1
Amino-acids

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Amino-acids continue to attract attention from all branches of chemistry and related disciplines. Unfortunately, this wide diversity of interest poses considerable problems in reviewing the year's literature since only in a limited number of cases can any particular development be considered in depth. However, there can be no doubt that this year some of the most interesting advances have occurred within the field of asymmetric synthesis, and these have been considered worthy of more detailed coverage. The pattern already established for this chapter is maintained with a few minor exceptions, and the emphasis remains on α-amino-acids. Regrettably, it has only been possible to cover biochemical aspects when they relate directly to the chemistry.

1 Naturally Occurring Amino-acids

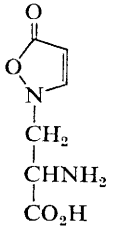
A. Introduction.—Amino-acids with novel structures continue to be isolated from natural sources both in the free state and from peptide and protein hydrolysates. Spectroscopic methods have played an important rôle in structure determination, in particular n.m.r. spectroscopy and mass spectrometry. However, it is interesting to note the increasing application of *X*-ray crystallographic analysis in cases where other spectroscopic methods have proved ambiguous (see Section 3). Those amino-acids whose structures have been confirmed by synthesis are presented in the list of newly synthesised amino-acids in Section 2.

The presence of amino-acids in marine sediments is attracting increasing attention. These acids are undoubtedly of natural origin since they are optically active, and the degree of racemisation observed with increasing age appears to offer an alternative method of dating.[1, 2]

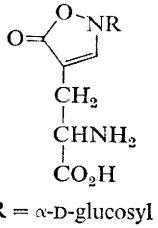
B. New Natural Free Amino-acids.—A number of new plant amino-acids have been described. Further work on the amino-acids formed in the early stages of germination of pea seedlings has resulted in the isolation of the isoxazolinones (1) and (2).[3, 4] The structures are based on extensive

[1] K. A. Kvenvolden, E. Peterson, and F. S. Brown, *Science*, 1970, **169**, 1079.
[2] J. L. Bada, B. P. Luyendyk, and J. B. Maynard, *Science*, 1970, **170**, 730.
[3] F. Lambein, N. Schamp, L. Vandendriessche, and R. van Parijs, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 375.
[4] F. Lambein and R. van Parijs, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 557.

spectroscopic data and degradative evidence. Treatment of (1) with mild base followed by acid hydrolysis affords αβ-diaminopropionic acid, whereas similar treatment of (2) gives D-glucose and glutamic acid. Both (1) and (2) are uncommonly sensitive to u.v. radiation and it has been suggested that they may play a rôle in some photobiological mechanism. It is also reported that they are present in the seedlings of several other leguminous plants.[4] γ-Cyano-L-α-aminobutyric acid has been identified

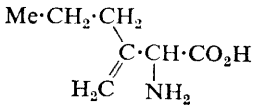


(1)



R = α-D-glucosyl

(2)



(3)

for the first time as a biological substance.[5] It accumulates when inorganic cyanide is administered to young cultures of *Chromobacterium violaceum*. β-Methylene-L-norleucine (3) has been isolated from the carpophores of *Amanita vaginata*[6] and is a further example of a dehydro-amino-acid produced by either a fungus or a micro-organism. *N*-Jasmonoyl- and *N*-dihydrojasmonoyl-isoleucine (stereochemistry at the amino-acid centre not defined) are produced by the fungus *Gibberella fujikuroi*[7] and 2-*N*,6-*N*-di-(2,3-dihydroxybenzoyl)-L-lysine has been isolated from an iron-deficient culture of *Azobacter vinelandii*.[8]

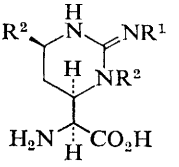
A number of new amino-acids have been detected in human urine.[9] Both *guanidino-NN*-dimethylarginine and *-NN'*-dimethylarginine were isolated in crystalline form; their structures were established by detailed spectroscopic analysis and chemical degradation to ornithine, and finally confirmed by synthesis. In addition, $N^{\varepsilon}N^{\varepsilon}$-dimethyl-lysine and $N^{\varepsilon}N^{\varepsilon}N^{\varepsilon}$-trimethyl-lysine were observed; although these had been previously obtained from certain protein hydrolysates, they had not been previously encountered in the free state.

β-Putreamine, a β-amino-acid, occurs in relatively large amounts in bovine brain tissue[10] and the quaternary β-amino-acids anodendrine and *allo*-anodendrine are present in the plant *Anodendron affine*.[11]

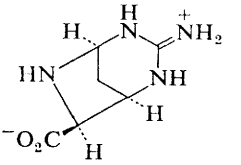
C. New Amino-acids from Peptide Hydrolysates.—Two new guanidino-amino-acids have been reported. Hydrolysis of the tuberculostatic anti-

[5] M. M. Brysk and C. Ressler, *J. Biol. Chem.*, 1970, **245**, 1156.
[6] R. Vervier and J. Casimir, *Phytochemistry*, 1970, **9**, 2059.
[7] B. E. Cross and G. R. B. Webster, *J. Chem. Soc.* (*C*), 1970, 1839.
[8] J. L. Corbin and W. A. Bulen, *Biochemistry*, 1969, **8**, 757.
[9] Y. Kakimoto and S. Akazawa, *J. Biol. Chem.*, 1970, **245**, 5751.
[10] T. Shiba, I. Kubota, and T. Kaneko, *Tetrahedron*, 1970, **26**, 4307.
[11] K. Sasaki and Y. Hirata, *Tetrahedron*, 1970, **9**, 2119.

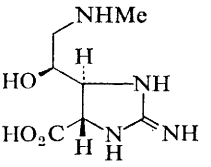
biotic tuberactinomycin affords tuberactidine (4) as well as viomycidine (6),[12] whereas hydrolysis of a new streptothricin-type antibiotic yields *N*-methylstreptolidine (7).[13] The relative and absolute configurations of stendomycidine have been established and are as shown (5).[14] It is noteworthy that the above-mentioned amino-acids, as well as enduracididine



(4) $R^1 = H$, $R^2 = OH$
(5) $R^1 = Me$, $R^2 = H$



(6)



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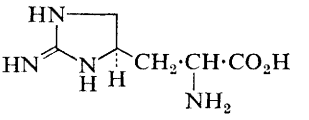


(8)

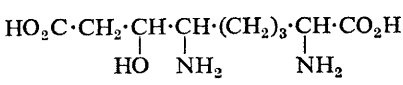
(8), reported last year, are all derived from microbial peptides and are related both structurally and stereochemically to L-arginine. But, as yet, there is no well-authenticated report of the isolation of arginine itself from microbial peptide hydrolysate.

The antibiotics edeine A and B, produced by a strain of *Bacillus brevis*, give on hydrolysis 2,6-diamino-7-hydroxyazelaic acid (9).[15] The stereochemistry has not been fully defined but it is suggested that the two amino-groups have the same relative chirality as in *meso*-pimelic acid. Acid hydrolysis of cycloheptamycin yields L-β-hydroxynorvaline [16] and *N*-methyl-*allo*-isoleucine, and L-β-hydroxyglutamic acid has been isolated from the hydrolysate of a peptide antibiotic complex.[17]

Further work on the hydrolysates of diatom cell walls has resulted in the isolation of $N^{\epsilon}N^{\epsilon}N^{\epsilon}$-trimethyl-δ-hydroxy-L-lysine (10). The structure has been determined by a detailed analysis of the spectral data, including 220 MHz ^{1}H n.m.r. spectra, and subsequently confirmed by synthesis.[18] Possible

[12] T. Nakamiya, T. Shiba, T. Kaneko, H. Sakakibara, T. Take, and J. Abe, *Tetrahedron Letters*, 1970, 3497.
[13] D. B. Borders, K. J. Sax, J. E. Lancaster, W. K. Hausmann, L. A. Mitscher, E. R. Wetzel, and E. L. Patterson, *Tetrahedron*, 1970, **26**, 3123.
[14] G. G. Marconi and M. Bodanszky, *J. Antibiotics*, 1970, **23**, 120.
[15] T. P. Hettinger and L. C. Craig, *Biochemistry*, 1970, **9**, 1224.
[16] W. O. Godtfredsen, S. Vangedal, and D. W. Thomas, *Tetrahedron*, 1970, **26**, 4931.
[17] J. Shoji and R. Sakazahi, *J. Antibiotics*, 1970, **23**, 418.
[18] T. Nakajima and B. E. Volcani, *Biochem. Biophys. Res. Comm.*, 1970, **39**, 28.

similarities between the structure and function of the cell-wall protein and collagen were noted. Sodium borohydride reduction of the intermolecular cross-links in collagen fibrils, followed by acid hydrolysis, affords N^{ε}-(5-amino-5-carboxypentyl)-δ-hydroxylysine (11) and its corresponding six-membered lactone,[19] as well as δε-dihydroxynorleucine.[20] It is debatable



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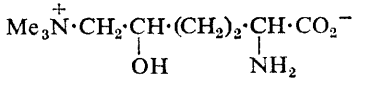
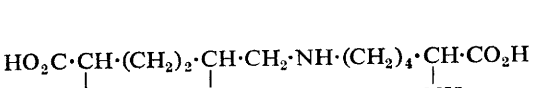
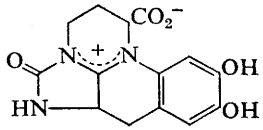


(10)



(11)



(12)

whether these can be classified as true natural products, but this method has proved valuable in locating the sites of cross-linking in collagen and it was felt that the structures are of sufficient interest to warrant inclusion. The same argument applies also to the isolation, and characterisation by an *X*-ray analysis, of (12) from the hydrolysate of a fluorescent peptide produced by iron-deficient cultures of *Azobacter vinelandii*.[21]

A number of β-amino-acids have been isolated for the first time from peptide hydrolysates: δ-hydroxy-β-lysine from tuberactinomycin;[12] iso-serine and β-tyrosine from edeine A and B;[15] and β-amino-β-phenyl-propionic acid from a cyclic tetrapeptide produced by the lichen *Roccella canariensis*.[22]

D. Occurrence of Known Amino-acids.—It has been decided to include in this section only those amino-acids which are rarely encountered or which exhibit interesting biological activity. The new basic amino-acids isolated from human urine have been described (see above); in fact the three possible N^{ε}-methyl derivatives of lysine were obtained, as well as glucosyl-galactosyl- and galactosyl-5-hydroxylysines.[9] The concentrations of the *N*-methyl derivatives of arginine and lysine were unchanged either by oral loading of these amino-acids or by a protein-free diet, and it was tentatively suggested that these compounds are derived from tissue protein. N^{ε}-Trimethyl-lysine has also been obtained from the hydrolysate of the cytochrome *c* derived from *Saccharomyces cerevisiae*.[23] It has previously been

[19] M. L. Tanzer and G. Mechanic, *Biochem. Biophys. Acta*, 1970, **207**, 548.
[20] G. Mechanic and M. L. Tanzer, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 1597.
[21] J. L. Corbin, I. L. Karle, and J. Karle, *Chem. Comm.*, 1970, 186.
[22] G. Bohman, *Tetrahedron Letters*, 1970, 3065.
[23] R. J. Delange, A. N. Glazer, and E. L. Smith, *J. Biol. Chem.*, 1970, **245**, 3325.

