *Calliandra angustifolia* and the sap of *Calliandra confusa*,[18] 4-methyleneglutamine and 4-methyleneglutamic acid in newly germinated peanut (*Arachis hypogaea*),[19] γ-methyl-L-glutamic acid (*erythro*/*threo* mixture) in *Mycena pura* and *Wynnea gigantea* fruiting bodies,[20] (2*S*,4*S*)-4-hydroxyglutamic acid as the predominant isomer of this amino-acid in *Phlox* species, the (2*S*,4*S*) isomer also found there being the first finding for this isomer in this species,[21] of which *Ledenbergia rose-aenea* and *Pandanus veitcheii* also contain 4-hydroxy-4-methylglutamic acid.[21]

Although non-tolerant seedling roots of *Armeria maritima* contain 1.5—3.0% L-proline, copper-tolerant strains contain extraordinarily high levels (11.0%) of this imino-acid, which seems to be a feature of the tolerance and not accumulated as a response to the stress.[22]

meso-Diaminopimelic acid predominates in the vegetative mycelium of the setamycin-producing actinomycete KM-6054, while the aerial mycelium contains predominantly the LL-diastereoisomer.[23]

Unusual amino-acid residues in proteins include N^{ε}-trimethyl-lysine, in two histone-like proteins from wheat germ,[24] and dihydroxylysinonorleucine, shown to be present in collagen when the other well established crosslinking residue pyridinoline is also present.[25]

New Natural Amino-acids.—Several new amino-acids have been discovered in fungal and bacterial cultures, some with antibiotic properties [L-2-(1-methylcyclopropyl)glycine from *Micromonospora miyakonensis*[26] and 4-oxo-5-amino-6-hydroxyhexanoic acid from *Bacillus cereus* 102 804[27]]. Clithioneine (3) is a new amino-acid betaine from *Clitocybe acromelalga*;[28] polyacetylenic amino-acids (4)



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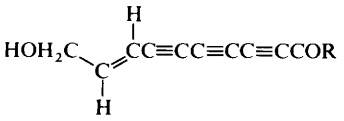
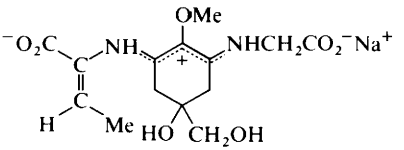
[16] E. A. Kean and C. E. Lewis, *Phytochemistry*, 1981, **20**, 2161.
[17] Q.-Z. Bao, H.-L. Zhang, S.-H. Xu, Y.-L. Hu, S.-X. Liu, and Y.-F. Ye, *Wei Sheng Wu Hsueh Pao*, 1981, **21**, 218 (*Chem. Abstr.*, 1982, **95**, 95 394).
[18] A. B. Bleecker and J. T. Romeo, *Phytochemistry*, 1981, **20**, 1845.
[19] H. C. Winter, G. K. Powell, and E. E. Dekker, *Plant Physiol.*, 1981, **68**, 588.
[20] S. Hatanaka and K. Takishima, *Sci. Pap. Coll. Gen. Educ., Univ. Tokyo*, 1981, **31**, 33 (*Chem. Abstr.*, 1982, **95**, 129 385).
[21] E. A. Bell, L. K. Meier, and H. Sörensen, *Phytochemistry*, 1981, **20**, 2213.
[22] M. E. Farago and W. A. Mullen, *Inorg. Nucl. Chem. Lett.*, 1981, **17**, 275.
[23] S. Omura, Y. Iwai, Y. Takahashi, K. Kojima, K. Otoguro, and R. Oiwa, *J. Antibiot.*, 1981, **34**, 1633.
[24] K. Motojima and K. Sakaguchi, *FEBS Lett.*, 1981, **132**, 334.
[25] Y. Kuboki, M. Tsuzaki, S. Sasaki, C. F. Liu, and G. L. Mechanic, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 119.
[26] J. Shoji, R. Sakazaki, T. Kato, K. Tori, Y. Yoshimura, and S. Matsuura, *J. Antibiot.*, 1981, **34**, 370.
[27] K. L. Perlman, U. Schoemer, T. H. Williams, and D. Perlman, *J. Antibiot.*, 1981, **34**, 483.
[28] K. Konno, H. Shirahama, and T. Matsumoto, *Tetrahedron Lett.*, 1981, **22**, 1617.



(4) R = OMe, Ala-OMe, or Gly-OMe



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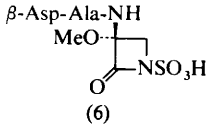
from *Fayodia bisphaerigera*,[29] L-α-amino-γ-nitraminobutyric acid from *Agaricus subrutilescens* (β-nitramino-L-alanine is also present),[30] and (5), a close relative of the palythene–mytilin group of amino-acid derivatives (see Vol. 12, p. 3, and Vol. 11, p. 4, respectively),[31] are also new natural products. Root nodule hydrolysates from *Lotus tenuis* (hosts to two strains of *Rhizobium* bacteria) contain 2,4-diamino-3-methylbutanoic acid, tentatively assigned the (2*R*,3*S*) configuration based on retention times on g.l.c. over a chiral stationary phase.[32] *N*-Acetyl-L-2-amino-3-(*o*-tolyloxy)propionic acid has been confirmed by synthesis as a metabolite of mephenesin.[33]

New Amino-acids from Hydrolysates.—The title of this section is to be interpreted this year to cover structure assignments to new amino-acid constituents of peptides and proteins. *Micromospora chalcea* produces the dipeptide *N*-(2,6-diamino-6-hydroxymethylpimelyl)-L-alanine,[34] while the novel antibiotic sulfazecin (from *Pseudomonas acidophila*) has the structure (6).[35]



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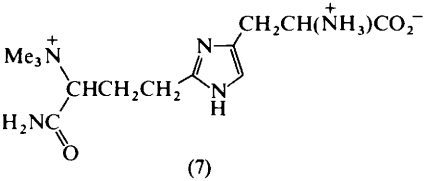

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The new amino-acid in baker's yeast protein elongation factor EF-2, which is ADP-ribosylated in the diphtheria toxin, has been christened diphthamide and assigned structure (7).[36] A protein containing hypusine [N^{ε}-(4-amino-2-hydroxybutyl)lysine] has been isolated from human lymphocytes treated with mitogen and grown in the presence of [^{3}H]putrescine or [^{3}H]spermidine.[37]

[29] M. Ahmed, M. Y. Jarrah, E. R. H. Jones, A. F. Magalhaes, M. G. Roberts, and V. Thaller, *J. Chem. Res. (S)*, 1981, 262.

[30] S. Hatanaka, *Nippon Kingakkai Kaiho*, 1981, **22**, 213.

[31] J. Kobayashi, H. Nakamura, and Y. Hirata, *Tetrahedron Lett.*, 1981, **22**, 3001.

[32] G. J. Shaw, P. J. Ellingham, and L. N. Nixon, *Phytochemistry*, 1981, **20**, 1853.

[33] N. Kosui, T. Kato, N. Izumiya, T. Kuhara, and I. Matsumoto, *Mem. Fac. Sci., Kyushu Univ., Ser. C*, 1981, **13**, 97.

[34] J. Shoji, H. Hinoo, T. Kato, K. Nakauchi, S. Matsuura, M. Mayama, Y. Yasuda, and Y. Kawamura, *J. Antibiot.*, 1981, **34**, 374.

[35] K. Kintaka, K. Kitano, Y. Nozaki, F. Kawashima, A. Imada, Y. Nakao, and M. Yoneda, *J. Ferment. Technol.*, 1981, **59**, 263; M. Asai, K. Haibara, M. Muroi, K. Kintaka, and T. Kishi, *J. Antibiot.*, 1981, **34**, 621.

[36] J. W. Bodley, B. G. Van Ness, and J. B. Howard, *Dev. Cell Biochem. (Amsterdam)*, 1980, **6**, 413; B. G. Van Ness, J. B. Howard, and J. W. Bodley, *J. Biol. Chem.*, 1980, **255**, 10710, 10717.

[37] H. L. Cooper and J. E. Folk, *Proc. Natl. Acad. Sci. U.S.A.*, 1981, **78**, 2869.

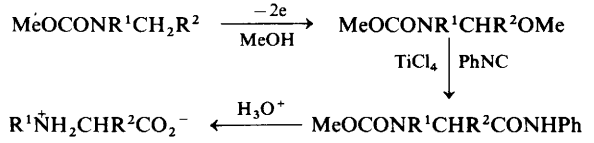
3 Chemical Synthesis and Resolution of Amino-acids

General Methods of Synthesis of α-Amino-acids.—Standard general syntheses and newer methods of potentially general character are collected in this section, although later sections also provide details of specific synthetic objectives achieved through the use of generally applicable methods.

Amino-acids may be synthesized in satisfactory yields from ammonia and the corresponding halogeno-acid, even though textbook warnings, that mixtures of amines result from this approach, linger in the minds of all organic chemists. DL-Alanine is obtained in 76% yield when a 10:1 molar ratio of NH_3 to α-chloropropionic acid is heated under pressure at 70 °C during 5 hours in aqueous solution.[38] A related approach, reaction of an alkali-metal cyanate with the halogeno-acid ester at 80—100 °C in dipolar aprotic solvents, has been developed further;[39] hydrolysis with dilute hydrochloric acid completes the synthesis in this variation.

Dialkyl acylamidomalonates $R^1CONHCH(CO_2R^2)_2$ continue to serve the need for reliable routes to α-amino-acids, in cases where the side chain to be introduced by alkylation of these substrates can withstand the conditions for cleavage and decarboxylation of the substituted malonate. Recent examples[40, 41, 46, 118, 123, 137] include a synthesis of 7-chloro-DL-tryptophan *via* $AcNHC(CO_2Me)_2CH_2CH_2CHO$[40] and of 4-methyleneglutamic acid from acetamidomalonate, HCHO, and diethyl oxalate.[41]

General routes to amino-acids could not reasonably be expected to develop from the reactions based on simple reactants, which have been studied as models of prebiotic synthesis. However, glow-discharge electrolysis of aliphatic amines[42] in aqueous solution gives amino-acids in addition to hydroxyalkylamines. While ethylamine gives glycine in this process, propylamine gives β-alanine and isoserine.[42] Reasonable yields of α-amino-acid amides are formed from urethanes, by anodic oxidation to give α-methoxyurethanes, followed by reaction with phenyl isocyanide, then hydrolysis:[43]



Ugi four-component condensation synthesis is gaining in favour because of variations that simplify the release of the amino-acid from the initial condensation product.[44,45,90] The product from *N*-benzyloxycarbonylglycine, 9-(aminomethyl)fluorene, benzaldehyde, and cyclohexylisocyanide carries the base-labile

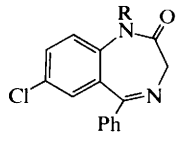
[38] Y. Ogata and M. Inaishi, *Kenkyu Hokaku-Asahi Garasu Kogyo Gijitsu Shoreikai*, 1980, **36**, 219 (*Chem. Abstr.*, 1982, **95**, 43613).
[39] F. Effenberger, K. Drauz, S. Förster, and W. Schöller, *Chem. Ber.*, 1981, **114**, 173.
[40] K. H. Van Pee, O. Salcher, and F. Lingens, *Liebigs Ann. Chem.*, 1981, 233.
[41] G. K. Powell and E. E. Dekker, *Prep. Biochem.*, 1981, **11**, 339.
[42] K. Harada, M. M. Nomoto, and H. Gunji, *Tetrahedron Lett.*, 1981, **22**, 769.
[43] T. Shono, Y. Matsumura, and K. Tsubata, *Tetrahedron Lett.*, 1981, **22**, 2411.
[44] C. F. Hoyng and A. D. Patel, *J. Chem. Soc., Chem. Commun.*, 1981, 491.
[45] P. Bukall and I. Ugi, *Heterocycles*, 1981, **15**, 381.

fluoren-9-ylmethyl group, removable with 1.1 equivalents 1,8-diazabicyclo-[5.4.0]undec-7-ene in pyridine.[44] Use of standard conditions for the four-component condensation in the synthesis of pyridylglycine derivatives has been described.[45]

The azlactone synthesis has been used for the preparation of metallocene analogues of phenylalanine, starting from the corresponding metallocene aldehydes.[46] 4-Substituted oxazolin-5-ones give the corresponding 4,4-disubstituted analogues through Michael addition to electron-deficient alkenes.[47]

Schiff-base alkylation usually gives the α-substitution product, but the anion formed from *N*-benzylidenealanine benzyl ester in conditions established by Stork and co-workers[48] in their general synthesis based on the glycine analogue (−78 °C, HMPA–THF, LDA) leads to a γ-alkylation product with t-butyl 2-bromo-3-phenylpropionate.[49] Although the influence of steric hindrance, solvent, and other rate-controlling factors must account for the predominance of γ-alkylation in this case, this adds a further uncertainty to the method, which has already been shown to give αα-dialkylation products in various proportions, with the desired mono-alkylation product in the case of glycine Schiff bases.

