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THE STEREOCHEMISTRY OF REACTION OF P-CHIRAL DIALKYL HYDROGEN PHOSPHOROTHIOATES WITH [^{18}O]-DIMETHYL SULPHOXIDE

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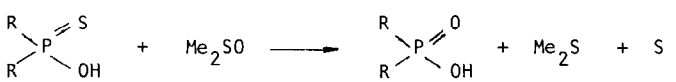
Abstract *Cis*- and *trans*-2-hydroxy-2-thiono-4-methyl-1,3,2-dioxaphosphorinanes are cleanly transformed by means of [^{18}O]- DMSO into corresponding 2-([^{18}O]-oxo)-derivatives with ca 90% *inversion* of configuration at phosphorus atom.

The use of nucleoside P-chiral phosphorothioates makes possible the determination of the stereochemical course of enzymatic processes involving phosphoryl and/or nucleotidyl transfer[1]. Recently a great improvement on this approach has been achieved by introducing phosphate models which are chiral by virtue of asymmetry of phosphorus caused by the presence of different oxygen isotopes surrounding this atom[2]. So far two methods of stereospecific introduction of stable oxygen isotopes into nucleotidyl phosphate group were employed. The first, applicable to the synthesis of monoalkyl (nucleoside) phosphates involves the use of specifically labelled five-membered P-chiral isotopomers of cyclic phosphates[3,4] or phosphoramidates[5] and their condensation with appropriate nucleoside or nucleotide. The second method, originally designed in our laboratory[6], employs diastereoisomeric nucleoside phosphoranilidates and their reaction with isotopically labelled carbonyl compounds such as benzaldehyde[7] or carbon dioxide[8]. Since nucleoside P-chiral phosphorothioates are themselves useful in enzymology and molecular biology[9], and their syntheses, mainly due to simplicity of their analysis, seem to be more straightforward than these leading to nucleoside P-chiral phosphates, we undertook studies of stereospecific conversion of phosphorothioates into phosphates. We have focused our attention on [^{18}O]-dimethyl sulphoxide (DMSO). The ability of DMSO to exchange sulphur for oxygen in phosphorothioate and thiocarbonyl moieties under relatively mild conditions was described in 1966 by Mikołajczyk[10].



R = alkyl, alkoxyl

Dialkyl hydrogen phosphorothioates (but not their salts) react with DMSO at room temperature to give corresponding oxo-acids, dimethyl sulphide and elemental sulphur. Excess of DMSO is neces-