observed in plant cytochromes but is absent in the cytochrome *c* produced by animal tissue. N^{ω}-Methylarginine has been detected in histones from rat-liver cell nuclei.[24]

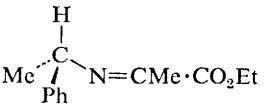
The West African legume *Griffonia simplicifola*, reputed to possess marked physiological activity, has been shown to contain relatively large amounts of 5-hydroxy-L-tryptophan in the free amino-acid pool.[25] N^{δ}-Acetylornithine has been isolated from the seeds of the bush bean, *Phaseolus vulgaris*.[26] Acid hydrolysis of a peptide produced by the plant *Canthium euryoides* gives *NN*-dimethyl-L-phenylalanine and L-*threo*-β-phenylserine,[27] whereas the antibiotic alamethicin, on hydrolysis, affords 2-amino-isobutyric acid.[28]

2 Chemical Synthesis and Resolution of Amino-acids

A. Introduction.—The problem of asymmetric synthesis of α-amino-acids has received considerable attention, and important advances have been made both as regards optical efficiency and yield of material. The majority of the work has centred on a continuation and extension of asymmetric syntheses of α-amino-acids from their corresponding α-keto-acids, for which several methods have already been employed. These include: (*a*) hydrogenation of C=N double bonds using an optically active catalyst; (*b*) reduction of Schiff bases derived from an α-keto-acid derivative and an optically active amine, and (*c*) reduction of Schiff bases obtained from an amine and an optically active α-keto-acid derivative. The earlier literature on hydrogenation using asymmetrically-modified catalysts has been reviewed [29, 30] and further work using Raney-nickel modified with histidine has been described.[31] A detailed investigation on the course of the reaction is reported, but in general the optical efficiency in the process is relatively poor. An extensive study on the sodium borohydride reduction of the Schiff bases of α-keto-esters with optically active α-ethyl- and α-methylbenzylamine has revealed that the optical purities of the resulting amino-acids are lower than those obtained by catalytic hydrogenation.[32] The effect of both temperature [33] and solvent [34] on the catalytic hydrogenation of the above-mentioned Schiff bases has been studied. Low-temperature hydrogenation of (13) and hydrolysis affords (*S*)(*i.e.* L)-alanine (optical purity 60%). The optical activity decreases sharply with a rise in the reaction

[24] W. K. Paik and S. Kim, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 224.
[25] L. E. Fellows and E. A. Bell, *Phytochemistry*, 1970, **9**, 2389.
[26] R. M. Zacharius, *Phytochemistry*, 1970, **9**, 2047.
[27] G. Boulvin, R. Ottinger, M. Pais, and G. Chiurdoglu, *Bull. Soc. chim. belges*, 1970, **78**, 583.
[28] J. Payne, R. Jakes, and B. S. Hartley, *Biochem. J.*, 1970, **117**, 757.
[29] Y. Izumi, *Tampakushitsu Kakusan Koso*, 196, **12**, 301.
[30] E. I. Klabunovskii and E. S. Levivina, *Uspekhi Khim.*, 1970, **39**, 2154.
[31] Y. Izumi, H. Takizawa, K. Nakagawa, R. Imamura, M. Imaida, T. Ninomiya, and S. Yajima, *Bull. Chem. Soc. Japan*, 1970, **43**, 1792.
[32] K. Harada and J. Okhashi, *Bull. Chem. Soc. Japan*, 1970, **43**, 960.
[33] K. Harada and T. Yoshida, *Chem. Comm.*, 1970, 1071.
[34] K. Harada and T. Yoshida, *Bull. Chem. Soc. Japan*, 1970, **43**, 921.

temperature, becoming zero at about 17 °C. Then the configuration is inverted and the optical activity of the resulting D-alanine increases steadily until it reaches a maximum (optical purity 43%) at about 50 °C, finally decreasing at higher temperatures. It is suggested that at lower temperatures the preferred conformation of the substrate on the catalyst



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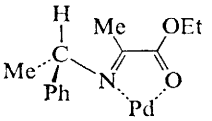

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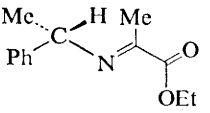
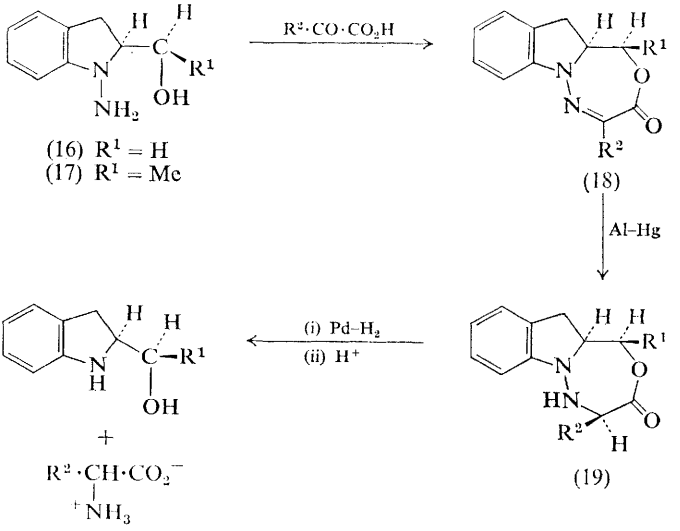


(15)

surface is as shown in (14) and that hydrogenation occurs from the least hindered side, generating an L-alanine derivative; at higher temperatures the conformer (15) predominates, which on reduction yields a D-alanine ester, and further increase in temperature results in complete conformational mobility and the consequent fall in optical activity. Similar changes in conformer population are invoked to account for the increase in optical efficiency in solvents with a low dielectric constant. It is claimed that with polar solvents there is an increase in the concentration of the conformer (15).

The inherent problem of conformational mobility of the substrate in all the previously described asymmetric syntheses has been elegantly solved in a new important synthesis which is essentially a combination of methods (*b*) and (*c*), and is outlined in Scheme 1.[35, 36] The chiral reagents (16) and (17) have been synthesised and resolved, so that both enantiomers can be employed in the synthesis. Condensation of either (16) or (17) with an α-keto-ester affords the corresponding cyclic hydrazono-lactone (18), a chiral compound with limited conformational mobility. Reduction of (18) cannot be achieved under catalytic conditions but, with aluminium amalgam, (19) is formed in high yield. As expected, the addition of the hydrogen, to the α-carbon atom occurs from the least hindered side, *i.e.* from a direction which is *cis* to the hydrogen at C-2 of the indoline. The stereochemical efficiency of the synthesis of (19) is 80—90% when (16) is employed but rises to 96—99% for (17). Reduction and hydrolysis of (19) in the manner indicated affords the optically pure amino-acid and regenerates the

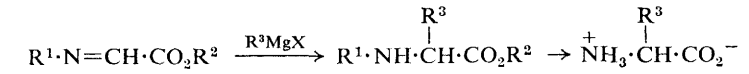
[35] E. J. Corey, R. J. McCaully, and H. S. Sachdev, *J. Amer. Chem. Soc.*, 1970, **92**, 2476.
[36] E. J. Corey, H. S. Sachdev, J. Z. Gougoutas, and W. Saenger, *J. Amer. Chem. Soc.*, 1970, **92**, 2488.



Scheme 1

chiral reagent. From a practical viewpoint this synthesis makes possible the facile and economical synthesis of 100% optically pure amino-acids.

A previously reported synthesis of optically active alanine,[37] employing a Strecker sequence from hydrogen cyanide and Schiff base between acetaldehyde and a chiral amine, has been extended to other amino-acids, and the optical efficiency and material yield have been substantially improved.[38] A new synthesis, involving the addition of a Grignard reagent to a Schiff base derived from an optically active amine and a glyoxylate ester, has been reported [39] (Scheme 2). The optical efficiency is not high but it offers a new general route to amino-acids.

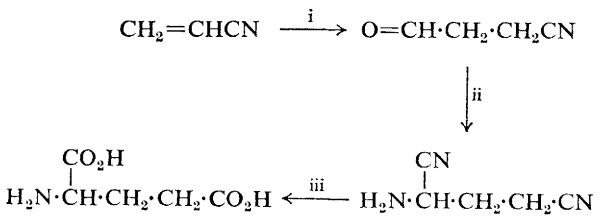


Scheme 2

Interest continues on the origin of amino-acids. Further work describing the synthesis of amino-acids in simulated primitive environments [40] and a speculative article on the origin of chiral compounds, with particular reference to amino-acids,[41] have been published.

[37] K. Harada and S. W. Fox, *Naturwiss.*, 1964, **51**, 106.
[38] M. S. Patel and M. Worsley, *Canad. J. Chem.*, 1970, **48**, 1881.
[39] J. C. Fiaud and H. B. Kagan, *Tetrahedron Letters*, 1970, 1813.
[40] A. Bar-Nun, N. Bar-Nun, S. H. Bauer, and C. Sagan, *Science*, 1970, **168**, 470.
[41] K. Harada, *Naturwiss.*, 1970, **57**, 114.

B. Protein and Other Naturally Occurring Amino-acids.—The methods described in the general introduction have, in many cases, been applied to the synthesis of protein amino-acids; in addition, further syntheses for D-alanine,[42] DL-aspartic acid,[43] and DL-glutamic acid [43] have been described. An industrial preparation of L-glutamic acid, although performed by a well-established route (Scheme 3),[44] is of considerable interest since it must



Reagents: i, CC–H_2; ii, NH_3,KCN; iii, H_3O^+

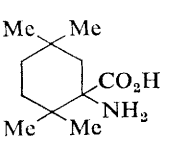
Scheme 3

presumably compete commercially with the naturally obtained material. The use of synthetic amino-acids in fortifying food materials is becoming increasingly important and has been reviewed.[45]

Interest in N^{ε}-methyl derivatives of lysine continues; a further synthesis of the trimethyl derivative has been reported,[46] as well as a new synthesis of the mono- and di-methyl derivatives.[9] The work on hydroxylation of phenylalanine to tyrosines under physiological conditions has been extended, and effective means of separation now appear to be available.[47] The unusual amino-acid α-amino-β-phenylbutyric acid, obtained from the hydrolysate of a microbial peptide, has been synthesised and the relative configuration determined.[48] Photocatalytic oxidation of glucose in the presence of a nitrate has been shown to give a variety of α-amino-acids.[49]

C. *C*-Alkyl- and Substituted *C*-Alkyl-α-amino-acids.—The synthesis of the novel cyclohexane-amino-acids (20) and (21) has been achieved by the Strecker procedure [50, 51] from the corresponding ketones and, in the case of (21), the *cis*- and *trans*-isomers have been separated.[51] The *cis*-isomer readily forms an anhydride, thus allowing a definitive assignment of relative stereochemistry. A novel general synthesis of perfluoroalkyl-α-amino-acids

[42] H. Matsuo, H. Kobayashi, and T. Tatsuno, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1693.
[43] S. Zen and E. Kaji, *Bull. Chem. Soc. Japan*, 1970, **43**, 2277.
[44] T. Yoshida, *Chem.-Ing.-Tech.*, 1970, **42**, 641.
[45] H. H. Ottenheym and P. J. Jenneskens, *J. Agric. Food Chem.*, 1970, **18**, 1010.
[46] J. Puskas and E. Tyihak, *Periodica Polytech.*, 1969, **13**, 261.
[47] M. Viscontini and G. Mattern, *Helv. Chim. Acta.*, 1970, **53**, 372.
[48] H. Arold, M. Eule, and S. Reissmann, *Z. Chem.*, 1969, **9**, 447.
[49] N. R. Dhar and S. K. Arora, *Proc. Nat. Acad. Sci., India*, 1969, **39**, 451.
[50] J. W. Cremlyn, R. M. Ellam, and T. K. Mitra, *J. Indian Chem. Soc.*, 1970, **8**, 218.
[51] J. D. Gass and A. Meister, *Biochemistry*, 1970, **9**, 842.