Like the oxazolinones and analogous thiazolines, when considered only as the Δ^2-isomer (other tautomeric forms do exist[50]), the benzodiazepinone (8) represents a cyclic Schiff base. Substitution products involving the methylene group have been reported,[51] including ^1H–^2H exchanged amino-acids obtained through alkaline hydrolysis of the substituted benzodiazepinone, apparently with no isomeric substitution product or di-substitution product.



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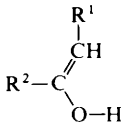

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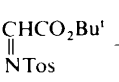

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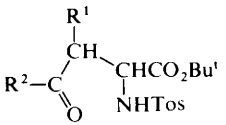


(10)

γ-Oxoalkyl-α-amino-acids (10) may be prepared through an ene reaction involving a *N*-toluene-*p*-sulphonylimide (9), followed by cleavage of the *N*-protecting group.[52]

General Methods of Synthesis of β- and Higher Homologous Amino-acids.—Routes to compounds carrying amino and carboxy groups separated by two or more carbon atoms are usually based on routine methods for introducing one of these functional groups into a compound already carrying the other. The Arndt–Eistert and Wolff rearrangement procedures provide an alternative approach, employing an α-amino-acid as starting material. New routes to β-amino-acids involve

[46] J. C. Brunet, E. Cuingnet, H. Gras, P. Marcincal, A. Mocz, C. Sergheraert, and A. Tartar, *J. Organomet. Chem.*, 1981, **216**, 73.
[47] H. Wegmann and W. Steglich, *Chem. Ber.*, 1981, **114**, 2581.
[48] G. Stork, A. Y. W. Leong, and A. Touzin, *J. Org. Chem.*, 1976, **41**, 3491.
[49] C. J. Harris, *Tetrahedron Lett.*, 1981, **22**, 4863.
[50] G. C. Barrett, *Tetrahedron*, 1980, **36**, 2023.
[51] E. Decorte, R. Toso, A. Sega, V. Sunjic, Z. Ruzic-Toros, B. Kojic-Prodic, N. Bresciani-Pahor, G. Nardin, and L. Randaccio, *Helv. Chim. Acta*, 1981, **64**, 1145.
[52] O. Achmatowicz and M. Pietraszkiewicz, *Tetrahedron Lett.*, 1981, **22**, 4323.

addition of a vinyloxyborane to a Schiff base,[53] aminomercuration of αβ-unsaturated esters followed by demercuration

$$R^1NHAr + Hg(OAc)_2 + R^2CH{=}CR^3CO_2R^4 \longrightarrow R^1NArCHR^2CR^3(HgOAc)CO_2R^4$$

with $NaBH_4$,[54] and condensation of *N*-thioacylurethanes with ethoxycarbonylmethylenetriphenylphosphorane

$$EtO_2CNHC(=S)R + Ph_3P{=}CHCO_2Et \longrightarrow EtO_2CNHCR{=}CHCO_2Et$$

followed by Pt-catalysed hydrogenation.[55] Yields in all these new methods are good or excellent. Wolff rearrangement of *N*-phthaloyl-D- or -L-valine (successive treatment with $SOCl_2$, diazomethane, and u.v. light) provides the β-leucine enantiomers in up to 68% yield.[56]

Asymmetric Synthesis of Amino-acids.—Extensions of established methods provide most of the references for this section. Enantioselective catalytic hydrogenation of α-acylamino-acrylates and -cinnamates continues to be studied by research groups already publishing prolifically in this area, which is also attracting newcomers.[57-59] Enantiomeric excess levels are remarkably uniform, around 75—80%, in all these reports in which open-chain substrates are used (although 84—99% levels were reported in one study[57]), in which there is the common feature of Rh–chiral phosphine complex homogeneous catalysis. More rigid systems (cyclodipeptides of α-aminoacrylates with an L-amino-acid) give 66—90% chiral induction of hydrogenation over palladized charcoal,[58] but reductive methanolysis of 2-methyl- and 2-phenyl-4-benzylideneoxazolin-5-ones in the presence of $PdCl_2$ and (*S*)-phenylethylamine gives less satisfactory results.[59] The *Z*-isomer leads predominantly to the L-phenylalanine derivative through this procedure, whereas hydrogenation of *Z*-α-benzamidocinnamic acid in the presence of (*S*)-phenylethylamine gives predominantly *N*-benzoyl-D-phenylalanine.[59] The chiral Schiff base formed between (*S*)-1-ferrocenylethylamine and pyruvic acid gives a mixture of *N*-substituted alanines in which the L-isomer predominates, through hydrogenation over palladized charcoal.[60] In an alternative approach based on Schiff bases, the glycine derivative (11) yields *threo*-β-hydroxy-α-amino-

[53] M. Otsuka, M. Yoshida, S. Kobayashi, M. Ohno, Y. Umezawa, and H. Morishima, *Tetrahedron Lett.*, 1981, **22**, 2109.

[54] J. Barluenga, J. Villamana, and M. Yus, *Synthesis*, 1981, 375.

[55] M. Slopianka and A. Gossauer, *Synth. Commun.*, 1981, **11**, 95.

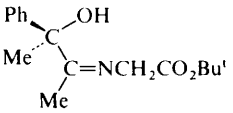
[56] S. R. Sylvester and C. M. Stevens, *Proc. West. Pharmacol. Soc.*, 1981, 24th Meeting, 117 (*Chem. Abstr.*, 1981, **95**, 13328).

[57] (*a*) H. Brunner, W. Pieronczyk, B. Schoenhammer, K. Streng, I. Bernal, and J. Korp, *Chem. Ber.*, 1981, **114**, 1137; (*b*) D. Lafont, D. Sinou, G. Descotes, R. Glaser, and S. Geresh, *J. Mol. Catal.*, 1981, **10**, 305; (*c*) G. L. Baker, S. J. Fritschel, J. R. Stille, and J. K. Stille, *J. Org. Chem.*, 1981, **46**, 2954; (*d*) P. A. MacNeil, N. K. Roberts, and B. Bosnich, *J. Am. Chem. Soc.*, 1981, **103**, 2273; (*e*) J. Irurre, A. Bosch, and J. Capdevila, *Afinidad*, 1981, **38**, 201; (*f*) U. Nagel, H. Menzel, P. W. Lednor, W. Beck, A. Guyot, and M. Bartholin, *Z. Naturforsch., Teil B*, 1981, **36**, 578; (*g*) D. Sinou, *Tetrahedron Lett.*, 1981, **22**, 2987; (*h*) J. Bakos, I. Toth, and L. Marko, *J. Org. Chem.*, 1981, **46**, 5427; (*i*) W. Bergstein, A. Kleemann, and J. Martens, *Synthesis*, 1981, 76.

[58] T. Kanmera, S. Lee, H. Aoyagi, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1980, **16**, 280.

[59] L. F. Godunova, E. S. Levitina, E. I. Kerpeiskaya, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1981, 815.

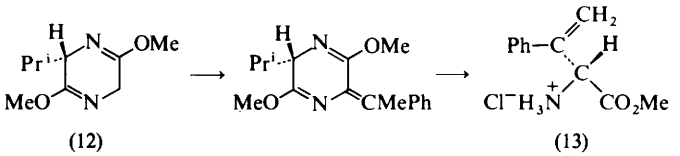
[60] A. Ratajczak and A. Czech, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1979, **27**, 661.



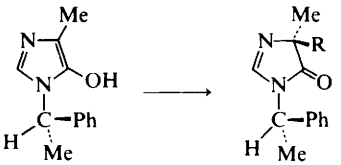
(11)

acids of high optical purity through addition of the derived anion to an aldehyde.[61]

Further uses of chiral dihydropyrazines (12) in the synthesis of a wide variety of α-amino-acids through enantioselective alkylation[62] and the methyl homologue of (12) in the synthesis of chiral α-methyl-α-amino-acids[63] have been reported (see also Vol. 13, p. 5). Condensation of (12) with PhCOMe and cleavage with HCl gives the βγ-unsaturated D-amino-acid (13) in 64% yield.[64] Extension of this approach to 1-chiral-substituted 4-methyl-2-imidazolin-5-ones (14), as a route to L-α-methylphenylalanine and analogues, achieves asymmetric induction at levels better than 95%.[65]



(12) (13)



(14)

A mixed-ligand cobalt(III) complex of D- or L-alanine with (6*R*,8*R*)-6,8-dimethyl-2,5,9,12-tetra-azatridecane, formed from the corresponding α-amino-α-methylmalonic acid complex through enantioselective decarboxylation (*cf.* ref. 66; Vol. 7, p. 5), has been shown[67] to release the amino-acid under conditions that do not cause racemization (a demerit of the earlier studies of this type).

Prebiotic Synthesis; Model Reactions.—Extensions of work featuring in all earlier volumes of this series are represented in near-u.v. irradiation of a suspension of Pt on TiO_2 in water under CH_4 and NH_3,[68] or Pt-catalysed decomposition of H_2O_2 in water under CH_4 and NH_3,[68] and hydrolysis of the reaction product of carbon

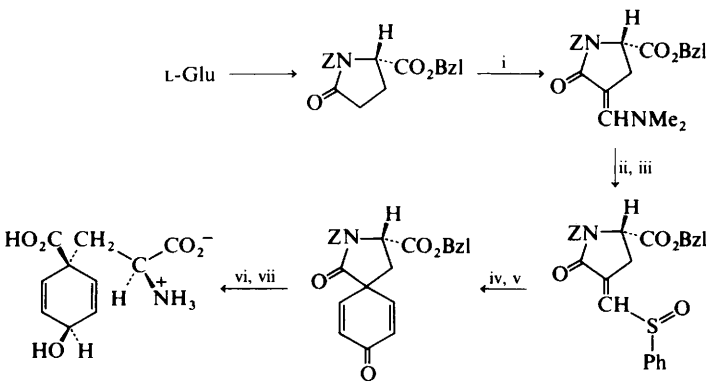
[61] T. Nakatsuka, T. Miwa, and T. Mukaiyama, *Chem. Lett.*, 1981, 279.
[62] U. Schöllkopf, U. Groth, and C. Deng, *Angew. Chem.*, 1981, **93**, 793.
[63] U. Schöllkopf, W. Hartwig, U. Groth, and K. O. Westphalen, *Liebigs Ann. Chem.*, 1981, 698.
[64] U. Schöllkopf and U. Groth, *Angew. Chem.*, 1981, **93**, 1022.
[65] U. Schöllkopf, H. H. Hausberg, M. Segal, U. Reiter, I. Hoppe, W. Sänger, and K. Lindner, *Liebigs Ann. Chem.*, 1981, 439.
[66] R. C. Job and T. C. Bruice, *J. Am. Chem. Soc.*, 1974, **96**, 809.
[67] M. Ajioka, S. Yano, K. Matsuda, and S. Yoshikawa, *J. Am. Chem. Soc.*, 1981, **103**, 2459.
[68] W. W. Dunn, Y. Aikawa, and A. J. Bard, *J. Am. Chem. Soc.*, 1981, **103**, 6893.

vapour (from a carbon-arc discharge) with ammonia.[69] The latter system creates glycine, alanine, β-alanine, *N*-methylglycine, serine, and aspartic acid, these last two amino-acids being formed with glycine and alanine in formaldehyde–hydroxylamine reaction mixtures [70] and arising from the reaction of the glycine with formaldehyde.[70]

Contact glow-discharge electrolysis of amines in aqueous solutions gives mixtures of amino-acids.[42]

Protein and Other Naturally Occurring Amino-acids.—The fermentative production and other biosynthetic routes to amino-acids continue to be well represented in the literature. Typical results cover the production of L-lysine by mutants of *Bacillus licheniformis*,[71] of L-arginine by mutant organisms resistant to L-arginine analogues,[72] and of L-aspartic acid by whole *Escherichia coli* cells immobilized on polyurethane, with ammonium fumarate as substrate.[73] *trans*-Cinnamic acid gives L-phenylalanine in nutrient media containing L-phenylalanine ammonia-lyase.[74] Familiar biosynthetic studies are represented in the establishment of L-pipecolic acid as an intermediate in the conversion of D-lysine into its enantiomer in *Nicotiana glauca*.[75]

A route from L-glutamic acid to L-tyrosine (Scheme 1) [76] involves a Diels–Alder addition to a vinyl sulphoxide as the key step and has been used in a total synthesis of pretyrosine (arogenic acid) and its epimer.[77]



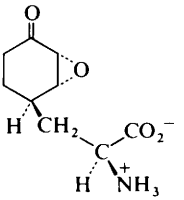
Reagents: i, HCHO, Me_2NH; ii, Ph_2S_2, Bu_3P; iii, *m*-chloroperbenzoic acid; iv, $MeOCH{=}CHC(=CH_2)OSiMe_3$; v, H_2O; vi, $NaBH_4$; vii, 2M NaOH–MeOH, 70 °C, 20—48 h

Scheme 1

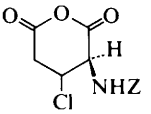
[69] P. B. Shevlin, D. W. McPherson, and P. Melius, *J. Am. Chem. Soc.*, 1981, **103**, 7006.
[70] F. Egami, *Origins Life*, 1981, **11**, 197.
[71] H. Hagino, S. Kobayashi, K. Araki, and K. Nakayama, *Biotechnol. Lett.*, 1981, **3**, 425.
[72] H. Yoshida, K. Araki, and K. Nakayama, *Agric. Biol. Chem.*, 1981, **45**, 959.
[73] M. C. Fusee, W. E. Swann, and G. J. Calton, *Appl. Environ. Microbiol.*, 1981, **42**, 672.
[74] S. Yamada, K. Nabe, N. Izuo, K. Nakamichi, and I. Chibata, *Appl. Environ. Microbiol.*, 1981, **42**, 773.
[75] N. Fangmeier and E. Leistner, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1769.
[76] S. Danishefsky, J. Morris, and L. A. Clizbe, *Heterocycles*, 1981, **15**, 1205.
[77] S. Danishefsky, J. Morris, and L. A. Clizbe, *J. Am. Chem. Soc.*, 1981, **103**, 1602.
[78] B. C. Laguzza and B. Ganem, *Tetrahedron Lett.*, 1981, **22**, 1483.

