703. *Peptides. Part XV.*[1] *4-Methyleneproline and 4-Hydroxymethylproline*

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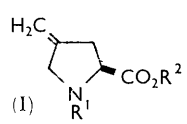
A stereoselective synthesis of *cis*-4-hydroxymethyl-L-proline (IIa) is based on reaction between di-(1,2-dimethylpropyl)borane and the diphenylmethyl ester (Ic) of *N*-benzyloxycarbonyl-4-methylene-L-proline. The product is identical with a natural amino-acid, and one of the intermediates (Ib) has been correlated with 4-methylene-DL-proline, isolated from loquat seeds.

PART XII [2] described stereospecific syntheses of *cis*- and *trans*-4-methylproline, both of which are natural products. In juices of apples and pears the latter is accompanied by 4-hydroxymethylproline.[3] As the configurations of the two asymmetric carbon atoms in

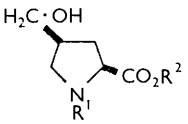
[1] Part XIV, D. F. W. Cross, G. W. Kenner, R. C. Sheppard, and C. E. Stehr, *J.*, 1963, **2143**.
[2] J. S. Dalby, G. W. Kenner, and R. C. Sheppard, *J.*, 1962, 4387.
[3] A. C. Hulme, *Nature*, 1954, **174**, 1055; G. Urbach, *ibid.*, 1955, **175**, 170; A. C. Hulme and F. C. Steward, *ibid.*, p. 171; L. F. Burroughs, *J. Sci. Food Agric.*, 1960, **11**, 14.

this amino-acid were undefined, we included it in our research, and Dr. L. F. Burroughs provided a sample isolated from perry. Reduction of the hydroxymethyl group to a methyl group could not be achieved with the small amount of natural product available, and a synthetic solution was sought. At this time Professor L. Fowden suggested that the configuration of 4-methyleneproline, recently isolated from seeds of loquat (*Eriobotrya japonica*),[4] should be investigated because the natural product had no detectable optical rotation, and he gave us a sample. We therefore devised a synthetic scheme to embrace both these natural products.[5]



(I)

	R^1	R^2
(a:	H	H)
(b:	$CO_2 \cdot CH_2Ph$	H)
(c:	$CO_2 \cdot CH_2Ph$	$CHPh_2$)



(II)

A convenient starting point of known configuration was *N*-benzyloxycarbonyl-4-oxo-L-proline.[6] The Wittig reaction [7] was carried out directly on this acid, employing an excess of methylenetriphenylphosphorane prepared by the sodamide technique,[8] and the product (Ib) was isolated as its dicyclohexylammonium salt. Removal of the benzyloxycarbonyl group was not attempted; in fact, the amino-acid (Ia), if needed, should be prepared from another derivative of 4-oxo-L-proline,[6] *e.g.*, the t-butyoxycarbonyl derivative. For us it was simpler to prepare the benzyloxycarbonyl derivative of the natural product,[4] and its dicyclohexylammonium salt. The two salts had different melting points, and their infrared spectra differed for the solids but not for chloroform solutions. That the salt derived from the natural product was the racemic form of the synthetic salt was confirmed by optical rotation. As the optical rotation of the synthetic salt was small, the natural amino-acid was hydrogenated; the rotation of the product was negligible, whereas in the same solvent, water, *cis*- and *trans*-4-methyl-L-proline have specific rotations of −85 and −57°, respectively. Recently, the natural amino-acid has been conclusively identified with totally synthetic 4-methylene-DL-proline.[9]

The second goal in synthesis was that of the *cis*-isomer (IIa) of 4-hydroxymethyl-L-proline, because the nuclear magnetic resonance spectrum of the natural amino-acid dissolved in deuterium oxide was remarkably similar to that of *cis*-4-methylproline as regards the complex pattern due to the protons directly attached to the five-membered ring, whilst *trans*-4-methylproline gave a quite different pattern.[10] This result was a little surprising because it had been shown [11] that the 4-methylproline, which accompanies 4-hydroxymethylproline in perry, is *trans*; indeed it was asserted [12] that the behaviour of natural 4-hydroxymethylproline in the mass spectrometer proved its *trans* nature. The *cis* assignment was nevertheless favoured by us, but in either case the question would be settled by stereoselective synthesis.