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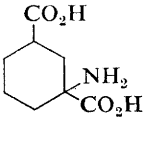
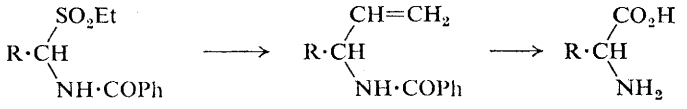


(21)

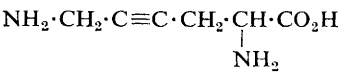
employs a perfluorocarboxylic acid anhydride as the starting material.[52] The anhydride is converted into the sulphone (22) *via* an oxazolone intermediate and treated with a Grignard reagent to give (23), which on oxidation and hydrolysis yields the required amino-acid (24). L-ω-Fluoro-*allo*-isoleucine has been prepared[53] and an improved synthesis of fluorinated



(22) (23) (24)

valine and norvaline derivatives reported.[54] Photochlorination of alanine affords a mixture of isomers, from which β-chloroalanine can be isolated.[55]

The D and L forms of the acetylenic acid (25) are conveniently prepared from 1,4-dichlorobutyne and acetamidomalonate.[56] It is suggested that (25) may be of value for the synthesis of lysine derivatives labelled in the

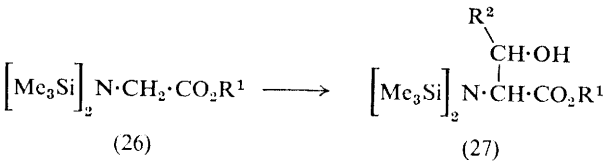


(25)

4- and 5-positions. Considerably enhanced yields of ornithino-alanine are obtained by condensing *N*-benzoyldehydroalanine with *N*-benzoylornithine and subsequent hydrolysis.[57]

D. α-Amino-acids with Aliphatic Hydroxy-groups in the Side-chain.—The synthesis of β-hydroxy-amino-acids by the reaction of a suitably protected glycine derivative with an aldehyde is a well-established method, but two interesting modifications have been reported. Treatment of *NN*-bis-(trimethylsilyl)glycine ester (26) with base, followed by reaction of the resultant carbanion with an aldehyde, affords (27).[58] Good yields are obtained if the aldehyde lacks α-hydrogen atoms, but enolisable aldehydes

[52] F. Weygand, S. Wolfgang, and W. Oettmeier, *Chem. Ber.*, 1970, **103**, 818.
[53] M. Hudlicky, V. Jelinek, K. Eisler, and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1970, **35**, 498.
[54] R. M. Babb and F. W. Bollinger, *J. Org. Chem.*, 1970, **35**, 1438.
[55] T. Zaima, K. Mitsuhashi, I. Sasaji, and T. Asahara, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1970, **73**, 319.
[56] A. C. A. Jansen, K. E. T. Kerling, and E. Havinga, *Rec. Trav. chim.*, 1970, **89**, 861.
[57] M. A. Febrer and P. Miro, *Invest. Inform. Text.*, 1969, **12**, 293.
[58] K. Rühlmann, K. D. Kaufmann, and K. Ickert, *Z. Chem.*, 1970, **10**, 393.



undergo aldol condensation under the basic conditions of the reaction. Similar observations had previously been made in the reaction between an aldehyde and copper bis glycinate. However, it is now claimed that, using the copper complex derived from the Schiff base of glycine and pyruvic acid, reaction occurs readily with mild bases and is applicable to a wide variety of aldehydes.[59]

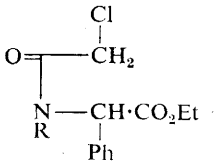
A number of *O*-glycosides of β-hydroxy-amino-acids have been prepared,[60–62] and also several phosphoglycerides of threonine.[63] Interest continues in L-homoserine and its derivatives [64] and a facile enzymic synthesis of *O*-alkyl-homoserines from *O*-acetyl-homoserine in the presence of an alcohol has been described.[65] An improved method for the synthesis of *threo*- and *erythro*-β-hydroxy-DL-aspartic acids from *cis*- and *trans*-epoxysuccinic acids has been claimed.[66]

E. Aromatic and Heterocyclic α-Amino-acids.—Interest continues in substituted phenylalanines because of their potential biological activity. Detailed accounts of the synthesis of various L-cyclodopa (5,6-dihydroxyindolin-2-carboxylic acid) derivatives by oxidative cyclisation of the corresponding L-3,4-dihydroxyphenylalanine have been published.[67, 68] The indane isostere of L-cyclodopa has been prepared,[69] as well as L-6-hydroxydopa by hydrobromic demethylation of the corresponding trimethoxyphenylalanine,[70] and L-*N*-bis-(2-chloroethyl)dopa.[71] Several new halogenated phenylalanines have been reported,[72] and in this context it is of interest to note that an earlier claim that *p*-chlorophenylalanine methyl ester is an aphrodisiac has been discounted.[73]

[59] T. Ichikawa, S. Maeda, Y. Araki, and Y. Ishido, *J. Amer. Chem. Soc.*, 1970, **92**, 5514.
[60] K. Kum, *Carbohydrate Res.*, 1970, **11**, 269.
[61] M. G. Vafina, E. M. Klimov, and T. G. Alieva, *Khim. Biokhim. Uglevodov, Mater. Vses. Konf., 4th* (1967) published 1969, p. 171.
[62] N. K. Kochetkov, V. A. Derevitskaya, L. M. Kikhosherstov, V. M. Kalinevich, and O. S. Novikova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 2509.
[63] J. W. Moore and M. Szelke, *Tetrahedron Letters*, 1970, 4423.
[64] A. Kase, K. Nakayama, and S. Kinoshita, *Agric. and Biol. Chem.* (*Japan*), 1970, **34**, 274, 282.
[65] Y. Murooka, K. Seto, and T. Harada, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 407.
[66] C. W. Jones, D. E. Leyden, and C. H. Stammer, *Canad. J. Chem.*, 1969, **47**, 4363.
[67] V. Wölke, A. Kaiser, W. Koch, and M. Scheir, *Helv. Chim. Acta*, 1970, **53**, 1704, 1708.
[68] N. Fischer and A. S. Dreiding, *Helv. Chim. Acta*, 1970, **53**, 1937.
[69] J. B. Taylor, J. W. Lewis, and M. Jacklin, *J. Medicin. Chem.*, 1970, **13**, 1226.
[70] B. A. Berkowitz, S. Spector, A. Brossi, A. Facella, and S. Teitel, *Experientia*, 1970, **26**, 982.
[71] M. N. Vasileva, V. S. Martynov, and A. Y. Berlin, *Zhur. org. Khim.*, 1970, **6**, 1677.
[72] R. E. Counsell, P. Desai, T. D. Smith, P. S. Chan, P. A. Weinhold, V. B. Rethy, and D. Burke, *J. Medicin. Chem.*, 1970, **13**, 1040.
[73] R. E. Whalen and W. G. Lutege, *Science*, 1970, **169**, 1000.

A number of *N*-uridyl-phenylalanine derivatives have been synthesised as analogues of naturally occurring nucleotides for an investigation into the mechanism of action of various enzyme systems.[74] Thyronine derivatives with an isopropyl group in the positions usually occupied by iodine atoms possess increased biological activity and it is claimed that this is related to the size of the isopropyl group. Several new derivatives have been prepared by standard methods and are listed.[75–77]

Intramolecular cyclisation of *N*-chloroacetyl-2-phenylglycine ester (28) with base produces the corresponding azetidinone (29) which undergoes



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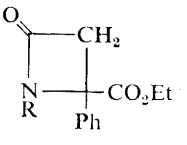
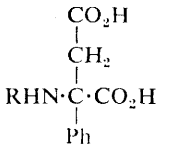


(29)



(30)

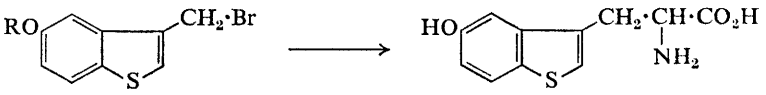
facile ring cleavage to yield a series of novel 2-phenylaspartic acid derivatives (30).[78] Reasonably high yields were obtained and it appears likely that this synthesis could be extended. An interesting modification of the *N*-formylaminomalonate route, which appears to offer a new general synthesis of substituted tryptophans, has been applied to the synthesis of a number of monofluorotryptophans. Alkylation of *N*-formylaminomalonate with morpholine and formaldehyde gives the expected Mannich base, which reacts with monofluoro-indoles to give, after hydrolysis, the required tryptophan in good yield.[79] A considerable number of new aromatic and heterocyclic amino-acids have been reported which were prepared by well-established synthetic routes, and those that have been synthesised for the first time are included in the list in Section H below.[80–84]

F. *N*-Alkyl-α-amino-acids.—Methylation of the amide nitrogen of *N*-benzyloxycarbonyl- and *N*-t-butoxycarbonyl-amino-acids with methyl iodide

[74] N. G. Shinskii, N. N. Preobrazhenskaya, Z. A. Shabarova, and M. A. Prokof'ev, *Zhur. obshchei Khim.*, 1970, **40**, 1114.
[75] T. Matsuura, T. Nagamachi, and A. Nishinaga, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 2176.
[76] E. C. Jorgensen and J. Wright, *J. Medicin. Chem.*, 1970, **13**, 745.
[77] E. C. Jorgensen and J. Wright, *J. Medicin. Chem.*, 1970, **13**, 367.
[78] T. A. Martin, W. T. Cower, C. M. Combs, and J. Q. Carrigan, *J. Org. Chem.*, 1970, **35**, 3814.
[79] M. Beutov and C. Roffman, *Israel J. Chem.*, 1969, **7**, 835.
[80] I. I. Grandberg, L. F. Morozova, V. A. Moskalenko, and A. N. Kost, *Khim. geterotsikl. Soedinenii*, 1969, 1049.
[81] S. J. Norton and P. T. Sullivan, *J. Heterocyclic Chem.*, 1970, **7**, 699.
[82] J. D. Milkowski, F. M. Miller, E. M. Johnson, and N. Zenker, *J. Medicin. Chem.*, 1970, **13**, 741.
[83] M. Y. Lidak, Y. Y. Shluke, S. Y. Poritere, and Y. P. Shvachkin, *Khim. geterotsikl. Soedinenii*, 1970, 529.
[84] V. V. Kiselev and G. P. Menshikov, *Zhur. obshchei Khim.*, 1970, **40**, 914.

and silver oxide in dimethylformamide gives the corresponding *N*-methyl-amino-acid derivatives in excellent yield.[85] The methylation occurs without racemisation of the α-centre and the reaction could no doubt be extended to other alkyl halides. Reference to the novel synthesis of *N*-alkyl-2-phenyl-aspartic acid derivatives has been made above, and several *N*-pyridyl derivatives of the same acid have been obtained by addition of an appropriate amine to a maleate ester.[86] Several new substituted proline derivatives have been reported.[87, 88]

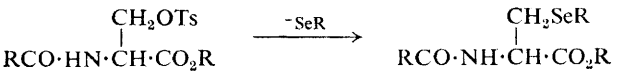
G. α-Amino-acids containing Sulphur or Selenium.—Alkylation of *N*-formyl-aminomalonate with the bromo-derivative (31) affords, after hydrogenolysis and hydrolysis, the novel benzothiophen isostere (32) of 5-hydroxytryptophan.[89] An extensive investigation into the synthesis of thialysine and its



(31) R = Bz (32)

sulphoxide and sulphone has been reported.[90] The antileukaemic activity of *S*-trityl-L-cysteine has led to the synthesis of a wide variety of these compounds by the condensation of the corresponding carbinol and cysteine in the presence of boron trifluoride;[91] most of these compounds are new but only a selection have been included in the list of newly synthesised amino-acids.