The first chiral total synthesis of anticapsin from L-tyrosine has been announced.[78] Although this natural amino-acid (15) seems eminently approachable from L-tyrosine, its sensitive epoxycyclohexanone moiety rules out conventional protection strategies. The success of the present routine is due to the use of a new acid- and base-resistant N-protecting group that is inert to nucleophilic attack.[78]

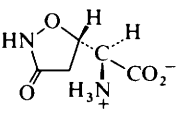
A simple synthesis of cysteine involves the condensation of chloroacetaldehyde with NH_3, acetone, and NaSH in aqueous solution at 0—10 °C, addition of HCN to the resulting 2-thiazoline, and hydrolysis with hydrochloric acid.[79] Another conversion of glutamic acid into proline *via* pyroglutamic acid employs successive treatment with P_4S_{10} and Raney nickel for the conversion of $>C{=}O$ to $>CH_2$;[80] it must be a relatively expensive operation for the purpose.



(15)



(16)



(17)

Routes to more distantly related naturally occurring amino-acids rarely involve the protein amino-acids as starting materials, though L-glutamic acid has been used[81] in a new stereospecific total synthesis of (α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazole-acetic acid, the anti-tumour compound AT-125 (see also Vol. 12, p. 9). The key step was Kollonitsch chlorination of the starting material to give β-chloroglutamic acid (as a 1 : 1 *erythro* : *threo* mixture) and conversion of the derived anhydride (16) into tricholomic acid (17), from which AT-125 has already been synthesized (Vol. 12, p. 9). Baldwin and co-workers have continued to explore synthetic routes to this amino-acid,[82] and an efficient synthesis starting from a protected dehydroglutamic γ-hydroxamate and ending eight steps later with hog kidney acylase I 'resolution' of the chloroacetylamino-acid has been reported.[82]

(−)-3-Aminocardicinic acid, a residue in the antibiotic nocardicin A, has been shown in two studies[83,84] to be accessible from an L-serine derivative through β-lactam formation using Ph_3P with CCl_4 and NEt_3 on L-serine *O*-benzylhydroxamate,[83] or Ph_3P and diethyl azodicarboxylate for the same purpose,[84] mimicking the biosynthesis of the four-membered ring. Homoserine derivatives feature in the synthesis of (+)-avenic acid A, $HO[CH_2CH_2CH(CO_2H)NH]_2CH_2CH_2CH(OH)CO_2H$,[85,86] and its azetidine analogue, 2′-deoxymugineic acid (2).[86,87] In these studies, L-*N*-(3-hydroxy-3-carboxypropyl)homoserine aldehyde was coupled

[79] J. Martens, H. Offermanns, and P. Scherberich, *Angew. Chem.*, 1981, **93**, 680.
[80] A. Kleemann, J. Martens, and K. Drauz, *Chem.-Ztg.*, 1981, **105**, 266.
[81] R. B. Silverman and M. W. Holladay, *J. Am. Chem. Soc.*, 1981, **103**, 7357.
[82] J. E. Baldwin, L. I. Kruse, and J.-K. Cha, *J. Am. Chem. Soc.*, 1981, **103**, 942.
[83] P. G. Mattingly and M. J. Miller, *J. Org. Chem.*, 1981, **46**, 1557.
[84] C. A. Townsend and L. J. Nguyen, *J. Am. Chem. Soc.*, 1981, **103**, 4582.
[85] Y. Ohfune and K. Nomoto, *Chem. Lett.*, 1981, 827.
[86] S. Fushiya, Y. Sato, S. Nakatsuyama, N. Kanuma, and S. Nozoe, *Chem. Lett.*, 1981, 909.
[87] Y. Ohfune, M. Tomita, and K. Nomoto, *J. Am. Chem. Soc.*, 1981, **103**, 2409.

with another homoserine derivative,[85, 86] while a longer route from L-malic acid to 2'-deoxymugineic acid (also accessible through the homoserine route[86]) has been described.[87]

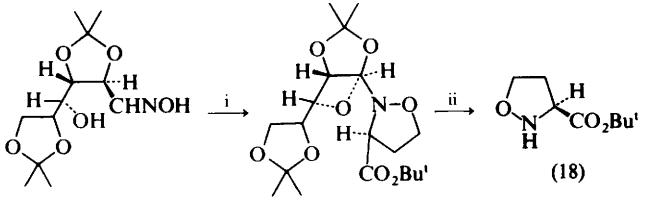
Standard routes have been employed in the syntheses of β-carboxyaspartic acid [from 2-bromo-1,1,2-tris(ethoxycarbonyl)ethane[88] and from 5-chlorohydantoin and diethyl malonate[89]], all isomers of furanomycin (2-methyl-2,5-dihydrofuran-5-ylglycine) through four-component condensation,[90] both diastereoisomers of (±)-2-amino-4-methyl-5-hexenoic acid from (2*R*,4*S*)-CH_2=CHCHMeCH$_2$-CHBrCO$_2$Me,[91] and (±)-dihydroviomycidine and (±)-viomycidine *via* alkylation of carboxylatoethylideneglycinatocopper with PhCH$_2$OCH$_2$CH(OEt)$_2$.[92]

An example of a new naturally occurring β-amino-acid derivative, and its synthesis by a straightforward method, is provided by 3-(*N*-methylamino)glutaric acid.[93]

α-Alkyl Analogues of Protein Amino-acids.—As well as the examples mentioned in the preceding paragraphs, α-alkylation of aspartic acid (protected as the *N*-formyl di-t-butyl ester) with an alkyl or allyl bromide or iodide in the presence of LDA in THF at −78 °C has been established.[94] Optical activity is preserved in this procedure, but substantial amounts of the β-alkylation product are also formed.

***C*-Alkyl and Substituted *C*-Alkyl Amino-acids.**—This section covers close relatives of the common amino-acids; several such examples have been included in other sections of this chapter. Hofmann rearrangement of Boc- or *Z*-L-asparagine using (CF$_3$CO$_2$)$_2$IPh gives L-2,3-diaminopropanoic acid.[95]

Several papers dealing with proline derivatives and analogues appear in the recent literature. *N*-Chlorination of esters of norvaline, *allo*-isoleucine, isoleucine, and leucine and base-induced Hofmann–Loeffler–Freytag cyclization give proline and *cis*- and *trans*-3- and -4-methylprolines.[96] 5-Oxaproline (18) has been obtained starting from D-mannose oxime (Scheme 2).[97] A 54% enantiomeric



(18)

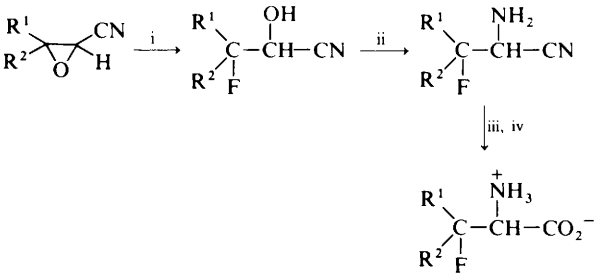
Reagents: i, Bu^{t}OCOCHO + CH$_2$=CH$_2$, 75 °C, 65 bar, 17 h; ii, H_3O^+

Scheme 2

[88] M. R. Christy, R. M. Barkley, T. H. Koch, J. J. Van Buskirk, and W. M. Kirsch, *J. Am. Chem. Soc.*, 1981, **103**, 3935.
[89] E. B. Henson, P. M. Gallop, and P. V. Hauschka, *Tetrahedron*, 1981, **37**, 2561.
[90] J. E. Semple, P. C. Wang, Z. Lysenko, and M. M. Jouillie, *J. Am. Chem. Soc.*, 1980, **102**, 7505.
[91] B. B. Snider and J. V. Duncia, *J. Org. Chem.*, 1981, **46**, 3223.
[92] T. Wakamiya, K. Konishi, H. Chaki, T. Teshima, and T. Shiba, *Heterocycles*, 1981, **15**, 999.
[93] R. E. Summons, *Phytochemistry*, 1981, **20**, 1125.
[94] D. Seebach and D. Wasmuth, *Angew. Chem.*, 1981, **93**, 1007.
[95] M. Waki, Y. Kitajima, and N. Izumiya, *Synthesis*, 1981, 266.
[96] S. L. Titouani, J. Lavergne, P. Viallefont, and R. Jacquier, *Tetrahedron*, 1980, **36**, 2961.
[97] A. Vasella and R. Voeffray, *J. Chem. Soc., Chem. Commun.*, 1981, 97.

excess of the L-isomer was obtained. 3-Methylglutamic acid diastereoisomers resulting from partial saponification and decarboxylation of diethyl 4-methylpyrrolidone-5,5-dicarboxylate have been described.[98]

Aliphatic Amino-acids Carrying Halogen Substituents in Side Chains.—First examples (Scheme 3) of uses of cyano-oxirans in the synthesis of β-fluoro-α-amino-acids have been described.[99] The route is analogous to that using aziridinecarboxylates (Vol. 12, p. 9); a further example is the synthesis of β-fluorophenylalanine from 2-phenyl-3-cyanoaziridine.[99]



Reagents: i, HF–py; ii, NH_3–MeOH; iii, H_3O^+; iv, MeOH–py

Scheme 3

γ-Fluoroisoleucine [prepared from MeCHFCOMe by Horner–Emmons–Wittig condensation with $(EtO)_2P(O)CH_2CO_2R$ followed by hydrogenation, α-bromination (Br_2/LDA) or α-iodination (I_2/LDA), substitution by azide ion, and hydrogenation of the derived α-azido-acid[100]] is a further close analogue of a protein α-amino-acid, which will be useful in metabolic studies. β-Fluoroalanine, already accessible through several routes, has been prepared as a test of a new general synthesis of α-amino-acids described earlier in this chapter.[51] There is no tradition in this Specialist Periodical Report of including citations from the patent literature since this is covered in concentrated form in Section 34 of *Chem. Abstr.* However, a route to β-chloroalanine, a compound first prepared around the turn of the century,[101] from the $ClCH_2CHO$–bisulphite adduct, HCN, and NH_3, is a typical example of continuing opportunities for patent applications in the amino-acid area.[102]

Aliphatic Amino-acids Carrying Hydroxy Groups in Side Chains.—Hydroxymethylation of benzylidene-amino-acid esters using HCHO/LDA followed by hydrolysis with boiling 6M hydrochloric acid proceeds in good yield.[103]

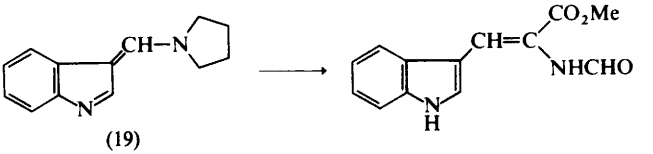
β-Hydroxyvaline has been prepared by a glycine-alkylation route, starting with $(PhCH_2)_2NCH_2CO_2Et$, with acetone and LDA/$MgBr_2$ as base.[104]

[98] A. B. Mauger, *J. Org. Chem.*, 1981, **46**, 1032.
[99] A. A. Ayi, M. Remli, and R. Guedj, *Tetrahedron Lett.*, 1981, **22**, 1505; *J. Fluorine Chem.*, 1981, **18**, 93.
[100] D. Butina and M. Hudlicky, *J. Fluorine Chem.*, 1980, **16**, 301.
[101] E. Fischer and K. Raske, *Berichte*, 1907, **40**, 3717.
[102] K. Nakayasu, O. Furuya, C. Inoue, and S. Moriguchi, *Ger. Offen.*, 3 021 566 (*Chem. Abstr.*, 1982, **95**, 25 629).
[103] A. Calcagni, D. Rossi, and G. Lucente, *Synthesis*, 1981, 445.
[104] A. I. Scott and T. J. Wilkinson, *J. Labelled Compd. Radiopharm.*, 1981, **18**, 347.