Hydroboronation [13] is the method of choice for preparing a primary alcohol, *e.g.*, (II), from an olefin, *e.g.*, (I), and the preference for the primary product can be increased by employing, instead of diborane, *e.g.*, "disiamylborane" [di-(1,2-dimethylpropyl)borane], with a large steric requirement.[14] It occurred to us that the steric requirement of this dialkylborane could be turned to additional advantage in our work through affording a

[4] D. O. Gray and L. Fowden, *Nature*, 1962, **193**, 1285.
[5] Preliminary communications: M. Bethell, G. W. Kenner, and R. C. Sheppard, *Nature*, 1962, **194**, 864; M. Bethell, D. Bigley, and G. W. Kenner, *Chem. and Ind.*, 1963, 653.
[6] A. A. Patchett and B. Witkop, *J. Amer. Chem. Soc.*, 1957, **79**, 185.
[7] U. Schöllkopf, *Angew. Chem.*, 1959, **71**, 260; S. Trippett, *Quart. Rev.*, 1963, **17**, 406.
[8] G. Wittig, H. Eggers, and P. Duffner, *Annalen*, 1958, **619**, 10.
[9] A. W. Burgstahler, M. L. Trollope, and C. E. Aiman, *Nature*, 1964, **202**, 388.
[10] R. J. Abraham, K. A. McLauchlan, S. Dalby, G. W. Kenner, R. C. Sheppard, and L. F. Burroughs, *Nature*, 1961, **192**, 1150.
[11] L. F. Burroughs, S. Dalby, G. W. Kenner, and R. C. Sheppard, *Nature*, 1961, **189**, 394.
[12] K. Biemann, G. G. J. Deffner, and F. C. Steward, *Nature*, 1961, **191**, 380.
[13] H. C. Brown, "Hydroboration," Benjamin, New York, 1962.
[14] H. C. Brown and G. Zweifel, *J. Amer. Chem. Soc.*, 1961, **83**, 1241.

stereoselective synthesis of the *cis*-isomer. Although the methylene and carboxyl groups are opposite in (I), a bulky esterifying group should hinder attack by the borane (*cis* addition [13]) from one side and thus favour production of the *cis*-isomer. An analogy is to be found in the formation of predominantly *cis*-4-methylproline by catalytic hydrogenation of 4-methyleneproline.[4] The diphenylmethyl group is very suitable for this purpose because the esters tend to crystallise well, they are easily prepared by means of diphenyldiazomethane, and the acid can be regenerated by hydrogenolysis.[15] The diphenylmethyl ester (Ic) of benzyloxycarbonyl-4-methylene-L-proline was converted smoothly into the crystalline 4-hydroxymethyl ester (IIc) by treatment with " disiamylborane " and subsequent oxidation. Hydrogenolysis of this ester gave 4-hydroxymethylproline, identical with the amino-acid from perry. This synthesis proved the L configuration, generally taken for granted, and supported assignment of the *cis* configuration, as in (IIa). Since the preliminary announcement of these results,[5] conclusive evidence for this view has been obtained by Untch and Gibbon.[16] They have repeated our work, confirming the data, and extended it to preparation of benzyloxycarbonyl-*cis*-4-hydroxymethyl-L-proline (IIb) which was lactonised by the action of dicyclohexylcarbodi-imide. Moreover, the minor product from the hydroboronation, the *trans*-isomer of the ester (IIc), was isolated by chromatography and converted into *trans*-4-hydroxymethyl-L-proline. The nuclear magnetic resonance spectrum of this amino-acid dissolved in deuterium oxide gave an excellent correlation with that of *trans*-4-methylproline, thus confirming the validity of our original deduction from n.m.r. spectra.[10] It should also be noted that a non-selective synthesis of the *cis–trans*, isomers of 4-hydroxymethyl-DL-proline has been described.[17] The *cis*- and *trans*-isomers were separated by crystallisation of a copper salt, and one of them was correlated with the natural product by mass and n.m.r. spectra; a lactone was also prepared from this DL-amino-acid, proving its *cis* configuration.[18]

The stereochemistry of all the 4-alkylprolines is now properly defined, but there remain questions about their inter-relation as natural products.[19] Evidently, *trans*-4-methyl-L-proline is not directly connected with the accompanying *cis*-4-hydroxymethyl-L-proline, although this might be a source of the *cis*-4-methyl-L-proline obtained from antibiotic 13959.[20,2] On the other hand, the configuration of the γ-carbon atom in γ-methyl-L-glutamic acid [21] is the same as at the 4-position in *trans*-4-methyl-L-proline, and thus the normal conversion *via* the glutamic semialdehyde [22] seems likely. Likewise, γ-methylene-L-glutamic acid is the probable source of 4-methyleneproline, and 4-hydroxymethylproline may be derived in turn by hydration in the anti-Markownikow direction.[4] However, the racemic character, demonstrated in this Paper, of 4-methyleneproline carefully isolated from loquat seeds is an awkward fact.