Optically active selenium-containing amino-acids can be prepared in good yield by nucleophilic displacement of a tosyl group by either a benzyl selenoate or selenide anion from a suitably protected *O*-tosylserine derivative (33).[93, 94] Compound (34) can be further modified to give a range of



(33) (34)

selenium-containing amino-acids. In this way L-selenocystine, L-seleno-lanthionine, L-selenomethionine, and L-selenoethionine have been prepared; it is claimed that the optical purities are greater than those previously obtained by alternative methods.

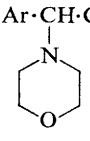
[85] R. K. Olsen, *J. Org. Chem.*, 1970, **35**, 1912.
[86] C. J. Abshire and R. Pineau, *Experientia*, 1970, **26**, 752.
[87] G. Gallina, F. Pentrini, and A. Romeo, *J. Org. Chem.*, 1970, **35**, 2425.
[88] A. J. Verbiscar and B. Witkop, *J. Org. Chem.*, 1970, **35**, 1924.
[89] E. Campaigne and A. Dinner, *J. Medicin. Chem.*, 1970 **13**, 1205.
[90] P. Hermann, K. Stalla, J. Schwimmer, I. Willhardt, and I. Kutschera, *J. prakt. Chem.*, 1970, **311**, 1018.
[91] K. Z. Cheng and C. C. Cheng, *J. Medicin. Chem.*, 1970, **13**, 414.
[92] C. Lee and G. S. Serif, *Biochemistry*, 1970, **9**, 2068.
[93] J. Roy, N. Gordon, I. L. Schwartz, and R. Walter, *J. Org. Chem.*, 1970, **35**, 510.
[94] C. S. Pande, J. Rudick, and R. Walter, *J. Org. Chem.*, 1970, **35**, 1440.

H. A List of α-Amino-acids which have been Synthesised for the First Time.—

Compound	*Ref.*
N^GN^G-dimethyl-L-arginine	9
$N^GN'^G$-dimethyl-L-arginine	9
$N^\varepsilon N^\varepsilon N^\varepsilon$-trimethyl-δ-hydroxy-L-lysine	18
N^ε-(5-amino-5-carboxypentyl)-5-hydroxy-L-lysine	19
δε-dihydroxy-L-norleucine	20
DL-1-amino-3,3,5,5-tetramethylcyclohexane-1-carboxylic acid	50
DL-1-aminocyclodecane-1-carboxylic acid	50
DL-1-aminocyclo-octane-1-carboxylic acid	50
DL-*cis*-1-amino-1,3-dicarboxycyclohexane (cycloglutamic acid)	51
DL-*trans*-1-amino-1,3-dicarboxycyclohexane	51
DL-3,3,4,4,4,-pentafluoro-2-aminobutyric acid	52
DL-3,3,4,4,5,5,5-heptafluoro-2-aminovaleric acid	52
ω-fluoro-DL- and L-*allo*-isoleucine	53
D- and L-2,6-diamino-4-hexynoic acid	54
O-(α-D-glucopyranosyl)-L-serine	60
O-(1-oleyl-glycero-3-phosphoryl)-L-threonine	63
O-(1,2-dioleyl-glycero-3-phosphoryl)-L-threonine	63
DL-2-amino-5,6-dihydroxyindan-2-carboxylic acid	69
N-bis-(2-chloroethyl)-3,4-dihydroxy-L-phenylalanine	71
DL-3-(*m*-fluorophenyl)-2-methylalanine	72
DL-3-(*m*-bromophenyl)-2-methylalanine	72
DL-3-(*m*-iodophenyl)-2-methylalanine	72
DL-2-[(*m*-iodophenyl)methyl]glycine	72
DL-4-(*m*-iodophenyl)-2-methyl-2-aminobutyric acid	72
3,5,3′-tri-isopropyl-DL-thyronine	75
3,5-dimethyl-3′-isopropyl-DL-thyronine	76
3,5-di-isopropyl-DL-thyronine	77
3,5-di-isopropyl-4′-amino-DL-thyronine	77
3,5-di-isopropyl-3′-bromo-DL-thyronine	77
3,5-di-isopropyl-3′-methyl-DL-thyronine	77
3,5-di-s-butyl-DL-thyronine	77
3,5-di-s-butyl-4′-amino-DL-thyronine	77
3,5-di-s-butyl-3′-bromo-DL-thyronine	77
3,5-di-s-butyl-3′-iodo-DL-thyronine	77
N-phenyl-2-phenyl-DL-aspartic acid	78
N-methyl-2-phenyl-DL-aspartic acid	78
N-benzyl-2-phenyl-DL-aspartic acid	78
4-fluoro-DL-tryptophan	79
5-fluoro-DL-tryptophan	79
6-fluoro-DL-tryptophan	79
β-(5-hydroxy-6-iodo-2-pyridyl-1-oxide)-DL-alanine	81
β-(5-hydroxy-6-iodo-2-pyridyl)-DL-alanine	81
β-(benzimidazol-5-yl)-DL-alanine	82
β-(2-amino-6-hydroxypurin-9-yl)-DL-alanine	83
β-(2-amino-6-mercaptopurin-9-yl)-DL-alanine	83
N-dicolchicidyl-L-lysine	84
N-(2-pyridyl)-DL-aspartic acid	86
N-(3-methyl-2-pyridyl)-DL-aspartic acid	86
N-(4-methyl-2-pyridyl)-DL-aspartic acid	86
N-(6-methyl-2-pyridyl)-DL-aspartic acid	86
cis-p-methoxybenzylmercapto-L-proline	88
trans-p-methoxybenzylmercapto-L-proline	88

Compound	*Ref.*
β-(5-hydroxy-3-benzo[*b*]thienyl)-DL-alanine	89
N^{ε}-acetyl-L-thialysine	90
L-thialysine sulphoxide	90
L-thialysine sulphone	90
S-[(diphenyl-α-naphthyl)methyl]-L-cysteine	91
S-[(diphenyl-β-naphthyl)methyl]-L-cysteine	91
S-(9-methyl-9-fluorenyl)-L-cysteine	91
DL-2-amino-6-(methylthio)caproic acid	92

I. Labelled Amino-acids.—An interesting new method for labelling aryl aldehydes with deuterium or tritium in the formyl group, which also provides a novel route to labelled amino-acids, has been described.[95] Aryl aldehydes are converted into the crystalline morpholinoacetonitrile derivatives (35). The benzylic hydrogen is readily exchanged with deuterium oxide or tritiated water, and hydrolysis with aqueous acid affords the formyl-labelled aldehydes in high yield without loss of the label. The utility of these aldehydes for the synthesis of labelled amino-acids has



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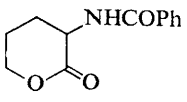


(36)

been demonstrated with synthesis of (±)-3,4-dihydroxy-[β-$^{2}H_{2}$]phenylalanine, but the wider synthetic potential of these aldehydes for specific labelling at prochiral centres is apparent. Tritioammonia has been employed to label 5-hydroxytryptophan, asparagine, and glutamic acid.[96] Reaction of the lactone (36) with radioactive cyanide in dimethylformamide, followed by reduction and hydrolysis, affords [6-^{14}C]-DL-lysine.[97] [2-^{14}C]- and [^{15}N]-DL-2-amino-6-(methylthio)caproic acid has been prepared from the correspondingly labelled aminomalonate,[92] and several DL-[^{35}S]thialysine derivatives have been synthesised by standard procedures.[90] An improved biochemically ^{14}C- and ^{35}S-labelled methionine has been reported[98] and incubation of *O*-acetylhomoserine in the presence of a radioactive alcohol gives the corresponding labelled *O*-alkylhomoserine.[65] It is claimed that the reaction of tyrosine with hydrochloric acid solutions of radioactive potassium iodide and potassium iodate gives good yields of [^{135}I]iodotyrosine,[99] but previous experience with this type of compound suggests that they are very unstable.

[95] D. J. Bennett, G. W. Kirby, and V. A. Moss, *J. Chem. Soc.* (*C*), 1970, 2049.
[96] K. Bloss, *J. Labelled Compounds*, 1969, **5**, 555.
[97] J. Pichat, J. Tostain, and C. Baret, *Bull. Soc. chim. France*, 1970, 1837.
[98] K. Samochocka and J. Kowalczyk, *Radiochem. Radioanalyt. Letters*, 1970, **4**, 131.
[99] M. El-Garhy, Y. M. Megahed, A. A. Kassem, and E. Abdullah, *Internat. J. Appl. Radiation Isotopes*, 1970, **21**, 240.

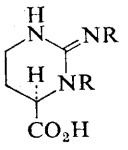
J. Resolution of α-Amino-acids.—It is well established that certain amino-acids can be resolved by paper adsorption chromatography, and further applications of this particular technique have been reported.[100] The possibility of employing alternative optically active adsorbents for resolution has recently attracted considerable attention. It is claimed that the ion-exchange resin, prepared by co-polymerisation of ethyl-*N*-acryloyl-L-polyglutamate and divinylbenzene, will resolve basic amino-acids such as lysine and ornithine with up to 90% efficiency.[101] Di- and tri-peptide derivatives of L-valine are also very effective stationary phases for the gas–liquid partition chromatographic separation of the enantiomers of racemic *N*-trifluoroacetyl-α-amino-esters.[102, 103] This technique is to some extent limited to neutral amino-acids and, as yet, is intended more for analytical application than for preparative use. (See also Section 5.)

Chemical and enzymic methods of resolution of synthetic racemates are still the most commonly employed, and detailed accounts of the use of ephedrine [104] and phenylethylamine [105] for the resolution of *N*-benzyloxycarbonyl-DL-amino-acids have been published.

3 Physical and Stereochemical Studies of Amino-acids

A. Determination of Absolute Configuration.—Lengthy chemical correlations for the establishment of absolute configuration are fortunately becoming less essential. The extent of application of o.r.d. and c.d. spectra and *X*-ray analytical techniques is increasing (see below), and chemical methods are only employed where direct correlation is relatively facile. Enzymic resolution of synthetic racemic amino-acids is, in effect, a method of determining the absolute configuration, and is constantly employed.