Reduction of γ-oxo-ornithine enantiomers, obtained from the corresponding histidines, gives all four stereoisomers of γ-hydroxyornithine, from which the corresponding γ-hydroxyarginines were obtained by standard methods.[105] Routes have been established to γ-hydroxyglutamic acid and analogous adipic acid and pimelic acid derivatives.[106]

2,3-*trans*-3,4-*trans*-3,4-Dihydroxy-L-proline and the 2,3-*cis*-epimer were obtained from the epoxide of *N*-Boc-L-Δ^3-pyrroline-2-carboxylic acid by conventional stages.[107] 3-Chloro- or -bromo-Δ^1-pyrroline-2-carboxylic acid has been used as the source of *cis*- and *trans*-3-acetoxyprolines and 3-heteroatom analogues.[108]

Unsaturated α-Amino-acids.—Conventional routes to αβ-unsaturated α-amino-acids are illustrated in condensation of a ketone with methyl isocyanoacetate and Bu^{t}OK [109] and in the equivalent dehydration of a β-hydroxy-α-amino-acid with disuccinimidyl carbonate [110] (the latter process conveniently provides the succinimido ester of the 'dehydroamino-acid'). Curtius rearrangement of ethyl (*E*)-2-azidocarbonylcarboxycinnamate has been applied for the synthesis of the *E*-dehydrophenylalanine derivative, although in the process a substantial amount of the *Z*-isomer was also formed.[111] Dehydrotryptophans can be obtained by the condensation of the enamine (19) with *N*-formylglycine methyl ester.[112] Deformylation was achieved by ethanolic HCl, a reaction that these authors have sought to optimize in the dehydroamino-acid series.[113]



(19)

Hydroxylamines $RCH_2CH(NHOH)CO_2Et$, formed by reduction of the corresponding oximes, give *N*-acetyldehydroamino-acid analogues through *N*-acetylation followed by treatment with base.[114]

Conversion of 2-isocyano-2-butenoates $MeCR^1{=}C(NC)CO_2R^2$ into the α-vinyl-α-isocyano-acids $CH_2{=}CR^1CR^3(NC)CO_2R^2$ through alkylation with R^3X following treatment with LDA in THF/HMPA provides a controlled route to βγ-unsaturated α-amino-acids.[115] γδ-Unsaturated analogues are obtained [116] through ene reaction of alkenes with (*E*)-$TsN{=}CHCO_2Bu$ [an equivalent route to γ-oxoalkyl α-amino-acids has been illustrated earlier in this chapter, (9) → (10) [52]].

[105] K. Mizusaki and S. Makisumi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 470.
[106] E. P. Kristensen, L. M. Larsen, O. Olsen, and H. Sörensen, *Acta Chem. Scand., Ser. B*, 1980, **34**, 497.
[107] J.-U. Kahl and T. Wieland, *Liebigs Ann. Chem.*, 1981, 1445.
[108] J. Haeusler, *Liebigs Ann. Chem.*, 1981, 1073.
[109] U. Schöllkopf and R. Mayer, *Liebigs Ann. Chem.*, 1981, 1469.
[110] H. Ogura, O. Sato, and K. Takeda, *Tetrahedron Lett.*, 1981, **22**, 4817.
[111] T. J. Nitz, E. M. Holt, B. Rubin, and C. H. Stammer, *J. Org. Chem.*, 1981, **46**, 2667.
[112] T. Moriya, N. Yoneda, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, 1982, **47**, 94.
[113] T. Moriya, K. Matsumoto, and M. Miyoshi, *Synthesis*, 1981, 915.
[114] J. D. M. Herscheid, H. P. H. Scholten, M. W. Tijhuis, and H. C. J. Ottenheijm, *Recl. Trav. Chim. Pays-Bas*, 1981, **100**, 73.
[115] I. Hoppe and U. Schöllkopf, *Synthesis*, 1981, 646.

Synthesis of Amino-acids with Aromatic and Heteroaromatic Side Chains.—Routes to L-tyrosine and L-dopa have been reviewed.[117] DL-2,5,6-Trifluorodopa has been synthesized starting from C_6F_6 (MeLi gives C_6F_5Me, then NaOMe gives 3,4-dimethoxytrifluorotoluene; photobromination gives the substituted benzyl bromide, used in a conventional acetamidomalonate preparation).[118] Reaction of dihydroxyphenylalanines with cystine in constant-boiling hydrobromic acid gave a variety of cysteinyldopas (*S*-cysteinylation of the phenolic moiety), while histidine gives the 2′-(*S*-cysteinyl)imidazole.[119] Other heteroaromatic amino-acids, 4′-pyridylglycines[45] and 7-chlorotryptophan,[40] were prepared by standard methods (Ugi four-component condensation and Fischer indolization, respectively).

α-Hydroxyamino-acids.—Alkylation of *N*-benzylidene-α-amino-acid methyl ester *N*-oxides $PhCH{=}N(=O)CHRCO_2Me$ with an alkyl or benzyl halide after carbanion formation with base gave the corresponding α-substituted amino-acid derivatives, which were converted into *N*-hydroxyamino-acids by hot ethanolic H_2NOH–HCl.[120] Details of the preparation of such compounds from oximes have been discussed in an earlier section.[114]

Synthesis of α-Amino-acids Containing Sulphur and Selenium.—Disulphide $AcSSCMe_2CH(NHAc)CO_2Me$, obtained from the DL-penicillamine derivative and $AcSCO_2Me$, gave the hydrodisulphide on treatment with methanol saturated with HCl.[121] Spontaneous dimerization gave the tetrasulphide, while the corresponding symmetrical trisulphide could be obtained through addition of penicillamine to the hydrodisulphide.[121]

S-Alkylation of cysteine derivatives may be an *in vivo* disposal mechanism for toxic arene oxides, and model studies involving styrene oxide and *N*-acetylcysteine show that attack at the most highly substituted oxiran carbon atom is preferred.[122]

β-(2-Amino-1,3-selenazol-4-yl)alanine has been prepared from 2-amino-4-chloromethylselenazole and diethyl formamidomalonate.[123]

Labelled Amino-acids.—This section covers syntheses of labelled amino-acids with some order imposed on the discussion by arrangement of the examples in order of increasing atomic number.

Synthetic routes allowing incorporation of ^2H have been described for [2,3-$^2H_2$]-L-glutamine [from 6-carboxy-3(2*H*)-pyridazinone],[124] (2*S*,5*R*)-[5-^2H]proline (from L-glutamic acid through enzymic decarboxylation in ^2H_2O, cyclization, condensation with diethyl oxalate, and conventional subsequent steps),[125] and [4,4′-$^2H_6$]-β-hydroxyvaline (synthesis from hexadeuterioacetone).[104]

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Bombardment of solid amino-acids with thermal (2000 K) and 0.6 keV-accelerated tritium atoms [126–129] causes 28% racemization of L-alanine accompanying 40% ^{3}H–^{1}H exchange at the α-carbon [126] and *ca.* 80% exchange at the β-carbon in phenylalanine.[127]

^{11}C-Labelled amino-acids continued to be prepared for tissue distribution studies. The short half-life of this isotope calls for rapid synthetic operations (Bücherer–Strecker synthesis within 40 min) for tryptophan [130] and for phenylalanine [131] (and a further 35 min for resolution using immobilized L- and D-amino-acid oxidases[131]). Even faster working (6—12 min) is needed for the synthesis of ^{13}N-labelled amino-acids using cyclotron-produced $^{13}NH_3$ and immobilized enzymes.[132] There is now a collection of reviews of ^{13}N-labelled [133] and ^{11}C- and ^{18}F-labelled amino-acids.[134]

(2*S*,4*S*)-[5-^{13}C]Leucine has been prepared using *E. coli* deficient in the synthesis of acetolactate and supplemented with (*RS*)-[2-$^{13}CH_3$]acetolactate;[135] silver picolinate oxidation to isovaleric acid, followed by conventional elaboration, leads to (2*RS*)-[4-^{13}C]valine.[135] ^{13}C-Labelled L-leucine has been synthesized [136] from $(^{13}CH_3)_2CO$ and $(EtO)_2P(O)CH_2{}^{13}CO_2Et$ by standard reactions ending with the use of the Strecker synthesis, and DL-[3-^{13}C,2-^{15}N]lysine has been obtained using ^{15}N-phthalimidomalonate and $Na^{13}CN$ at appropriate stages of conventional synthesis.[137] DL-Tyrosine-1-^{14}C,[138] 4-methylene-[2-^{14}C]-DL-glutamic acid,[41] β-*N*-phenyl-[U-^{14}C]-L-asparagine,[139] *O*-acetyl-[1-^{14}C]-L-serine,[139] *O*-benzoyl-[7-^{14}C)]-L-serine, benzyl-[7-^{14}C]- and [U-^{14}C]-glycinate toluene-*p*-sulphonate,[139] α-dimethylaminoisobutyric-[1-^{14}C]acid,[139] α-*N*-[methyl-^{14}C]-DL-asparagine,[140] and N^{G}-[monomethyl-^{14}C]-L-arginine [141] are likewise obtainable by standard methods.

Saponification of Boc-amino-acid methyl esters with NaOH–$^{17}H_2O$, followed by cleavage of the N-protecting group, gives ^{17}O-enriched amino-acids.[142]

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L-[^{35}S]Cysteinesulphinic acid,[143] ^{123m}Te-DL-α-amino-γ-(phenyltelluro)butyric acid,[144] [β-^{125}I]iodo-D- and -L-alanine,[145] and the introduction of a ^{125}I substituent into pteroyltyrosine (a folic acid analogue)[146] also appear in the recent literature.

Resolution of Amino-acids.—This section, as in previous volumes, covers the topic from the diverse viewpoints of theories of prebiotic enantioselection on the one hand and practical procedures for resolution on the other.

The origins of the exclusive dominance of L-amino-acids as protein constituents have been reviewed[147] with special reference to the putative role of chiral energy sources in this process. The Vester–Ulbricht theory (1959) proposing that the 'left-handed' electrons of naturally β-active elements (^{14}C and ^{40}K, for example) are the specific source of the asymmetry has received further support from theoretical analysis, which predicts a very small discrimination.[148] Other physical influences could also operate, and amino-acid solutions incubated with sodium montmorillonite at three different pH values lose slightly larger (0.5—2.0%) amounts of the L-enantiomer by adsorption onto this medium.[149] However, the selectivity does not seem to be conclusively demonstrated generally (it was not observed in some experiments[149]), and the observations of Bondy and Harrington (see Vol. 13, p. 17) on the selective bonding of L-amino-acids and D-sugars to bentonite, made using radioactively labelled amino-acids, may be better explained in terms of the effects of binding of the products of radiochemical decomposition.[150]

Resolution of *N*-benzyloxycarbonyl-DL-homoserine using L-tyrosine hydrazide has been carefully detailed;[151] the D-enantiomer is brought out of solution first when ethanol is used as solvent. Other conventional resolution procedures covered in the recent literature include a modified Pasteur method of visual sorting of crops, which separate from aqueous solutions of DL-amino-acids containing L-glutamic acid and other resolved amino-acids.[152] When DL-threonine separates from such mixtures, the D-enantiomer appears first in the form of long prisms, and the L-enantiomer follows in the form of a fine powder.[152] The preferential crystallization procedure has been studied further[153] with an assessment of the structural requirements for this phenomenon; these seem to be side chains of four atoms, as in methionine and norleucine.[153] Rates of crystallite growth in spontaneous resolution of L-glutamic acid are markedly suppressed by additions of impurities (L-aspartic acid[154]). A study of the characteristics of spontaneous crystallization of one enantiomer from a solution for DL-amino-acid arene-

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sulphonates as well as the influence of factors such as degree of supersaturation and temperature have been reported.[155]

Chromatographic methods hold considerable promise in this area, shown in circulation g.l.c. of amino-acid derivatives on *N*-stearoyl-L-valine t-butylamide [156] and in silica-gel separation of the diastereoisomers formed between DL-2-(2'-cyclopentenyl)glycine and (*R*)-2-acetamido-2-phenylethanol,[157] but particularly in the ligand-exchange procedure [L-hydroxyproline or L-phenylalanine modified polyacrylamide–polystyrene saturated with copper(II) ions [158] and similar systems on a silica support,[159] elution of L-enantiomers from DL-amino-acids adsorbed on reversed-phase silica-gel media by *NNN'N'*-tetramethyl-(*R*)-propane-1,2-diamine–copper(II) complex-containing eluant (H_2O:MeCN = 5:1),[160] and similar resolution of DL-dansylamino-acids using L-prolyl-n-octylamide–nickel(II) in the mobile phase [161]].