Experimental

Dicyclohexylammonium Salt of Benzyloxycarbonyl-4-methylene-L-proline.—All operations were carried out in an atmosphere of oxygen-free nitrogen. To freshly distilled liquid ammonia (*ca.* 170 ml.) was added sodium (1·29 g., 56 mmoles) and a trace of ferric chloride. When the blue colour had disappeared, methyltriphenylphosphonium bromide (20 g., 56 mmoles) was added with stirring. The ammonia was removed, and ether (75 ml.) and tetrahydrofuran (75 ml.) were added. The suspension was heated to 50° for 5 min. Benzyloxycarbonyl-4-oxo-L-proline (2·63 g., 10 mmoles),[6] dissolved in tetrahydrofuran (20 ml.), was added at room temperature with stirring during 10 min. The reaction mixture was refluxed for several hours.

[15] E. Hardegger, Z. El Heweihi, and F. G. Robinet, *Helv. Chim. Acta*, 1948, **31**, 439.
[16] K. G. Untch and G. A. Gibbon, *Tetrahedron Letters*, 1964, 3259.
[17] A. W. Burgstahler and C. E. Aiman, *Chem. and Ind.*, 1962, 1430.
[18] A. W. Burgstahler, cited in ref. 16.
[19] Cf. L. Fowden, *Ann. Rev. Biochem.*, 1964, **33**, 173.
[20] G. W. Kenner and R. C. Sheppard, *Nature*, 1958, **181**, 48.
[21] J. Blake and L. Fowden, *Biochem. J.*, 1964, **92**, 136.
[22] A. Meister, " Biochemistry of the Amino Acids," Academic Press, New York, 1957, pp. 288—293.

To the cooled mixture was added water, sodium hydrogen carbonate solution, and ether. After filtration from insoluble by-products, the sodium hydrogen carbonate layer was separated and extracted twice more with ether before acidification. The product was purified by repeated dissolution in sodium hydrogen carbonate, ether extraction, and subsequent reacidification, but it did not crystallise. Addition of dicyclohexylamine to the ethereal solution gave the *dicyclohexylammonium salt* (3·655 g., 84%), m. p. 155—158°, raised to 160—161° by recrystallisation from methyl cyanide, $[\alpha]_D^{19}$ −5·5° (*c* 2 in $CHCl_3$) (Found: C, 70·9; H, 8·6; N, 6·5. $C_{26}H_{38}N_2O_4$ requires C, 70·55; H, 8·65; N, 6·3%).

*Benzyloxycarbonyl-4-methylene-*DL*-proline.*—Natural 4-methyleneproline (127 mg.) was dissolved in acetone–water (10 ml.; 1 : 1 v/v) and heated at room temperature with benzyl chloroformate (0·2 ml.) in acetone (1·2 ml.) at pH 10·3 (autotitrator). Acetone was removed from the reaction mixture at room temperature, and the alkaline residue was extracted with ether. The aqueous layer was separated and acidified, the non-crystalline product being isolated in approximately quantitative yield by extraction with ethyl acetate. Treatment with excess of dicyclohexylamine in ether, and recrystallisation from methyl cyanide, gave the *dicyclohexylammonium salt* (95% overall yield), m. p. 139°, $[\alpha]_D^{19}$ −0·58° (*c* 3·0 in $CHCl_3$) (Found: C, 70·2; H, 8·5; N, 6·6%). In another experiment, natural 4-methyleneproline was dissolved in 90% methanol and hydrogenated at room temperature and atmospheric pressure with Adams platinum catalyst for 4 hr. (although the theoretical vol. of hydrogen was absorbed in a few min. and no more was taken up). The crystalline 4-methylproline obtained from the solution had $[\alpha]_D^{19}$ −0·5° (*c* 2·5 in H_2O). These two rotations were determined visually, and their difference from zero is insignificant.