The o.r.d. spectra of L-amino-acids exhibit a positive Cotton effect at about 225 nm.[106] This has now been shown to hold for amino-acids containing a second chiral centre and has been used to assign the L-configuration at the α-centre of stendomycidine (5). Oxidation of (5) with *N*-bromosuccinimide gives (37; R = Me), the o.r.d. curve of which is

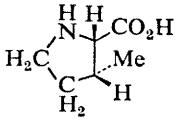


(37)

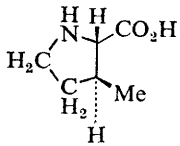
[100] R. Weichert, *Arkiv. Kemi*, 1970, **31**, 517.
[101] H. Suda, Y. Hosono, Y. Hosokawa, and T. Seto, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 1970, **73**, 1250.
[102] S. Nakaparkan, P. Birrell, E. Gil-Av, and J. Oro, *J. Chromatog. Sci.*, 1970, **8**, 177.
[103] B. Feibush and E. Gil-Av, *Tetrahedron*, 1970, **26**, 1361.
[104] K. Oki, K. Suzuki, S. Tuchida, T. Saito, and H. Kotake, *Bull. Chem. Soc. Japan*, 1970, **43**, 2554.
[105] E. Felder, D. Pitre, and S. Boveri, *Z. physiol. Chem.*, 1970, **351**, 943.
[106] J. P. Jennings, W. Klyne, and P. Scopes, *J. Chem. Soc.*, 1965, 294.

identical with that obtained from the hexahydropyrimidine derivative (37; R = H) derived from L-glutamic acid. The absolute chirality of stendomycidine is therefore as shown in (5)[14] and corresponds to that of capreomycidine and viomycidine at the α- and β-centres. The β-methyl-aspartic acid derived from the antibiotic amphomycin has been assigned the L-*threo*-configuration[107] and *N*-malonylmethionine, present in tobacco plants, is tentatively assigned the D configuration on the basis of the selective incorporation of D-methionine.[108]

Enzymic deacylation of the *N*-acetyl derivatives of ω-fluoro-DL-isoleucine and ω-fluoro-DL-*allo*-isoleucine affords the respective L-isomers which can be readily transformed into the corresponding *cis*-3-methyl-L-proline (38)



(38)

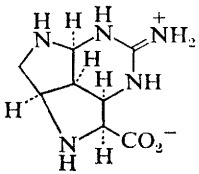


(39)

and *trans*-3-methyl-L-proline (39).[53] These are readily distinguished by their n.m.r. spectra, thus allowing a complete stereochemical assignment. A similar application of n.m.r. spectroscopy and enzymic resolution has allowed configurational assignments to be made to all the isomers of β-methyl-leucine and β-methylnorleucine.[109]

B. Crystal Structures of Amino-acids.—(See also Chapter 2, Part II, Section 2.) The crystal structures of L-isoleucine,[110] L-valine,[111] L-arginine hydrochloride,[112] DL-histidine hydrochloride,[113] 3,4-dihydroxy-L-phenyl-alanine[114] (L-dopa), and 5-hydroxy-DL-tryptophan,[115] as well as the derivatives *N*-chloroacetyl-DL-alanine[116] and *O*-phosphoryl-DL- and -L-serine,[117] have been published. A further *X*-ray analysis of the basic amino-acid viomycidine[12] and the details of the analysis of viocidic acid (40),[118] the other basic component isolated from the hydrolysate of the antibiotic viomycin, have been reported. The proposed structure and

[107] M. Bodanszky and G. G. Marconi, *J. Antibiotics*, 1970, **23**, 238.
[108] B. Ladešić, M. Pokorny, and D. Keglević, *Phytochemistry*, 1970, **9**, 2105.
[109] K. Okubi and Y. Izumi, *Bull. Chem. Soc. Japan*, 1970, **43**, 1541.
[110] B. Khawas, *Acta Cryst.*, 1970, **26B**, 1385.
[111] K. Torii and Y. Iitaka, *Acta Cryst.*, 1970, **26B**, 1317.
[112] J. Dow, L. H. Jensen, S. K. Mazumdar, R. Srinivasan, and G. N. Ramachandran, *Acta Cryst.*, 1970, **26B**, 1662.
[113] I. Bennett, A. G. H. Davidson, M. M. Harding, and I. Morelle, *Acta Cryst.*, 1970, **26B**, 1722.
[114] A. Mostad, T. Ottersen, and C. Romming, *Acta Chem. Scand.*, 1970, **24**, 1864.
[115] A. Wakahara, M. Kido, T. Fujiwara, and K. Tomita, *Tetrahedron Letters*, 1970, 3003.
[116] F. E. Cole, *Acta Cryst.*, 1970, **26B**, 622.
[117] M. Sundaralingam and E. Putkey, *Acta Cryst.*, 1970, **26B**, 782.
[118] P. Coggon, *J. Chem. Soc.* (*B*), 1970, 838.



(40)

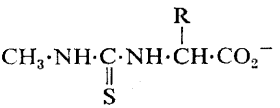
stereochemistry of the proline derivative obtained from diatom cell walls have been confirmed by *X*-ray crystallographic analysis.[119]

C. Optical Rotatory Dispersion (o.r.d.) and Circular Dichroism (c.d.).— (See also Chapter 2, Part III, Section 3B.) It has been confirmed that α-amino- and α-hydroxy-acids of the L-configuration exhibit, in addition to the strong positive c.d. maximum at 210—215 nm which is used for configurational assignments, a weak, negative, long-wavelength band at 235—240 nm.[120] The former is due to the n–π^* transition of the carboxy-group and the latter is attributed to the coupling of the non-bonding heteroatom with the chromophoric transition of the carbonyl,[121, 122] and not to intramolecular or intermolecular hydrogen-bonding as originally supposed. The fact that the hydrochlorides of L-α-amino-esters lack the weak, negative, long-wavelength band provides support for this proposal.[123]

The c.d. spectra of a number of α-methylamino-acids have been measured in acidic, alkaline, and neutral media and it appears probable that for the simple neutral amino-acids the shorter wavelength band can be used for configurational assignments.[124] Further work on the pH dependence of a number of aromatic amino-acids has led to the suggestion that the 220 nm band is due to the interaction of one of the transitions associated with the benzene ring and the π–π^* transition of the carbonyl, and not merely to a summation of the contributions of each chromophore.[125] *N-o*-Nitrobenzoyl derivatives of α-amino-acids exhibit a negative Cotton effect centred at 350 nm and it is claimed that they can be used for configurational assignments.[126]

In weakly alkaline solution α-amino-acids react with methyl isothiocyanate to give the derivatives (41). The c.d. data of the *N*-methylthiocarbamyl derivatives of all the common amino-acids with the L configuration exhibit positive Cotton effects centred around 260 nm.[127] These are

[119] I. L. Karle, *Acta Cryst.*, 1970, **26B**, 765.
[120] C. Toniolo, *J. Phys. Chem.*, 1970, **74**, 1390.
[121] J. C. Craig and W. E. Pereira, *Tetrahedron*, 1970, **26**, 3457.
[122] G. Barth, W. Voelter, E. Bunnenberg, and C. Djerassi, *Chem. Comm.*, 1969, 355.
[123] J. C. Craig and W. E. Pereira, *Tetrahedron Letters*, 1970, 1563.
[124] S. Yamada, K. Achiwa, S. Terashima, H. Mizuno, N. Takamara, and M. Legrand, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 2608.
[125] N. Sakota, K. Okita, and Y. Matsui, *Bull. Chem. Soc. Japan*, 1970, **43**, 1138.
[126] U. Nagai and M. Kurumi, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 831.
[127] C. Toniolo, *Tetrahedron*, 1970, 5479.



(41)

unaffected by the presence of a second chiral centre or a further dichroic centre. This method would appear to offer a very facile means of determining the configuration of amino-acids derived from microbial peptides. A similar, but more limited, approach employing isothiocyanate derivatives has also been reported.[128]

D. Nuclear Magnetic Resonance (n.m.r.) Spectra.—(See also Chapter 2, Part III, Section 4B.) The literature on the n.m.r. spectra of amino-acids has been critically reviewed[129] in an extremely valuable article which covers analysis and configurational determination, as well as the n.m.r. data of various derivatives including metal complexes. A detailed investigation of chemical shifts and spin–spin coupling constants of amino-acids, in relation to studies on peptide conformation, has been described[130] and rotational isomerism, as determined by variation of the vicinal couplings, has been shown to be dependent on solute–solvent and solute–solute interactions.[131]

As part of a more general programme related ultimately to the study of polypeptides, the ^{13}C n.m.r. spectra of ^{13}C-enriched amino-acids have been determined. The ^{13}C nuclei were noise decoupled from protons and the spread in the chemical shifts is sufficiently large for unambiguous assignment.[132] Interest in the application of ^{15}N n.m.r. data of amino-acids continues[133] and the vicinal coupling constants have been used to estimate conformer populations.[134] In addition to these more esoteric applications of n.m.r. spectroscopy, the technique is now generally employed for characterisation of new amino-acids and is particularly important in differentiating between diastereoisomers (see, *e.g.*, references 12, 14, 53, 66).

E. Mass Spectrometry.—The value of mass spectrometry as a structural tool appears to be increasing and it has been applied to at least two new naturally occurring amino-acids (see reference 10). Protection of amino-acids as either the *N*-trifluoroacetyl ester[135] or *N*-trimethylsilyl ester[136] continues to be the preferred method, but perhaps the most interesting

[128] B. Halpern, W. Patton, and P. Crabbé, *J. Chem. Soc.* (*B*), 1969, 1143.
[129] J. Rowe, M. Julian, J. Hinton, and K. L. Rowe, *Chem. Rev.*, 1970, **70**, 1.
[130] M. Nagai, A. Nishioka, and J. Yoshimura, *Bull. Chem. Soc. Japan*, 1970, **43**, 1323.
[131] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 1970, **92**, 1488.
[132] W. J. Horsley, H. Sternlicht, and J. Cohen, *J. Amer. Chem. Soc.*, 1970, **92**, 680.
[133] R. L. Lichter and J. D. Roberts, *Spectrochim. Acta*, 1970, **26**, 1813.
[134] R. L. Lichter and J. D. Roberts, *J. Org. Chem.*, 1970, **35**, 2806.
[135] M. S. Manhas, R. S. Hsieh, and A. K. Bose, *J. Chem. Soc.* (*C*), 1970, 116.
[136] K. Bergstrom, J. Gurtler, and R. Bloomstrand, *Analyt. Biochem.*, 1970, **34**, 74.

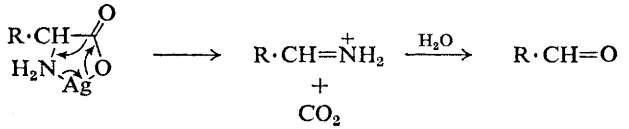
development in this area is the application of chemical ionisation mass spectrometry.[137] In this method ion formation is effected by protonation rather than loss of an electron. Consequently the resulting even-electron species is relatively stable and the quasimolecular ion at *m/e* ($M + 1$) is the most intense in the spectrum, except in the cases of glutamic acid or ornithine where cyclisation occurs, with loss of water and ammonia respectively. The technique could possibly be valuable for molecular weight determination since it avoids the inconvenient protection of amino-acids. A novel differentiation of *meso*- and racemic diaminopimelic acid, which involves an initial preferential thermal dehydration of the racemic form, has been reported[138] and it is suggested that the method can be extended to other diamino-acids. The mass spectra of trimethylsilyl derivatives of deuteriated[139] and ^{13}C-enriched[140] amino-acids have been determined, in relation to their possible application in biosynthetic studies.