Classical traditions are represented also in the resolution of DL-β-(2-thienyl)-alanine as the Boc derivative using (*R*)- or (*S*)-phenylethylamine,[162] of γ-methyl-leucine using leucine aminopeptidase to provide the L-enantiomer from the partially resolved amide,[163] and the same principle for *N*-chloroacetyl-DL-amino-acids using hog renal acylase I.[82]

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—Literature references are even more numerous for 1981, referring to *X*-ray analysis of amino-acids [DL-homocystine,[164] DL-proline hemihydrochloride,[165] L-thioproline hydrochloride,[166] *erythro*-β-fluoro-L-aspartic acid,[167] 5-nitro-L-histidine monohydrate,[168] 3,4-dehydro-L-proline and its *N*-Boc- and acetyl amide derivatives,[169] DL-β-(5-bromouracil-1-yl)alanine,[170] and the copper(II) complex of the novel metal-chelating mugineic acid (1) [171]] and of simple derivatives of amino-acids

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{*N*-Boc-DL-alanine,[172] *N*-Boc-D-glutamic acid monohydrate,[173] *N*-acetyl-L-tyrosine,[174] D-α-acetamidobutyric acid monohydrate,[175] *N*-acetyl-L-cystine,[176] *N*-acetyl-L-aspartic anhydride,[177] *N*-propionyl-L-proline,[178] di-*N*-benzyloxycarbonyl-δ-hydroxy-L-lysine lactone,[179] *N*-phosphonomethylglycine,[180] racemic *N*-[*S*-(methylethoxyphosphinyl)thioglycollyl]valine,[181] and L-proline benzyl ester hydrochloride[182]}. Points of interest from these studies include the revelation of a new self-association mode for *N*-Boc-DL-alanine in the solid state (ribbons of hydrogen-bonded cyclic dimers linked through carboxy groups)[172] and combination of the results of *X*-ray and of neutron diffraction to show the hydrogen-bonding patterns in *N*-acetyl-L-cystine (the —SH group is both donor —SH ... O and acceptor NH ... S).[176]

Crystal structures of amino-acids have been reviewed to study connections between side-chain conformations and the carboxy-group torsion angle χ_1.[183]

Nuclear Magnetic Resonance Spectrometry.—A review of the literature on n.m.r. spectroscopy of amino-acids, peptides, and proteins has appeared, covering the period 1977—1979.[184]

^{1}H n.m.r. studies of [β-^{2}H]-L-tyrosine and L-tryptophan derivatives (*N*-acetyl amino-acids and their esters and methylamides),[185] DL-tryptophan,[186] *S*-adenosyl-L-methionine and *S*-adenosyl-L-homocysteine,[187] L-histidine hydrochloride in water–DMSO,[188] L-histidine and its N^{α}-acetyl methylamide,[189] and *N*-acyl-L-phenylalanines[190] have been concerned with side-chain conformations,[185-187] effect of solvent on the chemical shifts of amide and α-protons in *N*-acylphenylalanines,[190] and proton-transfer studies.[188,189] When the mole fraction of DMSO in water–DMSO is greater than 42%, proton transfer from the imidazole NH group (N-3) to the carboxylate anion in L-histidine hydrochloride (the tautomerization $AH_2^{++-} \rightleftharpoons AH_2^{+}$) is facilitated.[188] The other study of histidine and its derivatives is also a good illustration of the unique contributions of n.m.r. spectroscopy, showing the pressure independence of imidazole p*K* values,

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and therefore suggesting that pressure-induced changes of catalytic activity or ligand-binding characteristic of proteins cannot be explained on this basis.[189] Lanthanide perturbations and nuclear Overhauser effects were part of one of these studies,[186] and complexation phenomena have also been the main feature of n.m.r. studies of Mg^{2+} and Ca^{2+} binding to aspartic acid and asparagine (only Mg^{2+} binds to the amino-group),[191] lanthanides to pyridoxalidene-aspartates,[192] aromatic side-chain complexation with cycloamyloses,[193, 222] and trifluoroacetic acid to *N*-acetyl-L-alanine methylamide (stoicheiometry 1 : 2).[194]

^{13}C and ^{15}N n.m.r. shows that a transition to *Z*/*E*-stereochemistry occurs on dissolution of (*Z*)-*N*-nitroso-*N*-alkyl-amino-acids.[195] Vicinal ^{15}N–^{13}C coupling constants of isotopically pure [^{15}N]-valine, -leucine, -isoleucine, and -threonine show a Karplus-type dihedral angle dependence for the γ-carbon atom, which may be applied to the conformational analysis of amino-acids and small peptides.[196] Other less conventional n.m.r. studies include solid-state quadrupole-echo ^{2}H n.m.r. of ring-deuteriated phenylalanine (showing rapid 180° flips of the phenyl group about the C^{β}–C^{γ} bond axis),[197] ^{13}C and ^{14}N n.m.r. of glycine (bond distances and bond angles from ^{13}C–^{13}C and ^{13}C–^{14}N dipolar splittings agree with neutron-diffraction results),[198] pulsed-n.m.r. study of ^{13}C,^{2}H-enriched glycine (longitudinal relaxation of the two ^{13}C nuclei),[199] and u.v.-excimer laser CIDNP of amino-acids histidine, tryptophan, or tyrosine at 360 MHz using quinoxaline and *p*-methoxyacetophenone as photoreagents (spin-polarized spectra may be interpreted in terms of molecular motion).[200]

^{17}O n.m.r. of four ^{17}O-enriched protein amino-acids is characterized by well resolved peaks.[142]

Optical Rotatory Dispersion and Circular Dichroism.—Use of these techniques to assign absolute configuration to α-amino-acids and small peptides has been reviewed.[201] Little scope remains for novel results on amino-acids themselves since all the common chromophores have been studied in this context. However, L-selenocystine shows Cotton effects centred at 320, 272, 232, and 211 nm, considered[202] to arise in the Se–Se chromophore. (*S*)-(+)-3-Amino-2-phenylpropionic acid shows o.r.d. and molecular-rotation changes accompanying variation in solvent composition, which follow well known empirical rules for α-amino-acids.[203]

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The semicarbazone chromophore in $Me_2C{=}NNHCONHCHRCO_2H$ is responsible for negative-Cotton-effect o.r.d. curves for compounds of the L-series.[204] This new example of the 'chromophoric derivative' approach must prove its worth against several well established alternatives, two of which are featured in configurational assignments to (2*S*,3*S*,4*S*)-2-amino-4-hydroxy-4-(5-hydroxy-2-pyridyl)-3-methylbutyric acid (the *N*-terminal amino-acid residue of the nikkomycins, showing a positive Cotton effect as its *N*-dithioethoxycarbonyl derivative;[205] *N*-dithioethoxycarbonyl-*N*-carboxymethyl-L-amino-acids also show positive Cotton effects[316]), *β*-amino-acids (as *N*-2,4-dinitrophenyl derivatives $DnpNHCHRCH_2CO_2H$; negative Cotton effect near 400 nm for *R*-configuration),[206] and 4-alkenyl and 4-alkynyl 2-amino-alkanoic acids (as Dnp derivatives; negative Cotton effect corresponds to the L-configuration).[207]

Calculated rotatory strengths of *N*-acetyl methylamides of L-alanine and L-serine as a function of conformation have been compared with measured c.d. spectra.[208]

Mass Spectrometry.—The major step forward represented by fast atom bombardment mass spectrometry has been demonstrated most spectacularly by spectra for amino-acids (glycine, L-valine, and phenylalanine) showing good $[M + 1]^+$ ions (a peak attributable to $[2M + 1]^+$ for valine) and detailed fragmentation patterns.[209] The study of thick layers of amino-acids (prepared by compression of the bulk solid on the probe, evaporation of a concentrated solution, or suspension of the solid in a liquid) by this technique and the excellent spectra contrast sharply with the well known problems of mass-spectrometric study of zwitterionic and involatile samples. Similar work[210] with leucine, either alone or in admixture with LiCl, on a silver probe, gives spectra containing $[M + 1]^+$, $[M + Ag]^+$, $[M + Li]^+$, $[M + 2Li - H]^+$, and $[M + Li + Ag - H]^+$ peaks, whose relative proportions depend on the structure of the amino-acid and other factors; 2.25 keV Ar^+ ion bombardment was used in this study.[210]

Chemical-ionization mass spectra of L-methionine and its derivatives in which the sulphide has been transformed into other simple sulphur functional groups have been fully discussed.[211]

The perils associated with thermal treatment of amino-acids on the probe in preparation for ionization are illustrated in the lack of reproducibility of isotopic abundances in [^{15}N]glycine spectra.[212]

Routine mass-spectrometric methods applied to the analysis of *γ*-aminobutyric acid as its trimethylsilyl derivative permit levels down to 25 pg to be reached.[213]

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Other Physical Studies.—This section covers other spectroscopic studies, as well as conventional physico-chemical techniques, applied to amino-acids.

Far-i.r. spectra (20—500 cm^{-1}) of solid layers of glycine and L-alanine represent one extreme application of this wavelength region,[214] while a combined i.r.–u.v. study of interactions between amino-acid esters and nucleotide bases in DMSO[215] represents the other extreme (with more directly usable information). In the latter study the orders (i) cytosine > adenine ≫ uracil ~ thymine and (ii) histidine > methionine > arginine > lysine > serine ~ glycine illustrate the relative hydrogen-bonding efficiencies. I.r. studies of *N*-acyl-α-amino-acids in $CHCl_3$ and CCl_4 to reveal hydrogen-bonding and solvation characteristics[172,216] continue long-established applications of this technique. The relative intensities of spectral lines in Raman spectra of phenylalanine and tyrosine have been compared, but attempts to determine the relative amounts of these amino-acids as residues in proteins, based on spectral lines specific to each, have low reliability.[217]

An i.r. spectral study of L-arginine adsorbed on apatitic calcium phosphate[218] is one of a number of reports of adsorption of L-amino-acids from aqueous solutions on to sodium montmorillonite,[149] α-zirconium phosphate,[219] and titanium(IV) oxide[220] or from aqueous NaF on to mercury.[221] Formation of inclusion complexes between phenylalanine and cyclohexa-, cyclohepta-, and cyclo-octa-amylose has received further detailed study.[193,222]

E.s.r. spectral characterization of semiquinone radicals generated by photolysis of dopa in aqueous solutions[223] and e.s.r.-established decay rates of radicals formed in glycine and β-alanine *X*-irradiated at 77 K[224] have been reported. The identification of alanine in fossil shells and bones is a novel application of e.s.r. in paleochemical studies.[225] Copper(II)-doped L-alanine single crystals at 4.2 K have been studied by the electron–nuclear double-resonance technique, to provide fundamental data, including assignment of the sp^2 state to the nitrogen atom in the amino-acid ligand.[226]

Potentiometric titration studies have been made of tyrosine and its *o*- and *m*-isomers,[227] histidine in water–DMSO,[228] and a series of amino-acids in CF_3CH_2OH.[229] Other physico-chemical studies deal with phase-equilibrium behaviour (solubilities of pairs of L-amino-acids in water[230] and distribution

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coefficients into 1-octanol–water [231]), viscosities of aqueous [232] and ethylene-glycol [233] solutions of amino-acids, osmotic coefficients of aromatic and hetero-aromatic amino-acids in water,[234] mechanism of reverse-osmosis separation of amino-acids through a cellulose acetate membrane,[235] partial molar volumes of α-amino-acids in water,[236] transport of benzoylamino-acids through a CH_2Cl_2 membrane separating two aqueous phases,[237] and molecular polarizabilities of α-amino-acids.[238] Thermodynamic studies cover enthalpies of combustion of L-cysteine and L-methionine,[239] integral enthalpies and densities of amino-acids in aqueous t-butanol,[240] and enthalpy and entropy of transfer of amino-acids from water to aqueous glycerol and its homologues.[241]

Molecular-orbital Calculations.—Solvation of glycine zwitterion in the gas phase [242] and interaction forces between a sodium ion and glutamate, aspartate, and alanine [243] and between purines and amino-acids [244] illustrate a continuing theme in theoretical studies of amino-acids. The last-mentioned study [244] suggests that both adenine and guanine preferentially hydrogen-bond to aspartic acid and asparagine; optimized geometries and conformational energies of these hydrogen-bonded complexes are given. Other conformational calculations reported deal with *N*-acetylglycine methylamide,[245] *N*-acetylglycine, glycine, and cysteine,[246] glycine methyl ester,[247] and L-histidine.[248]

Lattice energies of α-glycine, L-alanine, and DL-alanine crystals have been computed on the basis of a model which required modification after taking account of experimental data for these amino-acids.[249] Calculated proton-transfer energies and electrostatic-lattice energies of amino-acids indicate that lattice energies are greater for zwitterions than for non-zwitterions, but most of this energy surplus is cancelled by the proton-transfer energy. This explains why amino-acids and peptides often (but not always) crystallize as zwitterions.[250]

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[232] R. K. Wadi, A. Gupta, and D. V. S. Jain, *Indian J. Chem., Sect. A*, 1981, **20**, 21.
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[247] L. Schaefer, C. Van Alsenov, J. N. Scarsdale, V. J. Klimkowski, and J. D. Ewbank, *J. Comput. Chem.*, 1981, **2**, 410.
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5 Chemical Studies of Amino-acids

Racemization.—This topic is perhaps not a natural opening to this section at first sight, but it has occupied this position in this series from Volume 6 because it forms the basis of techniques providing age estimates of relatively young fossils and indications of average temperatures to which the fossils have been subjected. For these reasons mechanistic aspects of amino-acid racemization have been studied in some detail.

