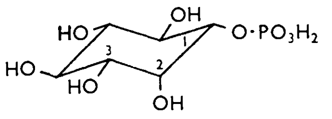
279. *Phospholipids. Part VIII.*[1] *Synthesis of* (+)-trans-2-*Hydroxycyclohexyl Phosphate, and a Calculation of the Configuration of the Myoinositol* 1-*Phosphate Residue in Monophosphoinositide.*

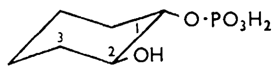
By D. M. Brown and B. F. C. Clark.

The absolute configuration of the myoinositol 1-phosphate derived from certain monophosphoinositides is confirmed, by calculations based on optical rotations.

Ballou and Pizer [2] defined the absolute stereochemistry of the myoinositol 1-phosphate obtained by degrading a soya-bean monophosphoinositide and relating it to a myoinositol galactoside (galactinol) of known configuration.[3] The same structure, L-myoinositol 1-phosphate has been established for the corresponding residue in horse-liver monophosphoinositide.[1,4] Before the appearance of Ballou and Pizer's stereochemical study, experiments had been begun with the object of deducing the configuration from the observed rotations. The method of calculation is based on assumptions first made by Whiffen [5] when attempting to relate optical rotation and geometrical structure. In accord with Whiffen's treatment, the molecular rotation, $[M]$, of a substituted cyclohexane, assumed to be in the chair form, can be expressed as a series of terms each of which is associated with interactions of chemical bonds and the substituents. These interactions give rise to rotations which are much larger than those due to asymmetric atomic groupings and the latter are therefore omitted from the calculations.



(I)



(II)

To calculate the molecular optical rotation of L-myoinositol 1-phosphate, it is assumed to be in the conformation (I) in which the phosphate and four hydroxyl groups are equatorial. This is supported by borate complex-formation of myoinositol and the fact that chemical and bacterial oxidation to myoinosose occurs at the 2-hydroxyl group, which should therefore be axial.[6] We view the molecule along the bonds and obtain ethane-like partial conformations; hydrogen, hydroxyl, and phosphate groups are designated by H, O, and P, respectively. Summation of parameters written out as in

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[1] Part VII, Brown, Clark, and Letters, *J.*, 1961, 3774.

[2] Ballou and Pizer, *J. Amer. Chem. Soc.*, 1959, **81**, 4745; 1960, **82**, 3333.

[3] Kabat, MacDonald, Ballou, and Fischer, *J. Amer. Chem. Soc.*, 1953, **75**, 4507.

[4] Brown, Clark, Hall, and Letters, *Proc. Chem. Soc.*, 1960, 212.

[5] Whiffen, *Chem. and Ind.*, 1956, 964.

[6] Angyal, *Quart. Rev.*, 1957, **11**, 212; Angyal and Anderson, *Adv. Carbohydrate Chem.*, 1959, **14**, 136.

Whiffen's treatment gives $[M]_m = 2(-\ P/O + P/H - H/O + O/O)$ where $[M]_m$ is the $[M]$ value of L-myoinositol 1-phosphate (I). Whiffen's sign conventions are adopted and $[M]$ refers to values obtained from an aqueous solution at a particular pH and 20° in light of wavelength 589 mμ.

The expression for $[M]_m$ involves parameters not discussed by Whiffen. Molecular rotations of simpler but related phosphates were therefore calculated to obtain parameters including the phosphate group. The configuration of (+)-*trans*-cyclohexane-1,2-diol was known,[7] and a consideration of the derived (+)-*trans*-2-hydroxycyclohexyl phosphate in the expected form (II) gave $[M]_c = P/O - H/P + H/H - O/H$. Since $O/O - 2\ O/H + H/H = 45°$ and $H/O = O/H$, $M_m = 2(45° - [M]_c)$.

The monophosphate ester of (+)-*trans*-cyclohexane-1,2-diol (II) was prepared and $[M]_c$ for the biscyclohexylammonium salt determined at pH 7 and pH 2 as +40·6° and +60·4°, respectively. Thus for measurements at pH 7, $[M]_m = +8{\cdot}8°$. The biscyclohexylammonium salt of the myoinositol 1-phosphate from horse-liver monophosphoinositide[4] had $[M]$ +10·3°. Less accurate measurements at pH 2 [the pH being adjusted by treatment with Dowex-50 (H^+ form)] gave a consonant result; the calculated value for $[M]_m$ is −30·8° and the observed value was −38·8°.

The results, therefore give strong support for the configurational conclusions drawn by Ballou and Pizer.[2] Moreover, the present determination of a series of parameters involving the phosphate group should allow conclusions to be drawn on the conformations or configurations of other cyclohexane derivatives substituted by a phosphate group, or of sugar phosphates. We are extending the study with this aim.