F. Other Physical and Stereochemical Studies.—The internal rotation in crystalline glycine has been estimated from heat capacity data[141] and the i.r. spectrum of matrix-isolated glycine supports the suggestion that the molecules are not in the zwitterion form in this state.[142] The conformations of a number of amino-acids have been calculated theoretically using extended MO theory.[143] These studies come within the framework of a broader approach to the conformation of polypeptides. The dissociation constants in deuterium oxide of several amino-acids have been determined[144] and a detailed study on the racemisation of α-amino-acids and their derivatives in acetic acid has been reported.[145–147]

4 Chemical Studies of Amino-acids

A. Oxidation and Reduction.—Oxidation of α-amino-acids by silver(II) picolinate gives almost quantitative yields of the nor-aldehyde, while the action of silver(II) oxide affords, in most cases, the nor-acid.[148] The α-keto-acid is not an intermediate in these reactions and the mechanism outlined in Scheme 4 has been proposed. Oxidation of α-amino-esters, under the

[137] G. W. A. Milne, T. Axenrod, and H. M. Fales, *J. Amer. Chem. Soc.*, 1970, **92**, 5170.
[138] H. Falter, M. Madaiah, and R. A. Day, *Tetrahedron Letters*, 1970, 4463.
[139] W. J. A. van den Heuvel, J. L. Smith, I. Patter, and J. S. Cohen, *J. Chromatog.*, 1970, **50**, 405.
[140] W. J. A. van den Heuvel, J. L. Smith, and J. S. Cohen, *Biochim. Biophys. Acta*, 1970, **208**, 251.
[141] R. C. J. Li and N. S. Berman, *J. Phys. Chem.*, 1970, **74**, 1643.
[142] Y. Grence, J. C. Lasseques, and C. Carrigou-Laqrange, *J. Chem. Phys.*, 1970, **53**, 2980.
[143] J. M. George and L. B. Kier, *Experientia*, 1970, **26**, 952.
[144] I. N. Gordon and B. M. Lowe, *Chem. Comm.*, 1970, 803.
[145] M. Satō, T. Tatsuno, and H. Matsuo, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1794.
[146] H. Matsuo, Y. Kawazoe, M. Satō, M. Ohnishi, and T. Tatsuno, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1788.
[147] M. Satō, T. Tatsuno, and H. Matsuo, *J. Pharm. Soc. Japan*, 1970, **90**, 1160.
[148] T. G. Clarke, N. A. Hampson, J. B. Lee, J. R. Morley, and B. Scanlon, *J. Chem. Soc. (C)*, 1970, 815.



Scheme 4

same conditions, yields the corresponding α-keto-esters. Alternatively, electrochemical oxidation of α-amino-acids at a silver electrode in aqueous electrolytes gives the corresponding nor-nitriles and small amounts of the nor-aldehyde.[149] In this case it is argued that the intermediate imine is formed and further oxidation to the nitrile occurs before it is released from the electrode surface.

Kinetic studies on oxidation using cobalt(III) in aqueous perchloric acid solution have not provided any evidence for the formation of chelate complexes in the course of reaction, and a radical mechanism is proposed for the formation of the nor-aldehyde.[150] Kinetic data have also been reported on the uncatalysed oxidation of glycine by potassium persulphate.[151] *N*-Bromosuccinimide oxidises aspartic acid to the corresponding nor-aldehyde, which then undergoes α-bromination and decarboxylation to give bromoform in good yield.[152] *N*-Acyl-β-hydroxyamino-acids (42) are cleaved on oxidation with lead tetra-acetate to *N*-acyl-hydroxyglycine

(42) (43)

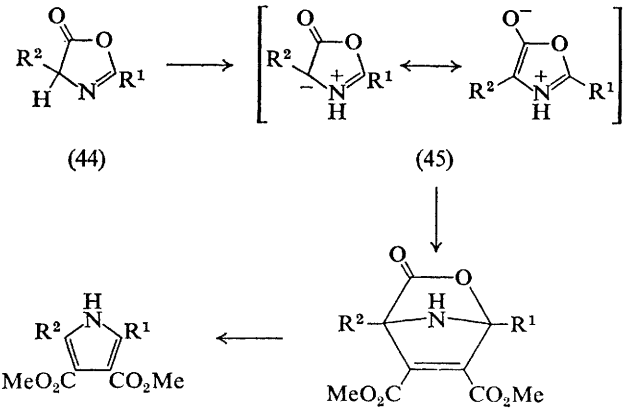
derivatives (43). The yields are high and this reaction offers a simple route to this interesting class of compounds.[153] A new general route has also been devised for the synthesis of *N*-phenylacetyl-β-alkoxyglycine derivatives by the reaction of 2-benzylidene pseudo-oxazolone with various alcohols.[154]

Reduction of α-amino-amides and esters with lithium aluminium hydride under a variety of conditions affords both primary alcohols and aldehydes in varying ratios,[155] whereas reduction of tertiary amides of amino-acids with sodium borohydride in pyridine gives the corresponding diamines in moderate yield.[156]

[149] N. A. Hampson, J. B. Lee, K. I. MacDonald, and M. J. Shaw, *J. Chem. Soc.* (*B*), 1970, 1766.
[150] R. A. Sheikh and W. A. Waters, *J. Chem. Soc.* (*B*), 1970, 988.
[151] K. Kumar and L. K. Saxena, *J. Indian. Chem. Soc.*, 1970, **47**, 435.
[152] W. L. Parker, C. Aklonis, and J. A. Last, *Experientia*, 1970, **26**, 242.
[153] W. Oettmeier, *Chem. Ber.*, 1970, **103**, 2314.
[154] G. Lucente, G. M. Lucente, F. Pantonella, and A. Romeo, *Ann. Chim.* (*Italy*), 1970, **60**, 259.
[155] M. P. Duhamel, L. Duhamel, and P. Siret, *Compt. rend.*, 1970, **270**, *C*, 1750.
[156] I. Saitō, Y. Kikugawa, and S. Yamada, *Chem. and Pharm. Bull.* (*Japan*), 1970, **18**, 1731.

B. General Reactions.—The literature on the protonation of amino-acids in strong acid solutions has been reviewed.[157] In superacids (*e.g.* fluorosulphonic acid–antimony pentafluoride) protonation of both the amino- and carboxy-functions occurs as well as at other basic sites in the molecule.[158] The protonated species have been studied by n.m.r. spectroscopy and are generally quite stable, unlike aliphatic carboxylic acids, which dehydrate to give the corresponding oxo-carbonium ions. The *N*-nitroso-derivatives of a number of common secondary amino-acids have been prepared in high yield under conditions approximating to those found in the mammalian stomach.[159] Evidence concerning the populations of the *syn*- and *anti*-conformers of these nitroso-amino-acids is derived from n.m.r. data. A previous report that the reaction of glucose with amino-acids affords nitrosamines has been refuted by a very extensive analytical investigation of the reaction products.[160]

α-Amino-acids continue to act as convenient starting materials for heterocyclic syntheses. The azlactones (44), prepared *in situ* from the corresponding *N*-acylamino-acid, react readily with dimethyl acetylenedicarboxylate to give substituted pyrrole-3,4-dicarboxylates.[161] The reaction proceeds as outlined in Scheme 5 *via* a 1,3-dipolar cycloaddition of the dimethyl acetylenedicarboxylate to the tautomeric oxazolium-5-oxide (45). An extensive investigation of this general type of reaction, employing a variety of dipolarophiles and azlactones, has been reported.[161–164] In

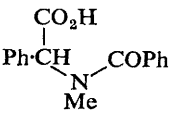


(44) (45)

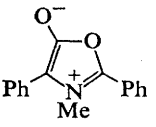
Scheme 5

[157] G. A. Olah, A. M. White, and D. H. O'Brien, *Chem. Rev.*, 1970, **70**, 561.
[158] G. A. Olah, D. L. Brydon, and R. H. Schlosberg, *J. Org. Chem.*, 1970, **35**, 317.
[159] W. Lijinsky, L. Keefer, and J. Loo, *Tetrahedron*, 1970, **26**, 5137.
[160] K. Heyns and H. Koch, *Tetrahedron Letters*, 1970, 741.
[161] H. O. Bayer, H. Gotthardt, and R. Huisgen, *Chem. Ber.*, 1970, **103**, 2356.
[162] R. Huisgen, H. Gotthardt, and H. O. Bayer, *Chem. Ber.*, 1970, **103**, 2368.
[163] H. O. Bayer, R. Huisgen, R. Knorr, and F. C. Schaefer, *Chem. Ber.*, 1970, **103**, 2581.
[164] R. Huisgen, H. Gotthardt, H. O. Bayer, and F. C. Schaefer, *Chem. Ber.*, 1970, **103**, 2611.

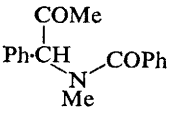
relation to these reactions, the mechanism of the Dakin–West reaction has also come under scrutiny.[165, 166] Reaction of (46) with acetic anhydride containing appreciable amounts of acetic acid yields (47) which then



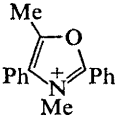
(46)



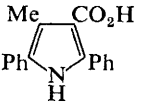
(47)



(48)



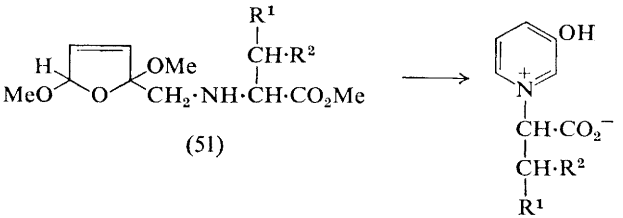
(49)



(50)

affords the normal Dakin–West product (48). However, if the reaction is conducted in acetic anhydride with a very low concentration of acetic acid a number of other products are formed, including the oxazolium salt (49) and the pyrrolecarboxylic acid (50). A reaction sequence involving acetylation of (47) followed by nucleophilic ring-opening is proposed to account for these products.[166]

N-Furfuryl-amino-acids have been synthesised by reductive alkylation of amino-acids using furfural. These compounds, on electrolytic oxidation in alcohol, yield compounds of the type (51), which readily rearrange in

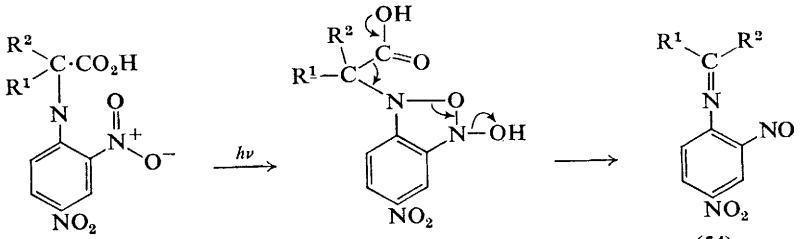


(51) (52)

acid solution to give the novel pyridinium derivative (52) of the starting amino-acid.[167] Treatment of *N*-benzoylserine methyl ester with phosgene gives the predicted oxazolidine in good yield.[168]

[165] N. I. Aronova, N. N. Makhova, and S. I. Zavialov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 1835.
[166] R. Knorr and R. Huisgen, *Chem. Ber.*, 1970, **103**, 2598.
[167] K. Unaheim and M. Gacek, *Acta Chem. Scand.*, 1969, 2488, 2475.
[168] T. Invi, S. Tanaka, and M. Takino, *Bull. Chem. Soc. Japan*, 1970, **43**, 1582.