New examples have been published of applications of D : L ratios of leucine,[251] aspartic acid,[252-254] isoleucine,[152] and methionine[254] measured for quaternary molluscs,[251] fossil bones and teeth from the Olduvai Gorge,[252] from archaeological sites in Italy,[253] and fossil trees of ages 3710, 9000—10 000, and *ca.* 10^5 y.[254] Whereas good agreement between the dates indicated by both racemization and ^{14}C values were obtained in the Italian samples,[253] this is not so for the mollusc samples unless unlikely temperatures through the Pleistocene age are assumed.[251] Samples older than *ca.* 10^5 y contain significant amounts of aspartic acid that has diffused into the fossils since deposition, and the isoleucine : *allo*-isoleucine ratio is a better dating index for such samples.[252]

The uncertainties associated with the amino-acid dating technique (see preceding volumes) continue to be pointed out,[251] and a further source of error not previously taken into account is the ready formation of dioxopiperazines from dipeptides and the faster racemization rates of amino-acid residues in these compounds.[255]

Acid-catalysed racemization of protein amino-acids[254, 256] (*e.g.* under the normal protein hydrolysis regime[254]) is insignificant for isoleucine, valine, serine, and threonine but substantial for aspartic acid, cysteine, proline, glutamic acid, and methionine (rates decreasing in this order).[256]

N-Acylated α-amino-γ-lactones give fully racemized ring-opening products with aqueous NaOH,[257] and *N*-urethane-protected amino-acid anhydrides, previously thought to be immune from racemization during peptide-coupling operations, give significant (*ca.* 5%) amounts of epimers when coupled using *p*-dimethylaminopyridine as base.[258]

Racemization accompanying thermal polymerization of amino-acids is often faster than the peptide-formation process.[259]

Specific Reactions of Natural Amino-acids.—Some of the chemistry described in this section might also apply to amino-acids in general (and this should be borne in mind when reading the following section), but most of the work cited here deals with reactions of the side-chain functional groups of the protein amino-acids.

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[257] K. Michl, *Liebigs Ann. Chem.*, 1981, 33.
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[259] K. Dose, *Origins Life*, 1981, **11**, 165.

Several papers have appeared describing uses of Ph_3P with diethyl azodicarboxylate to convert β-hydroxy-α-amino-acid amides into corresponding 3-amino-azetidin-2-ones[84, 260] (see also ref. 83). With *N*-benzyloxycarbonyl derivatives, no racemization accompanies this process,[260] but with other *N*-protecting groups aziridinecarboxylate derivatives may be obtained. *N*-Boc- and phthaloyl-serine and -threonine esters give corresponding dehydroamino-acids with Ph_3P and diethyl azodicarboxylate or, better, with a carbodi-imide.[261] Elimination on this basis is accompanied by reverse aldolization when β-hydroxyglutamic acid is in the presence of pyridoxal and metal ions.[262] α-Hydroxyglycine derivatives $R^1CONHCH(OH)CO_2R^2$ can be converted into α-chloro analogues using $SOCl_2$, thence into α-heteroatom analogues by nucleophilic substitution.[263]

Exposure of proline or lysine to superoxide anion H_2O_2 or $HO^{\cdot}$ leads to 3- and 4-hydroxyprolines (*cis–trans* mixtures) and 5-hydroxylysine, respectively.[264] Copper(I) autoxidation of cysteine in alkaline solutions gives cystine, H_2O_2, and H_2O, but no sulphur oxyacids.[265] The $Br_2^{-\cdot}$ ion, like $HO^{\cdot}$, gives a disulphide cation (λ_{max} 450 nm) with cystine and other disulphides;[266] electrochemical oxidation of cystine, cysteine, and methionine has been studied.[267] Irradiation with u.v. light ($\lambda < 320$ nm) converts a mixture of *NN'*-diacetylcystine and acetaldehyde into *NS*-diacetylcysteine.[268] The reversible Smiles rearrangement involving *S*- and *N*-Dnp-cysteines favours the *N*-substituted compound in MeOH in the presence of a base, but an equilibrium mixture is formed in DMF–DABCO.[269] Raney nickel desulphurization of common sulphur-containing amino-acids has received detailed study, methionine sulphone, cysteic acid, and homocysteinesulphonic acid being unaffected by the reagent whatever its method of preparation.[270] A novel sulphur-functional-group reaction of cysteine is represented by its conversion into tetrakis-(2-amino-2-carboxyethylmercapto)germane through heating with GeO_2 in water.[271] Protection of the cysteine SH group by the t-butyl group[272] or by using the diphenyl-4-pyridylmethyl group[273] has been described, the latter being particularly promising since it is cleaved easily using Zn–AcOH, $Hg(OAc)_2$, I_2, or electrochemical reduction.

N-Nitrosoproline is formed in solutions containing $NaNO_2$ and either L-citrulline or L-arginine under simulated human-stomach conditions; yields are 27.1 and 0.1%, respectively.[274] Proline, pipecolic acid, and 5-hydroxypipecolic acid are

[260] A. K. Bose, D. P. Sahu, and M. S. Manhas, *J. Org. Chem.*, 1981, **46**, 1229.
[261] R. Andruszkiewicz, J. Grzybowska, and H. Wojciechowska, *Pol. J. Chem.*, 1981, **55**, 67.
[262] K. Tatsumoto and A. E. Martell, *J. Am. Chem. Soc.*, 1981, **103**, 6203.
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[272] J. J. Pastuszak and A. Chimiak, *J. Org. Chem.*, 1981, **46**, 1868.
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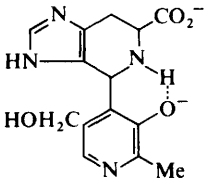
[274] T. Ishibashi and T. Kawabata, *J. Agric. Food Chem.*, 1981, **29**, 1098.

formed from the reaction of $Na_2Fe(CN)_5NO$ with ornithine, lysine, and 5-hydroxylysine, respectively.[275]

1,1-Diacetoxyiodobenzene reduces the amide group of N^{α}-benzyloxycarbonyl-asparagine to the primary amine, to an extent greater than 90%, but the possibility of using this reagent for protein modification is somewhat thwarted by the fact that tyrosine and the protein amino-acids carrying nitrogen or sulphur functional groups in the side chain all react with this reagent, as does the *N*-terminal NH_2 group.[276]

Glutamic acid γ-esters and *O*-acetylserine feature in studies of competitive hydrolysis and intramolecular aminolysis,[277] while ammonolysis of *N*-protected pyroglutamic acids to give glutamines is also in competition with cleavage of the *N*-protecting group.[278]

Apart from a study of *O*-alkylation of *N*-protected tyrosines by $MeSO_2OPr^i$ catalysed by 18-crown-6,[279] all the remaining papers in this section deal with heteroaromatic α-amino-acids. Histidine condenses with pyridoxal *via* the Schiff base to give (20);[280] full details of the proof that histidine decarboxylase catalysis



(20)

of the conversion of L-histidine in ^2H_2O to (1*R*)-[1-^{2}H]histamine involves retention of configuration have been published.[281] Protection of the histidine imidazole moiety by π-benzyloxymethylation or π-bromobenzyloxymethylation offers derivatives that are compatible with the stringent requirements of peptide synthesis.[282] Studies of reactions of tryptophan include sodium hypochlorite oxidation to 3-indoleacetaldehyde,[283] selective 5-hydroxylation *via* Fremy's salt oxidation of the cyclic tautomer (21) to the quinone-imine (22), and $NaBH_4$ reduction,[284] and attack by the trichloromethylperoxy radical to give a neutral tryptophan radical together with the radical adduct as major product.[285]

Pyridinoline, a crosslinking amino-acid residue in collagen, resists $NaBH_4$ reduction, and polarographic oxidation and reduction potentials indicate that its 3-hydroxypyridinium moiety is very difficult to reduce.[286]

[275] M. T. Beck, A. Katho, and L. Dozsa, *Inorg. Chim. Acta*, 1981, **55**, L55.
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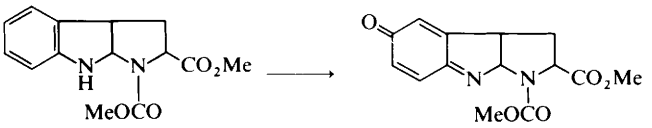

(21) (22)

The kinetic tritium isotope effect for the iodination of [5-^{3}H]-3-iodo-L-tyrosine at pH 7.5 is 4.29 for I_2 and 4.50 using Chloramine-T with KI.[287]

General Reactions.—KOBu^t-catalysed ^1H–^2H exchange of Piv-L-amino-acid dimethylamides in Bu^{t}OH occurs with small but real degree of retention and is the first example of stereospecific exchange at the α-carbon in an enolate-forming carbon acid.[288] Both ion pairing and the influence of the pivalyl group contribute to the stereospecificity.

Further examples of well established reactions of amino-acids include a spectroscopic study of the formation of melanoidins from glycine with glucose or fructose (Maillard reaction),[289] non-random sequence oligopeptides from the thermal polymerization of glycine, glutamic acid, and tyrosine,[259, 290] hydroxymethylation of polyfunctional amino-acids (fast but incomplete reaction with NH_2, guanidinyl, OH, indole, and imidazole side chains; fast and complete with SH; slow and complete with the α-NH_2 group) and condensation of HCHO with asparagine, threonine, histidine, and tryptophan to form cyclic derivatives (see Vol. 12, p. 29),[291] replacement of the α-NH_2 group by F using $NaNO_2$ in HF in the presence of pyridine, with retention of configuration,[292] Friedel–Crafts reactions with *N*-methoxycarbonylamino-acids (benzyloxycarbonyl analogues proved unsuccessful)[293] or ethoxycarbonyl or benzenesulphonyl analogues,[294] Strecker degradation (oxidation by hypochlorite) under u.v. irradiation (which promotes the already rapid initial *N*-chlorination step),[295] and effects of structure on rates of hydrolysis of leucine esters[296] and their copper(II) iminodiacetate complexes.[297]

Reduction of amino-acid esters to α-amino-alcohols with $NaBH_4$ has been developed into a convenient procedure,[298] while BH_3 in THF was found to be the best reagent for the reduction of *N*-protected α-amino-acids to corresponding alcohols in terms of avoidance of racemization and lack of disruption of *N*-terminal and side-chain protection.[299] The Boc-amino-alcohols obtained in this way were conveniently oxidized to the corresponding aldehydes with pyridinium

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[297] R. W. Hay and P. K. Banerjee, *J. Inorg. Biochem.*, 1981, **14**, 147.
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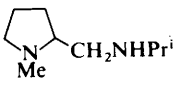
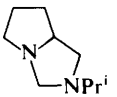
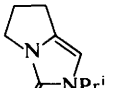
[299] C. F. Stanfield, J. E. Parker, and P. Kanellis, *J. Org. Chem.*, 1981, **46**, 4799.

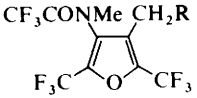
dichromate.[299] An unusual solvent effect on the reduction of *N*-benzyloxycarbonyl-L-proline isopropylamide by $LiAlH_4$ has been reported;[300] in ether the *N*-methyl analogue of the starting material is formed together with the fully reduced compound (23), while in THF (24) and (25) were formed.

