Synthesis and Circular Dichroism of Methyl (*R*)-[^{16}O,^{17}O,^{18}O]Phosphate

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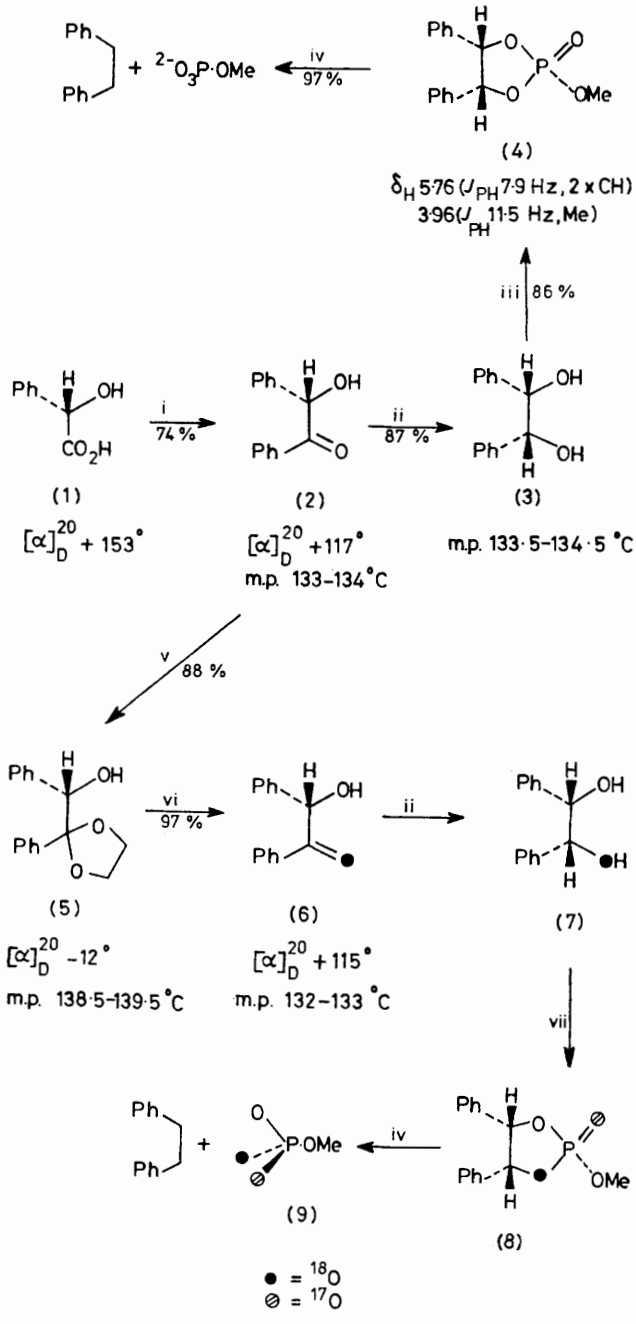
Summary A general method for the synthesis of chiral [^{16}O,^{17}O,^{18}O]phosphate monoesters has been developed; the preparation and chiroptical properties of methyl (*R*)-[^{16}O,^{17}O,^{18}O]phosphate are reported.

DELINEATION of the mechanisms of chemical and enzyme catalysed phosphoryl transfer reactions would be greatly assisted by a knowledge of the stereochemical fate of the phosphoryl group. Since phosphate monoesters chirally labelled with ^{17}O and ^{18}O would serve, in principle, to

establish whether inversion or retention of configuration had occurred, a general method for the synthesis of chirally labelled phosphate monoesters has been developed. Its utilisation for the synthesis of methyl (*R*)-[^{16}O,^{17}O,^{18}O]-phosphate is outlined in the Scheme.



SCHEME. Rotations were measured in acetone (*c ca.* 1) except for (*S*)-mandelic acid (H_2O, *c* 2). Reagents: i, PhLi; ii, $LiAlH_4$; iii, (a) $POCl_3$–C_5H_5N, (b) MeOH–C_5H_5N; iv, H_2–Pd; v, $HO.CH_2.CH_2.OH$–*p*-$Me.C_6H_4.SO_3H$; vi, $H_2{}^{18}O$–dioxan–*p*-$Me.C_6H_4.SO_3H$; vii, (a) $P^{17}OCl_3$–C_5H_5N, (b) MeOH–C_5H_5N.

(*R*)- and (*S*)-Benzoin have been prepared by a variety of methods,[1] but treatment of (*S*)-mandelic acid (**1**) with phenyl-lithium to form (*S*)-benzoin (**2**) represents the most direct and efficient method hitherto reported. Reduction of (*RS*)-benzoin with lithium aluminium hydride at 0 °C has been shown to give exclusively *meso*-hydrobenzoin.[2] Reduction of (*S*)-benzoin (**2**) with lithium aluminium hydride at 0 °C, likewise gives exclusively *meso*-hydrobenzoin (**3**). Transesterification of trimethyl phosphite with *meso*-hydrobenzoin has been reported to give a single diastereoisomer, *trans*-methyl *meso*-hydrobenzoin cyclic phosphite which on treatment with ozone gave *trans*-methyl *meso*-hydrobenzoin cyclic phosphate (*trans*-2-methoxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholan); both structures were firmly established by *X*-ray crystallography.[3] The *cis*-diastereoisomer (**4**) is obtained by treating *meso*-hydrobenzoin with phosphorus oxychloride in pyridine, which gives a single crystalline diastereoisomer of *meso*-hydrobenzoin cyclic phosphorochloridate,[4] which on treatment with methanol in pyridine gives *cis*-2-methoxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholan (**4**).[5] Catalytic hydrogenolysis of this crystalline triester gives methyl phosphate and 1,2-diphenylethane.