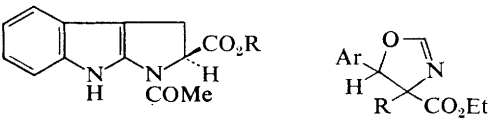
The use of orthoesters is still attracting considerable attention both for the acylation and esterification of amino-acids.[169] It is now possible to prepare the diazoketones from *N*-benzyloxycarbonyl or *N*-t-butoxycarbonyl amino-acids by the reaction of the mixed anhydride or carbodiimide adduct with diazomethane.[170] Previous attempts to prepare these compounds *via* the acid chloride had been unsuccessful. The suggested key step in the photolytic breakdown of *N*-2,4-dinitrophenyl derivatives of amino-acids is the formation of the intermediate (53) which then undergoes



(53) (54)

ring-opening and decarboxylation to (54). This intermediate can yield either 2-nitroso-4-nitroaniline and an aldehyde, or the benzimidazole-*N*-oxide, which are known products of photolysis, depending on the conditions of the reaction.[171]

C. Specific Reactions.—Oxidation of *N*-acetylated derivatives of tryptophan with t-butyl hypochlorite gives the acid-labile indole compounds (55), the cyclisation presumably occurring *via* the β-halogeno-indolenine.[172] Hydrogenolysis of aryl-2-oxazolines of the type (56) affords *N*-formylphenylalanine ethyl esters in good yield. Only a preliminary account of this work



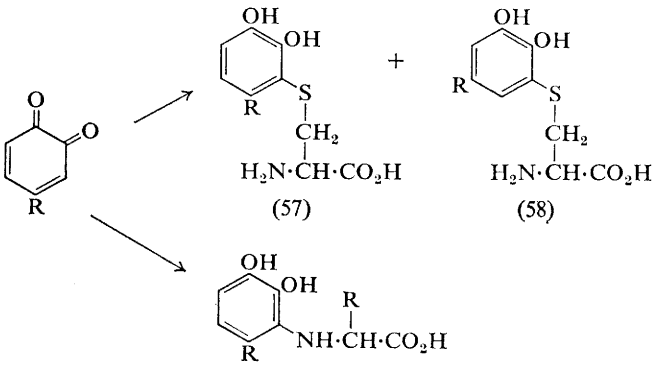
(55) (56) R = H
R = Me

has been published but it appears to offer an interesting new route to phenylalanines.[173] Interest in the reaction of L-cysteine with carbonyl compounds continues,[174] and several new thiazolidines have been prepared by the condensation of cysteine with monosaccharides.[175] Reduction of

[169] S. V. Rogozhin, Y. A. Davidovich, and V. V. Korshak, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 727, 956, 2858.
[170] B. Penke, J. Czombos, L. Baláspiri, J. Petres, and K. Kovács, *Helv. Chim. Acta*, 1970, **53**, 1057.
[171] O. Meth-Cohn, *Tetrahedron Letters*, 1970, 1235.
[172] M. Ohno, T. F. Spande, and B. Witkop, *J. Amer. Chem. Soc.*, 1970, **92**, 343.
[173] U. Schöllkopf and D. Hoppe, *Angew. Chem.*, 1970, **82**, 459.
[174] N. Hellström, S. Almqvist, and M. Aamisepp, *J. Chem. Soc.* (*B*), 1969, 1103.
[175] R. Bognar, L. Somogyi, and Z. Gyorgydeak, *Annalen*, 1970, **738**, 68.

α-nitro-acrylates [176] and condensation of an amide with an α-keto-acid [177] are still the main sources of dehydro-amino-acids.

D. Non-enzymic Models of Biochemical Processes Involving Amino-acids.— The reactions of *o*-quinones with amino-acids continue to attract attention in relation to the biosynthesis of products resulting from quinone–amino-acid and quinone–protein interactions. It is now fairly well established that phaeomelanins are formed *in vivo* by the 1,6-addition of cysteine to dopaquinone produced by enzymic oxidation of tyrosine.[178, 179] The first step is stated to involve the formation of 2-*S*-cysteinyldopa (57) and 5-*S*-cysteinyldopa (58) in the ratio 95 : 5 (Scheme 6) which then undergo oxidative cyclisation. These proposals are supported by the observation that the model compound (59) is oxidised by oxygen in buffered solution to give a high yield of the dihydrobenzothiazine (60).[180] It is claimed that the *o*-quinone produced by oxidation of caffeic acid reacts with the α-amino-group of amino-acids [181] (Scheme 6). The *in vitro* oxidation of L-dopa with



Scheme 6

p-benzoquinone under physiological conditions leads to the same products as are obtained from inorganic oxidants and phenol oxidase.[182]

The interaction of amino-acids with pyridoxal can be conveniently followed by n.m.r. spectroscopy and appears to be a valuable probe for examining structure and equilibria, as well as the reactivity of the azomethine bond towards various functional groups in polyfunctional amino-acids.[183] The formation of a thiazolidine derivative from cysteine is readily observed, as is the formation of a bis-Schiff-base by homocystine.

[176] C. Shin, M. Masaki, and M. Ohta, *Bull. Chem. Soc. Japan*, 1970, **43**, 3219.
[177] A. Kaneda and R. Sudo, *Bull. Chem. Soc. Japan*, 1970, **43**, 2159.
[178] G. Mizuraca, R. A. Nicolaus, G. Prota, and G. Ghiara, *Experientia*, 1969, **25**, 920.
[179] L. Minale, E. Fattorusso, S. De Stefano, and R. A. Nicolaus, *Gazzetta*, 1970, **100**, 461.
[180] G. Prota, S. Crescenzi, G. Misuraca, and G. A. Nicolaus, *Experientia*, 1970, **26**, 1058.
[181] C. H. Brieskorn and A. Mosande, *Tetrahedron Letters*, 1970, 109.
[182] A. Hikosaka and J. Kumanotani, *Bull. Chem. Soc. Japan*, 1970, **43**, 2620.
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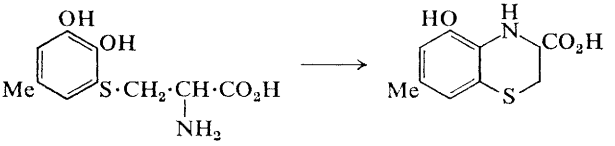
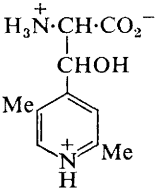


(59) (60)

Cystine is known to degrade when it reacts with pyridoxal phosphate, giving thiocysteine, ammonia, and pyruvic acid, but homocystine is stable under these conditions, giving, as observed by n.m.r. spectroscopy, a bis-Schiff-base.[184]

Transamination reactions conducted with 1-methyl-3-hydroxy-4-formylpyridinium chloride indicate that quaternisation of the ring nitrogen in pyridoxal models markedly increases the amount of transamination.[185] The decarboxylation of aminomalonic acid has been studied as a function of pH and in the presence of 5-deoxypyridoxal.[186] In acid solution, (61)

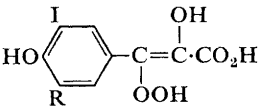


(61)

is formed by the interaction of aminomalonic acid with two molecules of 5-deoxypyridoxal.

The non-enzymic addition of ammonia to fumaric acid has been shown to be non-stereospecific, unlike the enzymic reaction.[187]

The hydroperoxide (62) is still regarded as the precursor of thyroxine in non-enzymic model systems, involving the oxidative coupling of di-iodotyrosine. Further work supports this claim and indicates that (63) is the precursor of the other thyroid hormone, 3,5,3′-tri-iodothyronine.[188]

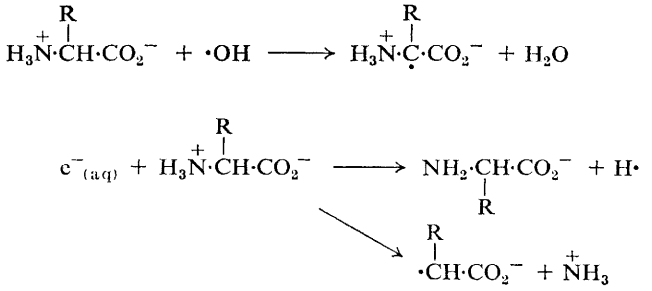


(62) R = H
(63) R = I

[184] A. Rinaldi and C. De Marco, *Arch. Biochem. Biophys.*, 1970, **138**, 697.
[185] J. R. Maley and T. C. Bruice, *Arch. Biochem. Biophys.*, 1970, **136**, 187.
[186] J. W. Thanassi, *Biochemistry*, 1970, **9**, 525.
[187] J. L. Bada and S. L. Miller, *J. Amer. Chem. Soc.*, 1970, **92**, 2774.
[188] H. J. Cahanmann and K. Funakoshi, *Biochemistry*, 1970, **9**, 90.

A direct conversion of di-iodotyrosine to thyroxine can be achieved by aerial oxidation in the presence of glyoxylic acid and copper acetate.[189] It is suggested that 4-hydroxy-3,5-di-iodophenylpyruvic acid is formed *in situ* by a transamination reaction with the glyoxylic acid and that this is oxidised to the intermediate (62). A kinetic investigation of the iodination of tyrosine suggests that a non-polar environment favours di-iodination relative to mono-iodination and it is concluded that this may be significant in relation to the internal environment of thyroglobin.[190]

E. Effects of Electromagnetic Radiation on Amino-acids.—There continues to be much activity in research on the effect of ionising radiation on amino-acids, and the observed radiolytic products and postulated reaction mechanisms have recently been reviewed.[191] Although a number of studies in the solid state have been reported,[192–195] the emphasis again remains on reactions in solution. For radiolysis of neutral amino-acids in aqueous solution, the products formed by their reaction with (·OH) radicals and hydrated electrons and the presumed mechanistic pathways are shown in Scheme 7. An e.s.r. study of the deamination step by hydrated electrons

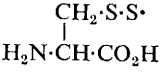


Scheme 7

has been reported.[196] Irradiation with high-energy electrons was carried out directly in the e.s.r. cavity and either ethyl formate or methanol was used as an (·OH) radical scavenger in order to eliminate its reacting with the amino-acids. General confirmation of the pathways outlined in Scheme 7 has been obtained from the transient optical absorption spectra of the intermediates in the dehydrogenation and deamination steps.[197]

[189] T. Shiba, M. Kajiwara, Y. Kato, K. Inoue, and T. Kaneko, *Arch. Biochem. Biophys.*, 1970, **140**, 90.
[190] W. E. Mayberry and T. J. Hockert, *J. Biol. Chem.*, 1970, **245**, 697.
[191] W. M. Garrison in 'Current Topics in Radiation Research', ed. M. Ebert and A. Howard, North Holland, Amsterdam, 1968, p. 43.
[192] V. G. Pasoyan, M. K. Pulatova, and L. P. Kavush, *Biofizika*, 1970, **15**, 12.
[193] V. G. Krivenko and M. K. Pulatova, *Biofizika*, 1969, **14**, 986.
[194] T. M. Chen, *Photochem. and Photobiol.*, 1970, **12**, 81.
[195] M. D. Sevilla, *J. Phys. Chem.*, 1970, **74**, 2096.
[196] P. Neta and R. W. Fessenden, *J. Phys. Chem.*, 1970, 2263.
[197] P. Neta, M. Simic, and E. Hayon, *J. Phys. Chem.*, 1970, **74**, 1214.