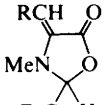

(23)


(24)


(25)

The preparation of *N*-(2,2,2-trichloroethoxycarbonyl)-L-amino-acids has been described,[301] and a polymer thiolacetate has been used for the synthesis of *N*-acetyl derivatives of amino-acids.[302] Instead of the expected *N*-trifluoroacetyl derivatives or mesoionic oxazolinones, *N*-methylamino-acids yield mainly furans (26) and the oxazolidinones (27) by reaction with trifluoroacetic anhydride.[303]


(26)


(27)

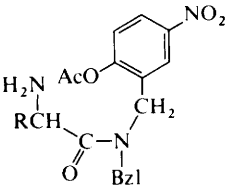
Further results (see Vol. 12, p. 26) on the cyclization of *N*-alkoxycarbonylamino-acids to 2-alkoxyoxazolin-5-ones, and the reaction of these with the starting material to give symmetrical anhydrides, have been reported;[304] it is the anhydride that is formed from this starting material, not the *O*-acylisourea, on treatment with di-isopropyl carbodi-imide.[305] The anhydrides can be converted into the oxazolinones through treatment with a tertiary amine or a carbodi-imide.[306] The oxidative decarboxylation of benzoylphenylglycine esters by O_2 in the presence of an alkali-metal alkoxide and HMPA or DMSO ($ArCONHCHArCO_2Et \rightarrow ArCONHCOAr$)[307] probably involves an oxazolinone intermediate.

Products are formed from amino-acids and ferrous salts in phosphate buffer, which yield a chromogen with thiobarbituric acid, similar to the chromogen formed with malondialdehyde, and iron(II)-catalysed aerial oxidative breakdown is assumed to be taking place;[308] clearly, further studies and rational confirmation of this conclusion are needed. Iridium(III) and iridium(III)–manganese(II)-catalysed oxidative decarboxylation and deamination by cerium(IV) have been achieved for representative amino-acids.[309]

[300] S. Kiyooka, F. Goto, and K. Suzuki, *Chem. Lett.*, 1981, 1429.
[301] J. F. Carson, *Synthesis*, 1981, 268.
[302] M. Gosselet and B. Sebille, *J. Chem. Technol. Biotechnol.*, 1981, **31**, 341.
[303] U. Hess and W. A. König, *Liebigs Ann. Chem.*, 1981, 1606.
[304] N. L. Benoiton and F. M. F. Chen, *Can. J. Chem.*, 1981, **59**, 384.
[305] N. L. Benoiton and F. M. F. Chen, *J. Chem. Soc., Chem. Commun.*, 1981, 543.
[306] N. L. Benoiton and F. M. F. Chen, *J. Chem. Soc., Chem. Commun.*, 1981, 1225.
[307] C. Yijima, F. Hino, and K. Suda, *Synthesis*, 1981, 610.
[308] J. M. C. Gutteridge, *FEBS Lett.*, 1981, **128**, 343.
[309] Y. R. Serma and P. K. Saiprakash, *Indian J. Chem., Sect. A*, 1981, **19**, 1175.

Mutagenic *N*-acetyl-*N*-nitroso-α-amino-acids formed from an *N*-acetylamino-acid and $NO^+BF_4^-$ rearrange into α-diazoalkanoic acids and give 2-methoxy- and 2-hydroxy-alkanoic acids in MeOH and H_2O, respectively.[310]

Reactions through the amino group are involved in: diazo transfer from $CF_3SO_2N_3$ to give corresponding α-azido-acids of high optical purity,[311] stereo-selective addition of amino-acids to chiral *N*-acylimines to give αα-diamino-acid derivatives,[312] reaction of α-amino-acids[313] and esters[314] with malondialdehyde to give enaminals, formation of *N*-phosphovaline in 3.2% yield by reaction of L-valine with cyclotetraphosphate,[315] preparation of *N*-(carboxymethyl)amino-acids using bromoacetic acid,[316] and nine- and twelve-membered ring formation followed by intramolecular *ON*-acyl transfer in (28).[317]



(28)

Enantioselectively catalysed hydrolysis of *N*-protected D- or L-amino-acid *p*-nitrophenyl esters (D:L = 5.68) has been demonstrated, using an L-α-aminohydroxamate as catalyst.[318] Full details of enantioselective deacylation of long-chain acylated α-amino-acid *p*-nitrophenyl esters using co-micelles of *N*-acyl-L-histidine and a cationic surfactant have been published.[319]

Following similar studies with β-hydroxy-α-amino-acid amides reported in 1981,[83, 84, 260] β-amino-acids have been shown to give high yields of β-lactams using Ph_3P and 2,2′-dipyridyl disulphide.[320]

Effects of Electromagnetic Radiation on Amino-acids.—As in all previous volumes, the photochemistry of tryptophan provides most of the papers eligible for this section. Flash photolysis of aqueous tryptophan at 265 nm with simultaneous excitation at other wavelengths causes ionization from a short-lived pre-fluorescent state.[321] Radical cations formed in similar studies[322] in aqueous acidic solutions were the mono- and di-protonated species. Dye-sensitized photo-oxidation of tryptophan in aqueous micellar dispersions[323] and in aqueous

[310] Y. L. Chow and J. Polo, *J. Chem. Soc., Chem. Commun.*, 1981, 297.
[311] J. Zaloom and D. C. Roberts, *J. Org. Chem.*, 1981, **46**, 5173.
[312] C. Shin, H. Ohmatsu, Y. Sato, and J. Yoshimura, *Chem. Lett.*, 1981, 701.
[313] V. Nair, D. E. Vietti, and C. S. Cooper, *J. Am. Chem. Soc.*, 1981, **103**, 3030.
[314] D. J. Pietrzyk and J. Stodola, *Anal. Biochem.*, 1981, **117**, 245.
[315] M. Tsuhako, N. Fujita, A. Nakahama, T. Matsuo, M. Kobayashi, and S. Ohashi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 289.
[316] T. Miyazawa, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 2555.
[317] D. S. Kemp, D. J. Kerkman, S.-L. Leung, and G. Hanson, *J. Org. Chem.*, 1981, **46**, 490.
[318] S. Ono, H. Shosenji, and K. Yamada, *Tetrahedron Lett.*, 1981, **22**, 2391.
[319] K. Ohkubo, K. Sugahara, H. Ohta, K. Tokuda, and R. Ueoka, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 576.
[320] S. Kobayashi, T. Iimori, T. Izawa, and M. Ohno, *J. Am. Chem. Soc.*, 1981, **103**, 2406.
[321] L. I. Grossweiner, A. M. Brendzel, and A. Blum, *Chem. Phys.*, 1981, **57**, 147.
[322] B. Finnstroem, S. Claesson, and E. A. Cherniak, *Chem. Scr.*, 1980, **16**, 65.
[323] E. Rossi, A. Van de Vorst, and G. Jori, *Photochem. Photobiol.*, 1981, **34**, 447.

solutions[324] gives 3a-hydroperoxypyrroloindole as labile precursor to the eventual product, *N*-formylkynurenine. Fluorescence-decay kinetics as a function of pH[325] and emission wavelength[326] (collisional quencher H^+ and $H_2C{=}CHCONH_2$, respectively) and temperature dependence of tryptophan and tyrosine luminescence in poly(vinyl alcohol) films[327] have also been studied. Fluorescence analysis confirms that γ-radiolysis of aqueous phenylalanine gives dityrosine, from tyrosine, in amounts determined by the interplay of many factors.[328] Pyridoxal amino-acid methyl esters undergoing transamination can photorearrange to a *p*-quinonoid structure whose lifetime and other parameters have been determined.[329]

6 Analytical Methods

Gas–Liquid Chromatography.—This technique is the basis of most of the data discussed earlier in this chapter concerning enantiomer ratios for amino-acids.[149, 251–255] The technique has also been studied for its own sake, with the intention to optimize the derivatization preliminaries[330] and to investigate new instrumentation.

Derivatives employed in recent reports are all familiar combinations of *N*-trifluoroacetyl,[331–335] *N*-pentafluoropropionyl,[335] *N*-heptafluorobutyryl,[335, 336] or *N*-isobutoxycarbonyl,[337] as acylating group, which is introduced after esterifying the amino-acids with methanol[335–337] (esterification achieved using BF_3–MeOH[336]), n-propanol,[332] isopropanol,[333] n-butanol,[331, 334, 335] or hexafluoropropanol.[335] Trimethylsilylation has been used for derivatization of ^{14}C-labelled amino-acids for g.l.c. analysis.[338] Glass-capillary-column g.l.c. has been advocated for this purpose,[333, 339] including whisker-walled columns (one column coated with a chiral stationary phase followed by another coated with the enantiomer of the same chiral stationary phase) for the analysis of enantiomer ratios.[333] Several other papers cited in this chapter describe the use of g.l.c. for the analysis of partly racemized amino-acids.[340]

[324] M. Nakagawa, S. Kato, S. Kataoka, S. Kodato, H. Watanabe, H. Okajima, T. Hino, and B. Witkop, *Chem. Pharm. Bull.*, 1981, **29**, 1013; M. Nakagawa, S. Kato, K. Nakano, and T. Hino, *J. Chem. Soc., Chem. Commun.*, 1981, 855.

[325] E. Gudgin, R. Lopez-Delgado, and W. R. Ware, *Can. J. Chem.*, 1981, **59**, 1037.

[326] M. R. Eftink and C. A. Ghiron, *Photochem. Photobiol.*, 1981, **33**, 749.

[327] R. Sakúrovs and K. P. Ghiggino, *Aust. J. Chem.*, 1981, **34**, 1367.

[328] G. Boguta and A. M. Dancewicz, *Nukleonika*, 1981, **26**, 11.

[329] J. W. Ledbetter, J. M. Hankel, and T. J. Cornish, *Photochem. Photobiol.*, 1981, **34**, 115.

[330] J. Drozd, 'Chemical Derivatization in Gas Chromatography', Elsevier, Amsterdam, 1981, p. 126.

[331] F. Boudah, K. Abdeddaim, and M. H. Guermouche, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, 1981, **4**, 247.

[332] E. Mussini, L. Cotellessa, L. Colombo, D. Cani, P. Sfondrini, F. Marcucci, and F. Poy, *J. Chromatogr.*, 1981, **224**, 91.

[333] T. Hobo, M. Yamada, S. Suzuki, S. Araki, A. Shimoyama, and C. Ponnamperuma, *Bunseki Kagaku*, 1981, **30**, T71 (*Chem. Abstr.*, 1982, **95**, 220 275).

[334] A. L. Lapidus, Y. B. Yan, and S. Ya. Grabovenko, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1981, 429.

[335] K. Murayama, N. Shindo, R. Mineki, and K. Ohta, *Biomed. Mass Spectrom.*, 1981, **8**, 165.

[336] Y. Okano, M. Kataoka, T. Miyata, H. Morimoto, K. Takahama, T. Hitoshi, Y. Kase, I. Matsumoto, and T. Shinka, *Anal. Biochem.*, 1981, **117**, 196.

[337] S. Matsuura, S. Yamamoto, and M. Makita, *Anal. Biochem.*, 1981, **114**, 371.

[338] C. Pertzsch, L. Bender, and K. H. Neumann, *Z. Pflanzenernaehr. Bodenkd.*, 1981, **144**, 231.

[339] I. M. Moodie and J. Bunger, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, 1981, **4**, 218.

[340] M. H. Engel, T. K. Sawyer, M. E. Hadley, and V. J. Hruby, *Anal. Biochem.*, 1981, **116**, 303.

It is rare to find a report in which some of the available variations of the g.l.c. technique are compared, and an attempt to simplify g.l.c. analysis (omitting desalination in analysis of glutamic acid and γ-aminobutyric acid at the femtomole level in Ringer's solution) has been described.[335] Dimethyl *N*-pentafluoropropionyl-L-glutamate and hexafluoropropyl *N*-trifluoroacetyl γ-aminobutyrate were found to be superior combinations in terms of sensitivity at these levels, compared with the other groups mentioned in the preceding paragraph. The effects of various salts in either facilitating or inhibiting the esterification and acylation steps are discussed in this report.[335] The objectives of the other reports mentioned in this section were comparisons of different poly(siloxane) stationary phases using amino-acid derivatives,[331] analysis of N^{π}-methylhistidine in physiological fluids,[332] estimation of pipecolic acid and proline at picomole levels,[336] estimation of γ-carboxyglutamic acid,[337] and analysis of alkali-treated frog skins to test whether the observed increase in melanotropic activity is related to partial racemization of amino-acid residues in the constituent proteins.[340]

Ion-exchange Chromatography.—The well established procedures represented in estimations of methylated basic amino-acids in urine [341] and of N^{τ}-methylhistidine (using either fluorescamine[342] or the ninhydrin–*o*-phthalaldehyde reagent after separation from other amino-acids [343]) are typical of a much larger number of more routine studies that have been excluded by considerations of economy of space. New internal standards for routine amino-acid analyser use, γ-carboxyglutamic acid and [^{14}C]cysteic acid, have been proposed.[344]

Thin-layer Chromatography.—The coverage is restricted to representative examples illustrating trends in favour of particular techniques, as well as novel procedures.