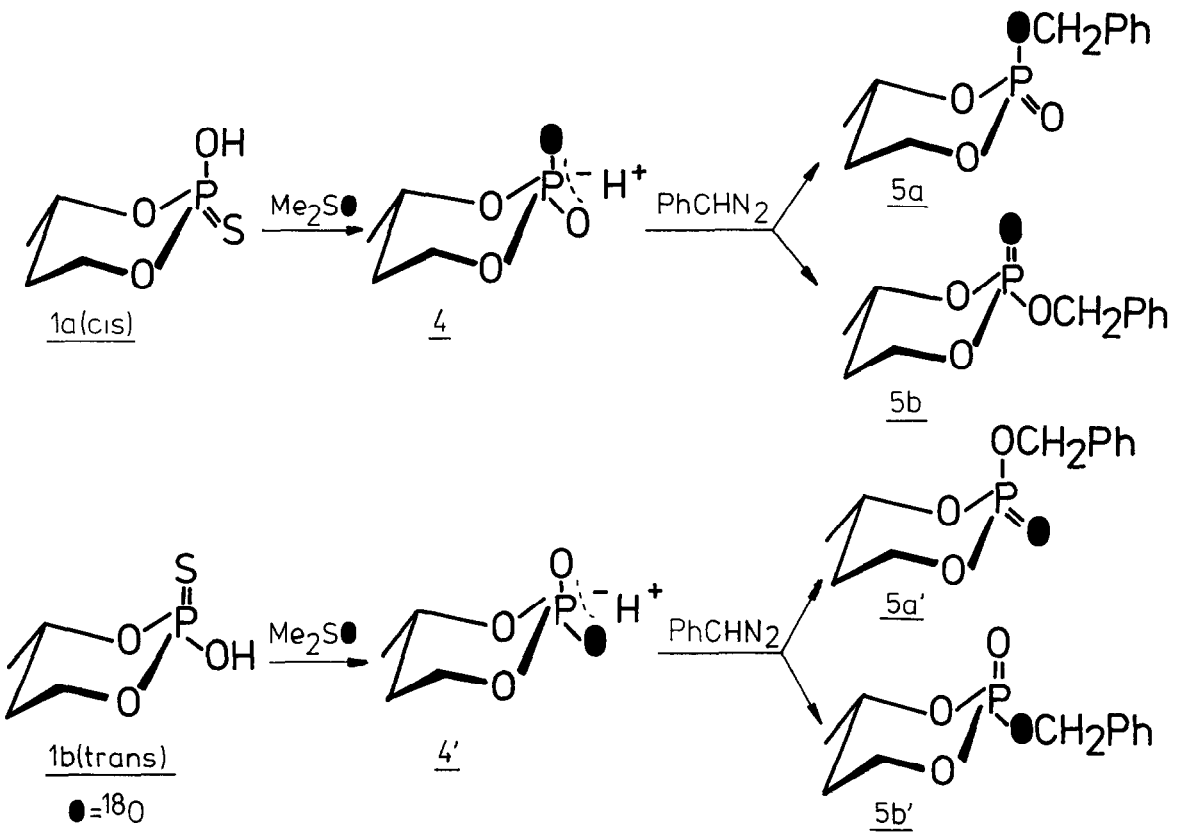
sary since otherwise the corresponding oxophosphorane disulphides are formed as undesired side-products. The additional arguments speaking for the choice of [^{18}O]-DMSO as the reagent for converting phosphorothioates into [^{18}O]-phosphates are as follows *i* - excellent solubility of the majority of organophosphorus compounds in DMSO, *ii* - possibility of recovery of its excess from the reaction mixture, *iii* - easy preparation of [^{18}O]-DMSO using [^{18}O]-H_2O as the source of oxygen-18[11].

For the preliminary stereochemical studies of *sulphur - oxygen exchange* we have chosen diastereoisomeric *cis*-(1a) and *trans*-2-hydroxy-2-thiono-4-methyl-1,3,2-dioxaphosphorinane (1b). Compounds 1a and 1b possess the same ring system as that existing in cAMPS[12] and therefore are suitable models for a studies of the reaction in question. The *cis*- and *trans*-isomers of 1 were obtained according to the procedure described by Mikołajczyk and Łuczak[13] and stored in the form of crystalline dicyclohexylammonium salts[14]. The free acids were liberated before use by passing the salt through an ion-exchange Dowex 50WX8 column.

The preliminary experiments, followed by ^{31}P-NMR, showed the dramatic difference in the reactivity of isomers of 1 towards DMSO. At room temperature the reaction of compound 1b with a 9-fold excess of DMSO is completed within two days whereas isomer 1a under analogous conditions requires 7 days for its disappearance from the reaction mixture. In the case of 1b the product, 2-hydroxy-2-oxo-4-methyl-1,3,2-dioxaphosphorinane [4, δ_{31P}=-7.4 ppm (DMSO)] was accompanied by the corresponding disulphide [ca 20%, δ_{31P}=11.4 ppm (DMSO)][15]. In the reaction of 1a with DMSO the formation of corresponding unsymmetrical pyrophosphorothioate [ca 10%, δ_{31P_S}=45.1 ppm, δ_{31P_O}=-21.2 ppm, $^2J_{PP}$=26 Hz (DMSO)][16], in addition to phosphate 4', was observed. The products 4 and 4' obtained from the reaction of 1a and 1b, respectively, with [^{18}O]-DMSO were analysed in the form of benzyl esters 5a,b and 5a',b' prepared by treatment of a crude reaction mixture with ethereal solution of phenyldiazomethane. This transformation (4 → 5) allowed us to apply the method of analysis of ^{18}O-distribution in 4 and 4' by the procedure reported from this laboratory[7] (see Scheme). The diastereomeric esters 5a (5a') [δ_{31P}=-7.2 ppm ($CHCl_3$), R_f=0.26] and 5b (5b') [δ_{31P}=-5.0 ppm ($CHCl_3$), R_f=0.12], which are formed in the ratio of 2 3, respectively, were separated by preparative TLC[17]. The quantitative analysis of Electron-Impact mass spectra (70 eV) performed for both molecular ion (m/z 242-244) and the fragment-ion resulting from the loss of benzaldehyde (m/z 136-138) allowed us to assign the position of oxygen-18 in 4 and 4' and hence to calculate the stereoselectivity of reaction (see Table).