*Benzyloxycarbonyl-4-methylene-*L*-proline Diphenylmethyl Ester.*—Benzyloxycarbonyl-4-methylene-L-proline (522 mg.), liberated from its dicyclohexylammonium salt by treatment with hydrochloric acid, was dissolved in dry methyl cyanide (15 ml.) and treated with diphenyldiazomethane in methyl cyanide (5·2 ml. of 0·39M). The reaction mixture was kept overnight at room temperature and evaporated *in vacuo*. The residual oil was taken up in ethyl acetate and washed with aqueous sodium hydrogen carbonate. Removal of the solvent afforded the *ester* (699 mg., 82%), m. p. 68—71°, raised to 74—75° by several recrystallisations from methanol and benzene–hexane, $[\alpha]_D^{20·5}$ −31·7° (*c* 1·4 in $CHCl_3$) (Found: C, 75·9; H, 5·7; N, 3·3. $C_{27}H_{25}NO_4$ requires C, 75·9; H, 5·9; N, 3·3%).

*Benzyloxycarbonyl-*cis*-4-hydroxymethyl-*L*-proline Diphenylmethyl Ester.*—The hydroboronation was carried out in an atmosphere of dry, oxygen-free nitrogen. Benzyloxycarbonyl-4-methylene-L-proline diphenylmethyl ester (550 mg.) was added with stirring to a solution in diglyme (15 ml.) at 0° of "disiamylborane," prepared *in situ* from sodium borohydride (5 ml. of 0·95M-solution in diglyme), 2-methylbut-2-ene (1·5 ml.), and boron trifluoride etherate (1·0 ml.). After 12 min. the ice-bath was removed and the reaction mixture was allowed to warm up for 15 min. Excess "disiamylborane" was destroyed by addition of water (20 ml.). The reaction mixture was cooled to 0° again, and the pH adjusted to 8 with 3N-sodium hydroxide. Hydrogen peroxide (3 ml. of 30%) was added and the mixture was maintained at 0° for 10 min. The ice-bath was removed for 10 min., during which time more hydrogen peroxide (1·0 ml.) was added, the pH being maintained at 8 by further addition of sodium hydroxide. The reaction mixture was finally brought to pH 6 and separated between water and ether. The ether layer was washed repeatedly with water containing a little glycerol, dried ($MgSO_4$), and evaporated to give *benzyloxycarbonyl-*cis*-4-hydroxymethyl-L-proline diphenylmethyl ester* (322 mg., 56%), m. p. 154—155°, raised to 159—160° by recrystallisation from aqueous ethanol and methanol, $[\alpha]_D^{18·5}$ −58·2° (*c* 2·05 in $CHCl_3$) (Found: C, 72·5; H, 6·05; N, 3·1. $C_{27}H_{27}NO_5$ requires C, 72·8; H, 6·1; N, 3·1%).

cis-4-*Hydroxymethyl-*L*-proline.*—A slow stream of hydrogen was passed for several hours over a magnetically stirred solution of benzyloxycarbonyl-*cis*-4-hydroxymethyl-L-proline diphenylmethyl ester (290 mg.) in ethanol (25 ml.) containing 5% palladised charcoal (400 mg.). The reaction mixture was diluted with water, the catalyst filtered off, and diphenylmethane extracted by ether from the concentrated solution before evaporation. Crystallisation from a little methanol gave cis-4-*hydroxymethyl-*L*-proline* (78 mg., 83%), m. p. 257—258° (decomp.), $[\alpha]_D^{19·5}$ −75·6° (*c* 1·65 in H_2O) (Found: C, 49·7; H, 7·7; N, 9·6. $C_6H_{11}NO_3$ requires C, 49·6; H, 7·6; N, 9·65%). This product was identified with the natural product by infrared spectrum (KBr disc),[3] n.m.r. spectrum (D_2O),[10] mixed m. p., and paper chromatography [butan-1-ol–acetone–water–dicyclohexylamine (10 : 10 : 5 : 2), descending overnight].

The optical rotation of the natural amino-acid, which has not been recorded hitherto, was determined by Mr. J. Beacham, with a photoelectric polarimeter, on the minute sample remaining, as $[\alpha]_D$ $-73° \pm 4°$ (*c* 1·8 in H_2O).

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