Polyamide t.l.c. features in several studies,[345-347] being particularly suitable for separation of dansyl derivatives at low levels;[345, 346] samples extracted from regions of the polyamide layer can be identified by mass spectrometry.[347]

Conventional t.l.c. techniques (including two-dimensional t.l.c.[348]) have been applied to diazoaminobenzenesulphonylthiohydantoins [348] and *N*-diphenylindenonesulphonyl amino-acids.[349]

Novel materials for t.l.c. include activated carbon (visualization involving ninhydrin colour formation on 'prints' taken by pressing filter paper on the developed plates) [350] and ammonium tungstophosphate and molybdophosphate (mainly adsorption but partly an ion-exchange medium).[351]

[341] M. F. Lou and M. Siena, *Biochem. Med.*, 1981, **25**, 309.
[342] A. J. Murray and F. J. Ballard, *Anal. Biochem.*, 1981, **116**, 537.
[343] H. Vielma, J. Mendez, M. Druckenmiller, and H. Lukaski, *J. Biochem. Biophys. Methods*, 1981, **5**, 75.
[344] Y. Ishikawa and R. E. Wuthier, *Anal. Biochem.*, 1981, **114**, 388.
[345] G. Ulmar, *Adv. Biochem. Psychopharmacol.*, 1981, **29**, 105.
[346] R. Roeser, *Mikrokosmos*, 1981, **70**, 152.
[347] R. Kraft, A. Otto, A. Makower, and G. Etzold, *Anal. Biochem.*, 1981, **113**, 193.
[348] C. Marriq, M. Rolland, and S. Lissitzky, *Anal. Biochem.*, 1981, **116**, 89.
[349] Yu. Vladovska, *Dokl. Bolg. Akad. Nauk*, 1981, **34**, 51.
[350] H. Isono, S. Miyaura, and R. Mikawa, *Eisei Kagaku*, 1981, **27**, 38 (*Chem. Abstr.*, 1981, **95**, 20603).
[351] L. Lepri, P. G. Desideri, and D. Heimler, *Ann. Chim. (Rome)*, 1981, **71**, 89.

High-performance Liquid Chromatography.—More space is provided for this topic, which, though developing routine aspects, continues to provide opportunities for pioneering studies.

Electrochemical detection methods lower the sensitivity limits in several areas, for example to 500 and 50 pg levels for dopa and 5-hydroxytryptophan, respectively, in brain tissue [352] (this particular area has been reviewed [353]). Use of a copper tubular electrode as a potentiometric detector for h.p.l.c. of amino-acids has been described.[354]

Most procedures now use fluorescence detection, illustrated in the assay of dansyl thyroxine at femtomole levels,[355] but particularly in the use of *o*-phthalaldehyde–thiol reagents for pre-column fluorescent labelling of amino-acid mixtures.[356–359] Analysis at the 25 picomole level is feasible using this approach.[359] 7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole has been used as a pre-column fluorescent labelling reagent for amino-acids[360] including proline and hydroxyproline,[360, 361] which can be estimated in fluids containing 0.08 and 0.04 nmol ml^{-1}, respectively. The ninhydrin–*o*-phthalaldehyde reagent system, with effluent monitoring at 405 nm, has been applied to the assay of N^{τ}-methyl histidine.[362]

Standard procedures have been followed in h.p.l.c. analysis of tryptophan and its metabolites,[363] α-methyldopa in plasma,[364] and serotonin and related compounds.[365] A review of h.p.l.c. of amino-acids has appeared.[366] Most of the h.p.l.c. studies in the amino-acid area have been concerned with derivatives, and current examples are *N*-dimethylaminoazobenzenesulphonylamino-acids (one study [367] claiming 0.3 picomole detection limits, another from the same research group [368] claiming 2—5 picomole limits), dansyl amino-acids,[369] phenylthiohydantoins,[370] and diphenylindenoylthiohydantoins.[371] The h.p.l.c. behaviour of particular derivatives [*N*-acetylaspartic acid [372] and *cis*- and *trans*-$CF_3CONHC(CO_2Et)_2CH{=}CHCO_2Et$ [373]] has been described.

[352] S. P. Arneric, D. B. Goodale, J. R. Flynn, and J. P. Long, *Brain Res. Bull.*, 1981, **6**, 407.
[353] G. C. Davis, D. D. Koch, P. J. Kissinger, C. S. Bruntlett, and R. E. Shoup in 'Liquid Chromatography in Clinical Analysis', ed. P. M. Kabra and L. M. Marton, Humana, Clifton, New Jersey, 1981, p. 253.
[354] P. W. Alexander, P. R. Haddad, G. K. C. Low, and C. Maitra, *J. Chromatogr.*, 1981, **209**, 29.
[355] R. Bongiovanni, K. D. Burman, R. K. Garis, and J. Boehm, *J. Liq. Chromatogr.*, 1981, **4**, 813.
[356] M. Kuwada and K. Katayama, *Anal. Biochem.*, 1981, **117**, 259.
[357] M. J. Drescher, J. E. Medina, and D. G. Drescher, *Anal. Biochem.*, 1981, **116**, 200.
[358] B. N. Jones, S. Paabo, and S. Stein, *J. Liq. Chromatogr.*, 1981, **4**, 565.
[359] B. R. Larsen and F. G. West, *J. Chromatogr. Sci.*, 1981, **19**, 259.
[360] Y. Watanabe and K. Imai, *Anal. Biochem.*, 1981, **116**, 471.
[361] K. Imai and Y. Watanabe, *Anal. Chim. Acta*, 1981, **130**, 377.
[362] L. C. Ward, M. Miller, and S. Hawgood, *J. Chromatogr.*, 1981, **223**, 417.
[363] J. B. Tarr and J. Arditti, *New Phytol.*, 1981, **88**, 621.
[364] D. A. Jenner, M. J. Brown, and F. J. M. Lhoste, *J. Chromatogr.*, 1981, **224**, 507.
[365] D. E. Mais, P. D. Lahr, and T. R. Bosin, *J. Chromatogr.*, 1981, **225**, 27.
[366] K. Hara, E. Uenishi, C. Ishii, H. Egawa, and K. Murata, *Rinsho Byori*, 1981, **29**, 868 (*Chem. Abstr.*, 1982, **96**, 31012).
[367] J.-Y. Chang, R. Knecht, and D. G. Braun, *Biochem. J.*, 1981, **199**, 547.
[368] J.-Y. Chang, P. Martin, R. Bernasconi, and D. G. Braun, *FEBS Lett.*, 1981, **132**, 117.
[369] S. Weiner and A. Tishbee, *J. Chromatogr.*, 1981, **213**, 501.
[370] F. Lottspeich, *Hoppe-Seyler's Z. Physiol. Chem.*, 1980, **361**, 1829.
[371] I. Mancheva, R. Nikolov, and J. Pfletschinger, *J. Chromatogr.*, 1981, **213**, 99.
[372] K. Lenda, *J. Liq. Chromatogr.*, 1981, **4**, 863.
[373] R. Westwood and P. W. Hairsine, *J. Chromatogr.*, 1981, **219**, 140.

Two studies aimed at the determination of enantiomer ratios through conversion of partly racemic amino-acids into diastereoisomeric dipeptides (by reaction with *N*-carboxyanhydrides derived from L-phenylalanine or L-leucine)[374] or into *N*-Boc derivatives by reaction with the *N*-hydroxysuccinimide ester of *N*-Boc-L-leucine[375] are, respectively, a broad study of 20 common amino-acids (except proline and hydroxyproline)[374] and a specific application to the analysis of thyroxine in serum.[375]

A porous polystyrene–divinylbenzene copolymer has been found to be comparable with alkyl-modified silicas in the liquid chromatography of amino-acids and their derivatives.[376]

Other Analytical Methods.—Fluorescence spectrometry has been used for estimation of total amino-acids in serum, after treatment with *o*-phthalaldehyde–mercaptoethanol,[377] a widely used fluorogenic reaction that has been studied by the fluorescence stopped-flow technique.[378]

Several reviews in a symposium proceedings volume[2] cover the more esoteric variations of standard techniques, for example[379] derivative spectroscopy in the analysis of aromatic amino-acids.

Determination of Specific Amino-acids.—Whereas most papers considered for this section describe colorimetric assays and other methods based on long-established analytical techniques, much routine work is excluded. Enzymatic methods, although also based on familiar principles, are given more space here.

Analysis of mixtures of methionine and cysteine can be achieved in two stages.[380] First, reaction with formaldehyde converts cysteine into thiazolidinecarboxylic acid, which does not interfere with the assay for methionine based on K_2PtI_6 complex formation and colorimetry; the cysteine content is determined using 3,3′-dithio-bis(5-nitrobenzoic acid). Nitroprusside colorimetry is used in an assay for γ-carboxyglutamic acid after conversion into a yellow proline derivative by reaction with acetaldehyde.

N-Hydroxyproline can be assayed at levels of $4\,\mu mol\,l^{-1}$ after alkaline hydrolysis and spectrophotometric estimation of derivatives of products of the resulting reverse aldol reaction.[382] Estimation of *N*-hydroxymethylmethionine similarly involves hydrolysis and separate assays for formaldehyde (chromotropic acid) and methionine (iodometric titration).[383]

Enzymatic methods have little competition in terms of their selectivity, illustrative examples being: an assay of L-cysteine based on catalysis by L-methionine γ-lyase (from *Pseudomonas putida*) of its conversion into pyruvate, estimated colorimetrically (this reaction can also be exploited in an assay of L-

[374] T. Takaya, Y. Kishida, and S. Sakakibara, *J. Chromatogr.*, 1981, **215**, 279.
[375] G. Leb, E. P. Lankmayr, R. Goebel, H. Pristantz, F. Nachtmann, and G. Knapp, *Klin. Wochenschr.*, 1981, **59**, 861.
[376] Z. Iskandarani and D. J. Pietrzyk, *Anal. Chem.*, 1981, **53**, 489.
[377] O. F. Velikanova and Yu. V. Galaev, *Lab. Delo*, 1981, 701 (*Chem. Abstr.*, 1982, **96**, 16869).
[378] E. Trepman and R. F. Chen, *Arch. Biochem. Biophys.*, 1980, **204**, 524.
[379] A. F. Fell in ref. 2, p. 86.
[380] F. Holz, *Landwirtsch. Forsch.*, 1981, **34**, 35 (*Chem. Abstr.*, 1982, **96**, 31091).
[381] L. Pecci and D. Cavallini, *Anal. Biochem.*, 1981, **118**, 70.
[382] G. Szymanowicz and G. Laurain, *Anal. Biochem.*, 1981, **113**, 58.
[383] K. Ranfft, *Landwirtsch. Forsch.*, 1981, **34**, 13 (*Chem. Abstr.*, 1982, **96**, 31192).

methionine),[384] radiochemical assay of *S*-adenosyl-L-homocysteine and L-homocysteine using *S*-adenosyl-L-homocysteine hydrolase and labelled adenosine,[385] and assay of L-serine based on its conversion into phosphoserine, using pyrophosphate and L-serine *O*-phosphotransferase, and spectrometric or fluorimetric estimation of the product.[386] The 0.2 nanomole detection limit compares favourably with that which can be achieved with the amino-acid analyser.[386]

Details of the assembly and use of a tyrosinase electrode for estimation of L-dopa have been published.[387] The potentiometric technique has also been used for estimation of L-phenylalanine in serum, based on a lactate electrode with immobilized *Leuconostoc mesenteroides*.[388]

[384] H. Tanaka, H. Imahara, N. Esaki, and K. Soda, *Agric. Biol. Chem.*, 1981, **45**, 1021; *J. Appl. Biochem.*, 1980, **2**, 439.
[385] N. M. Kredich, H. E. Kendall, and F. J. Spence, *Anal. Biochem.*, 1981, **116**, 503.
[386] R. D. Hurst, K. Lund, and R. W. Guynn, *Anal. Biochem.*, 1981, **117**, 339.
[387] J. L. Iborra, E. Vilanova, and J. A. Lozano, *Biochem. Educ.*, 1981, **9**, 51.
[388] T. Matsunaga, I. Karube, N. Teraoka, and S. Suzuki, *Anal. Chim. Acta*, 1981, **127**, 245.