The radiolysis of aqueous solutions of cysteine and of cystine, and of phenylalanine and tyrosine, involves qualitatively different reactions from those outlined, due to the comparatively higher reactivity of the thiol group and the benzene ring towards hydroxyl radicals, and of sulphide and thiol group towards hydrated electrons. Several further studies on cysteine and cystine have been reported.[198–200] The radiolysis products of these amino-acids have been attributed to the breakdown or reaction of the sulphur radical of cysteine, but evidence for the participation of the radical (64) in the radiolysis of cystine has now been given.[201]



(64)

Chemically produced hydroxyl radicals continue to be used to simulate the effects of radiation. The generation of hydroxyl radicals (by the reaction of titanous chloride with hydrogen peroxide) in the presence of amino-acids leads to the same free radicals as are formed by radiolysis, and these can be conveniently studied by e.s.r. spectroscopy.[202, 203] The free radicals produced in the reaction between amino-acids and peptides with ninhydrin are very much dependent on the water and oxygen concentrations in the reaction mixture. Analysis of the e.s.r. spectra reveals hyperfine structure which, it is suggested, permits their possible use in identifying certain amino-acids and peptides.[204]

The mechanism for the photochemical addition of L-cysteine to uracil is believed to proceed through the triplet excited state of uracil, which can abstract an hydrogen atom from cysteine to form (65) (see Scheme 8).[205] The photo-oxidation of methionine to methionine oxide has been investigated using acetone[206] and proflavine[207] as sensitizers. In the case of proflavine it is suggested that the triplet state of the sensitizer is an intermediate and a mechanism is proposed in which methionine reacts with the first singlet state of oxygen, produced by energy transfer from the triplet sensitizer. A detailed scheme for the sensitized photo-oxidation of histidine

[198] D. Giles and D. W. Grant, *Chem. and Ind.*, 1970, 1437.
[199] L. I. Grossweiner and Y. Usui, *Photochem. and Photobiol.*, 1970, **11**, 53.
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[201] C. J. Dixon and D. W. Grant, *J. Phys. Chem.*, 1970, **74**, 941.
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[203] P. Smith, W. M. Fox, D. J. McGinty, and R. D. Stevens, *Canad. J. Chem.*, 1970, **48**, 480.
[204] V. P. Yuferov, W. Froncisz, I. G. Kharitonenkov, and A. E. Kalmanson, *Biochem. Biophys. Acta*, 1970, **200**, 160.
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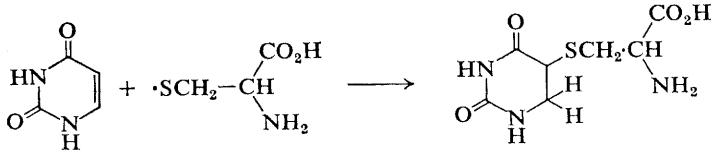
[206] G. Gennari and G. Jori, *F.E.B.S. Letters*, 1970, **10**, 129.
[207] G. Jori and G. Cauzzo, *Photochem. and Photobiol.*, 1970, **12**, 231.



(65)

Scheme 8

and *N*-benzoylhistidine to aspartic acid and urea has been proposed and substantiated to a considerable degree by the isolation of a number of the proposed intermediates.[208]

5 Analytical Methods

The number of papers on the subject of amino-acids which are devoted to analytical methods continues to run at approximately a quarter of the total published. As pointed out in previous Reports, these mainly cover modifications of established techniques or the determination of specific amino-acids under certain conditions (*e.g.* amino-acids in biological fluids). The improvements and developments in amino-acid analysis in relation to structural studies of proteins and peptides are also covered elsewhere in this Report (Chap. 2, Part I, Section 2A). Therefore the majority of references will be presented under the appropriate heading without discussion, and only a few advances of general interest will be dealt with more fully.

A. Gas–Liquid Chromatography.—The application of g.l.c. for amino-acids protected either as the *N*-trifluoroacetyl n-butyl esters or the trimethylsilyl derivatives is now a well-established technique and is being used more often for routine analysis.[209–211] Per(methylsilyl) derivatives have been employed extensively [212–214] and the various methods of silylation have been reviewed.[215] The difficulties imposed by injection of the derivatives onto the column while in solution have to some extent been circumvented either by direct solid injection [216] or by preparation of the derivative on a solid support followed by direct introduction on to the column.[217] The separation of enantiomers of racemic *N*-trifluoroacetyl-α-amino-esters by g.l.c. has been described earlier, but several more detailed accounts have

[208] M. Momita, M. Irie, and T. Ukita, *Biochemistry*, 1969, **8**, 5149.
[209] D. J. Casagrande, *J. Chromatog.*, 1970, **49**, 537.
[210] R. W. Zumwalt, D. Roach, and C. W. Gehrke, *J. Chromatog.*, 1970, **53**, 195.
[211] W. Parr, C. Yang, J. Pleterski, and E. Bayer, *J. Chromatog.*, 1970, **50**, 510.
[212] C. W. Gehrke, H. Nakamoto, and R. Zumwalt, *J. Chromatog.*, 1969, **45**, 24.
[213] C. W. Gehrke and K. Leimer, *J. Chromatog.*, 1970, **53**, 195.
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[216] A. Darbre and A. Islam, *J. Chromatog.*, 1970, **49**, 293.
[217] B. Teuwissen and A. Darbre, *J. Chromatog.*, 1970, **49**, 298.

been reported.[218–220] A further study on g.l.c. separation of methylthiohydantoins has been presented.[221]

B. Ion-exchange Chromatography.—Hydrolysis of proteins with a mixture of oxalic and hydrochloric acids in a sealed tube is claimed to be more efficient than the classical procedure, and analysis can be conducted without initial neutralisation.[222] A large number of improvements in instrumentation and techniques,[223–226] as well as alternative buffer systems,[227–229] for automatic amino-acid analysers have been described. The ninhydrin colour-constant for *N*-methylamino-acids can be increased if the eluting buffer flow is slowed down, and the optical purity can be established by analysing the diastereoisomeric dipeptides obtained by coupling L-alanine *N*-carboxy-anhydride with the *N*-methylamino-acid.[229] The increasing application of computers for handling automatic analysis data is widely apparent and the whole area has been critically reviewed.[230] Alternative internal standards for amino-acid analysis have been reported.[231]

C. Thin-layer Chromatography.—An English translation of a well-known handbook on the t.l.c. of amino-acids is now available.[232] A new method of detection of amino-acids and amines on thin-layer chromatograms uses the fact that primary amines readily condense with 2,5-dimethoxytetrahydrofuran to yield *N*-substituted pyrroles which with *p*-dimethylaminobenzaldehyde in acid solution give an intense violet-red colour.[233]

A further modification of the Dragendorff reaction [234] for the visualisation of amino-acids and detailed investigations on techniques for rapid separation with various absorbents and detection with various reagents have been reported.[235, 236] Other papers on t.l.c. have described an improved technique for determining dansyl derivatives,[237] iodo-amino-acids,[238] and

[218] S. Nakaparkan, P. Birrell, E. Gil-Av, and J. Oro, *J. Chromatog. Sci.*, 1970, **8**, 177.
[219] W. A. Koenig, W. Parr, H. A. Lichenstein, E. Bayer, and J. Oro, *J. Chromatog. Sci.*, 1970, **8**, 193.
[220] W. Parr, C. Yang, J. Pleterski, and E. Bayer, *J. Chromatog.*, 1970, **50**, 510.
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[224] S. Jacobs, *Analyst*, 1970, **95**, 370.
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[227] R. G. Redman, *J. Chromatog.*, 1970, **46**, 107.
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[229] J. R. Coggins and N. L. Benoiton, *J. Chromatog.*, 1970, **52**, 251.
[230] B. Sheldrick, *Quart. Rev.*, 1970, **24**, 454.
[231] J. F. Cavins and M. Friedman, *Analyt. Biochem.*, 1970, **35**, 489.
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[233] P. Haefelfinger, *J. Chromatog.*, 1970, **48**, 184.
[234] E. Tyihak and D. Vagujfalvi, *J. Chromatog.*, 1970, **49**, 343.
[235] J. G. Heathcote, R. J. Washington, C. Haworth, and S. Bell, *J. Chromatog.*, 1970, **51**, 267.
[236] D. T. N. Pillay and R. Mehdri, *J. Chromatog.*, 1970, **47**, 119.
[237] P. Nedkov and K. Gaucev, *Pharmazie*, 1970, **25**, 159.
[238] C. Wu and R. C. Ling, *Analyt. Biochem.*, 1970, **37**, 313.

peptide hydrazides.[239] A preliminary account of the separation of ^{14}C-labelled amino-acids and their detection by autoradiography on indium oxide plates indicates that this could be a valuable technique for the separation of very small amounts.[240]

D. Other Methods.—High-voltage electrophoresis continues to be a valuable analytical technique.[241] A new electrolytic procedure for the detection of L-amino-acids, employing an electrode covered with L-amino-acid oxidase, has been developed.[242] A preliminary account of the possible application of isotachophoresis of amino-acids in the presence of formaldehyde suggests that the technique may have some qualitative application.[243] Many other topics have been discussed, including fluorimetric determinations,[244] Sephadex chromatography,[245] and ion-exchange paper electrophoresis.[246]

E. Determination of Specific Amino-acids.—Papers on the determination of the following amino-acids have appeared: L-leucine,[247] L-phenylalanine,[248] L-glutamic acid,[249] L-tryptophan,[250] arginine,[251, 252] cystine,[253] hydroxylysine,[254] and lysine.[255]

[239] H. J. Goren and M. Fridkin, *J. Chromatog.*, 1970, **47**, 519.
[240] E. Cremer and E. Seidl, *Monatsh.*, 1970, **101**, 1614.
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[245] A. D. Williams, D. E. Freeman, and W. H. Florsheim, *J. Chromatog.*, 1969, **45**, 391.
[246] E. Selegny, J. C. Fenyo, G. Broun, F. Matray, and C. De Bernady, *J. Chromatog.*, 1970, **47**, 552.
[247] S. Pande and M. L. Verma, *Microchem. J.*, 1970, **15**, 6.
[248] E. Jellum, V. A. Close, W. Patton, W. Pereira, and B. Halpern, *Analyt. Biochem.*, 1969, **31**, 227.
[249] A. Zamorani and G. Roda, *J. Chromatog.*, 1970, **47**, 261.
[250] F. Lingens and B. Sprössler, *Analytische Chemie*, 1970, **252**, 232.
[251] C. Saxena, *Microchem. J.*, 1970, **15**, 391.
[252] K. Bahadur and M. H. Verma, *Microchem. J.*, 1969, **14**, 547.
[253] P. Grisonni, *Biochim. appl.*, 1970, **15**, 227.
[254] E. Moczar and M. Moczar, *J. Chromatog.*, 1970, **54**, 277.
[255] O. A. M. Lewis and B. M. G. Shanley, *J. Agric. Food. Chem.*, 1970, **18**, 1178.