Although (*S*)-mandelic acid and (*S*)-benzoin were shown to be configurationally stable under conditions which would allow acid-catalysed ^{18}O-exchange from [^{18}O]water into the carboxy and keto groups, the dilution of isotope which would accompany exchange made incorporation of ^{18}O into (*S*)-benzoin by way of its acetal (**5**) more attractive. It was established that the acid-catalysed acetalisation of (*S*)-benzoin with ethylene glycol and the acid-catalysed hydrolysis of the acetal back to (*S*)-benzoin could be achieved without loss of chirality. Acid-catalysed hydrolysis of the acetal (**5**) using [^{18}O]water (99·5 atom % ^{18}O) gave (*S*)-[^{18}O]benzoin (**6**). The configurational stability of (*S*)-benzoin under the conditions used for ^{18}O-exchange, effectively excluded the possibility of ^{18}O-exchange into the hydroxy group. This however was confirmed by reducing (*S*)-[^{18}O]benzoin with lithium aluminium deuteride, the mass spectrum of [^{2}H,^{18}O]hydrobenzoin showing a molecular ion peak at *m/e* 217 and base peaks of equal intensity at 107 and 108 ($PhCH{=}O^+H$ and $[PhCH_2OH]^+$) and 110 and 111 ($PhC^2H{=}^{18}O^+H$ and $[PhCH^2H{-}^{18}OH]^+$)† clearly indicating that the ^{2}H and ^{18}O were borne by the same carbon atom and hence that (*S*)-[^{18}O]benzoin was exclusively labelled in the carbonyl group.

Reduction of (*S*)-[^{18}O]benzoin (**6**) with lithium aluminium hydride at 0 °C gave (1*R*,2*S*)-1,2-[1-^{18}O]dihydroxy-1,2-diphenylethane (**7**) which owes its chirality solely to isotopic dissymmetry. On treatment with phosphorus [^{17}O]-oxychloride (derived from phosphorus pentachloride and 1 equiv. of water containing 44·0 atom % ^{17}O, 1·8 atom % ^{16}O, and 54·2 atom % ^{18}O) in pyridine, followed by methanolysis in pyridine of the resulting cyclic phosphorochloridate gave (2*S*,4*S*,5*R*)-2-methoxy-2-[^{17}O]oxo-4,5-diphenyl-1,2,3-[1-^{18}O]dioxaphospholan (**8**) as a single crystalline stereoisomer. On catalytic hydrogenolysis methyl (*R*)-[^{16}O,^{17}O,-^{18}O]phosphate (**9**) (and 1,2-diphenylethane) was obtained and isolated as its sodium salt.

As expected the 1,2-diphenylethane showed no optical activity, but methyl (*R*)-[^{16}O,^{17}O,^{18}O]phosphate disodium

† *meso*-Hydrobenzoin shows a molecular ion peak at *m/e* 214 and base peaks of equal intensity at 107 and 108.

salt does possess chiroptical properties; the c.d. spectrum is shown in the Figure. The correspondence between the u.v. absorption [inflection at *ca.* 208 (ϵ 24)] and c.d. spectra, together with the fact that unlabelled methyl phosphate obtained by the route outlined in the Scheme did not show circular dichroism, excludes the possibility that a trace impurity could be responsible for the observed c.d. spectrum. Since the ^{17}O content of the water used to prepare methyl (*R*)-[^{16}O,^{17}O,^{18}O] phosphate was only 44 atom % (highest enrichment available) the true c.d. maxima can be calculated to be 208 nm ($\Delta\epsilon + 2{\cdot}7 \times 10^{-3}$).

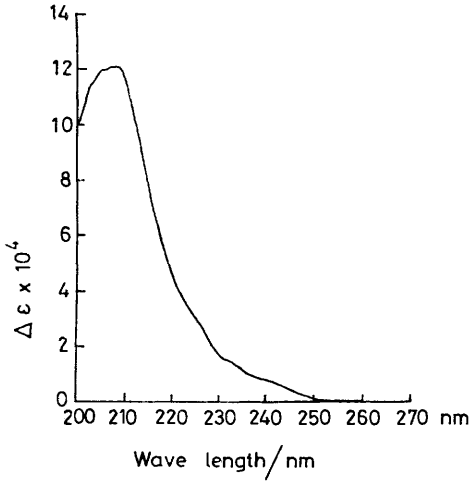


FIGURE. C.d. spectrum of methyl (*R*)-[^{16}O,^{17}O,^{18}O]phosphate disodium salt in deuterium oxide (0·03 M).

Methyl (*R*)-[^{16}O,^{17}O,^{18}O]phosphate is the first example of a molecule exhibiting circular dichroism due solely to the chiral disposition of three isotopes. Moreover it establishes that chiroptical techniques offer a convenient physical method for determining the configuration of chirally labelled phosphoryl groups and hence the stereochemical course of phosphoryl transfer reactions.

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