The optically active *trans*-2-hydroxycyclohexyl phosphate (II) was prepared as follows. Treatment of cyclohexene oxide with (−)-menthyloxyacetic acid gave *trans*-2-hydroxycyclohexyl menthyloxyacetate. Fractionation, by Winstein and Heck's procedure,[8] gave the pure (−)-ester that with diphenyl phosphorochloridate in pyridine afforded a high yield of the crystalline, lævorotatory, diphenyl phosphate ester. Hydrogenolysis of the phenyl groups, followed by basic hydrolysis of the menthyloxyacetate group gave (+)-*trans*-2-hydroxycyclohexyl phosphate (II) which was isolated as its biscyclohexylammonium salt.

EXPERIMENTAL

(−)-trans-2-*Hydroxycyclohexyl Menthyloxyacetate*.—This was prepared from (−)-menthyloxyacetic acid and cyclohexene oxide by a method based on that of Winstein and Heck.[8] It formed feathery needles, m. p. 126°, from light petroleum (b. p. 60—80°) and had $[\alpha]^{20}_{589}$ −30·4° (*c* 3·2 in ethanol). (Lit.,[9] m. p. 126—127°; $[\alpha]_{589}$, −32·7°.) The ester yielded (+)-*trans*-cyclohexane-1,2-diol, m. p. 108°, on hydrolysis.

(−)-*Diphenyl* trans-2-*Menthyloxyacetoxycyclohexyl Phosphate*.—Diphenyl phosphorochloridate (1·05 g.; 3·9 mmoles) was added with shaking, at room temperature, to a solution of the above menthyloxyacetate (0·53 g.; 1·7 mmoles) in pyridine (8 ml.; dried over calcium hydride). After 14 hr., water (1 ml.) was added dropwise with shaking. After 10 min. benzene (40 ml.) was added and the solution washed successively with water (20 ml.), N-hydrochloric acid (15 ml.), saturated sodium hydrogen carbonate solution (20 ml.), and water (20 ml.), and finally dried ($MgSO_4$). The solution was filtered and concentrated *in vacuo*, and then ethanol was added and removed *in vacuo* thrice. The product was dissolved in ethanol (4 ml.) and kept at −15° for 18 hr. The *diphenyl phosphate* separated in needles (820 mg.; 89%), m. p. 76·5° (Found, in material dried at room temp. for 8 hr./0·1 mm. over P_2O_5: C, 66·4; H, 7·7. $C_{30}H_{41}O_7P$ requires C, 66·2; H, 7·5%).

The substance had $[\alpha]^{20}_{589}$ −14·4° (*c*, 2·8 in ethanol). Optical rotatory dispersion measurements gave $[M]^{20}$ values: −74·5° (600 mμ), −78·4° (589), −87·5° (540), −106·5° (500), −133·9° (450), −168·0° (400), −233·5° (350), and −242·5° (330).

(+)-trans-2-*Hydroxycyclohexyl Phosphate*.—The above diphenyl phosphate (774 mg.;

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[7] Posternak, Reymond, and Freidli, *Helv. Chim. Acta*, 1955, **38**, 205.
[8] Winstein and Heck, *J. Amer. Chem. Soc.*, 1952, **74**, 5584.
[9] Wilson and Read, *J.*, 1935 1269.

1·4 mmoles), dissolved in ethanol (40 ml.), was hydrogenated over platinum dioxide (126 mg.) at room temperature and pressure for 17 hr. Hydrogenation was essentially complete after 3 hr. After removal of catalyst the solution was evaporated under reduced pressure. The gum was dissolved in 0·5N-sodium hydroxide (20 ml.), and the solution kept at 70° for 30 min., then at room temp. for 9 hr. Water (40 ml.) was added and the suspension shaken with Dowex-50 (H^+-form) and ether (25 ml.) to remove menthyloxyacetic acid and cations. The aqueous phase, after removal of resin, was extracted with ether (2 × 30 ml.). The ether extracts were washed with water (30 ml.), and the combined aqueous phases brought to pH 11 with cyclohexylamine. Excess of cyclohexylamine was removed by ether extraction, and the aqueous solution was evaporated to dryness. The residue was crystallised from ethanol, giving *biscyclohexylammonium* (+)-trans-2-*hydroxycyclohexyl phosphate* (220 mg.; 40%) in needles, m. p. 200—205° (decomp.) (Found, in material dried at 25°/0·1 mm./8 hr. over P_2O_5: C, 50·4; H, 10 1; N, 6·15. $C_{18}H_{39}N_2O_5P,2H_2O$ requires C, 50·25; H, 10·0; N, 6·5%).

The compound was indistinguishable from the synthetic racemic material on paper chromatograms and by infrared spectroscopy.

Optical rotation measurements gave $[\alpha]_{589}^{20}$ +10·3° (*c* of anhydrous cyclohexylammonium salt, 1·74 in water). Values of $[M]^{20}$ at pH 7—8 were +40·6° (589 mμ), +46·9° (540), +55·7° (500), +71·2° (450), +94·2° (400), +125·0° (350). After acidification of the solution to pH 2 with Dowex-2 (H^+) resin, values were +60·4° (589 mμ), +70·3° (540), +80·2° (500), +105·0° (450), +135·0° (400), +184·2° (350).

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