The results listed in the Table clearly show that replacement of S by ^{18}O in cyclic six-membered ring hydrogen phosphorothioates occurs with predominant *INVERSION* of configuration at phosphorus. The stereoselectivity depends on the reaction conditions. The best results (92.4% inversion and 7.6% retention) were obtained when reaction was performed at room temperature with nine-fold molar excess of [^{18}O]-DMSO.

The work on the application of the *PS → P^{18}O conversion* caused by [^{18}O]-DMSO to the synthesis of P-chiral nucleoside phosphates labelled with oxygen isotopes is in progress. It should be mentioned that when the work described in this communication was underway three reports on the stereospecific conversion of nucleoside phosphorothioates by means of BrCN/$H_2{}^{18}O$ (Frey et al.)[19], NBS/$H_2{}^{18}O$ (Eckstein et al.)[20] and Br_2/$H_2{}^{17}O$ (Lowe et al.)[21] into oxygen labelled P-chiral phosphates have appeared.



Substrate	Reaction conditions [a]			Stereoselectivity [b]	
	Temp (°C)	Time	Molar ratio [^{18}O]-DMSO/1	% of inversion	% of retention
1a	80	2 h	2.5 1	79 5	20.5
1b	80	1 h	2.5 1	83.0	17 0
1a	25	6 days	2.5 1	85.7	14.3
1b	25	2 days	2.5 1	86.2	13.8
1a	80	2 h	10 1	88 1	11.9
1b	80	1 h	10 1	92.4	7.6

a/ *The chloroform solution of acid 1 (1 mmol) was added to the chloroform solution of Me_2SO (69% ^{18}O-enrichment). Chloroform was removed in vacuo and the mixture was left to stay for the period indicated in the Table. The excess of Me_2SO was recovered by vacuum-line technique [18] and the residue was dissolved in THF (2 ml). Ethereal solution of phenyldiazomethane was added dropwise to the point when the mixture acquired a permanent orange colour. After evaporation crude esters 5 were separated by preparative TLC.*

b/ *No loss of isotopic enrichment in the product, as compared with starting [^{18}O]-DMSO was observed.*

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14. 1a (salt) mp. 194-6°, δ_{31P}=54.0 ppm (MeOH)(lit.[13] mp 194-6°, δ_{31P}=53.5 ppm), 1b (salt) mp. 209-11°, δ_{31P}=51.1 ppm (MeOH) (lit [13] mp. 208-11°, δ_{31P}=50.5 ppm). Positive ^{31}P-NMR chemical shift values correspond to compounds absorbing at lower field than 85% H_3PO_4
15. For the *trans-trans* bis-(2-oxo-2-thio-4-methyl-1,3,2-dioxaphosphorinan-2-yl)disulphide the chemical shift δ_{31P}=11.4 ppm is reported (E.Krawczyk, Ph.D. Thesis, Łódź 1982)
16. For the *trans-cis* unsymmetric pyrophosphorothioate following ^{31}P-NMR data are reported δ_{PS}=46.5 ppm, δ_{PO}=-21.6 ppm, $^{2}J_{PP}$=26 8 Hz (E Krawczyk, Ph.D. Thesis, Łódź 1982)
17. TLC was performed on a precoated silicagel plates 60 F_{254} with ether-chloroform (v/v 3 1) as developing system
18. The DMSO recovered did not show any loss of ^{18}O-enrichment
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