The Mechanism of Iodine-Water Oxidation of *H*-Phosphonate Diesters

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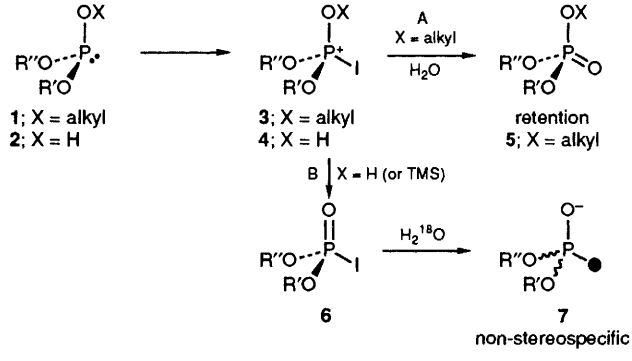
Using ^{18}O-labelling the oxidation of 5-*tert*-butyl-2-hydroxy-1,3,2-dioxaphosphorinane **8** and **9** with iodine-water has been shown to proceed non-stereospecifically, *via* a mechanism presumed to involve the formation of an iodophosphate intermediate which, under the reaction conditions, epimerises at phosphorus.

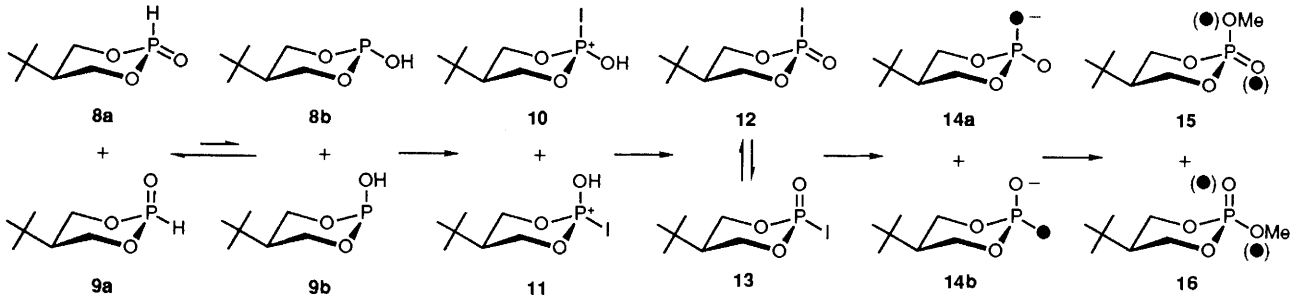
The oxidation of phosphites is a crucial reaction in the well established P^{III} synthesis of oligonucleotides.[1] We have previously reported that the oxidation of trialkyl phosphites with iodine-water and with *m*-chloroperbenzoic acid proceeds stereospecifically with retention of configuration[2] and this was later confirmed by others.[3] This reaction provides one of the most convenient routes for the introduction of ^{18}O or ^{17}O labels into the non-bridging positions of internucleotidic linkages in oligonucleotides; such species finding widespread application in the determination of the stereochemical course of enzymatic cleavage reactions.[4] Despite superficial similarities between the iodine-water oxidation of phosphite triesters and that of *H*-phosphonates, we report here that this latter class of reactions proceed non-stereospecifically. A mechanism involving an iodophosphate intermediate is proposed to account for this.

The mechanism of iodine-water oxidation of trialkyl phosphites 1, shown in Scheme 1 (pathway A), was studied in both cyclic and acyclic phosphites triesters.[2,3] The observation of overall retention of configuration can be rationalised if the displacement of iodide from the phosphonium salt proceeds with retention of configuration.

Recent work by Seela and Kretschmer,[5] has drawn an analogy between our earlier work on dinucleoside phosphite triesters and their more recent work on the oxidation of the corresponding dinucleoside *H*-phosphonates. However, we do not believe that this analogy is justified. Extending the mechanism for phosphite triester **1** shown in Scheme 1 pathway A, to the oxidation of *H*-phosphonates we suggest that oxidation of *H*-phosphonates probably proceeds *via* the minor three-coordinate hydroxyphosphite **2**, but that the



Scheme 1



Scheme 2

intermediate phosphonium salt **4** can, in this case, lose a proton to give an iodophosphate intermediate **6**, Scheme 1, pathways B. It would not be surprising if this iodophosphate **6** were configurationally unstable leading to epimerisation of the phosphoryl centre. Furthermore, the stereochemical course of the displacement of iodide from the iodophosphate **6** will differ from that for the iodophosphonium salt **3**.

To establish this mechanism we have prepared 5-*tert*-butyl-2-hydroxy-1,3,2-dioxaphosphorinane **8** and **9** and subjected this mixture to oxidation in iodine–$H_2{}^{18}O$, Scheme 2. The resulting ^{18}O-labelled 5-*tert*-butyl-2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane as its pyridinium salt **14** was methylated directly with diazomethane and the location of the ^{18}O determined by ^{31}P NMR spectroscopy.[6] 5-*tert*-Butyl-2-hydroxy-1,3,2-dioxaphosphorinane **8** and **9** was prepared by literature methods[7] and could be shown to be substantially enriched (*ca.* 5 : 1) in the *cis* isomer **8**. Simple silica gel chromatography failed to separate these diastereoisomers and vacuum distillation gave rise to equilibration leading to an approximately 1 : 1 ratio of the diastereoisomers **8** and **9**. Controlled heating generated mixtures intermediate in composition. The I_2–$H_2{}^{18}O$ oxidation of samples of **8** and **9** with diastereoisomeric ratios 5 : 1, 2.5 : 1 and 1 : 1 were conducted under identical conditions.† For each separate reaction the phosphate diester was isolated by aqueous extraction and methylated with diazomethane. High-field NMR spectra of the resulting axial and equatorial 5-*tert*-butyl-2-methoxy-2-oxo-1,3,2-dioxaphosphorinane **15** and **16** and their ^{18}O isotopomers established the ^{18}O distribution in the 5-*tert*-butyl-2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane arising from the oxidation. Regardless of the diastereoisomeric ratio of 5-*tert*-butyl-2-hydroxy-1,3,2-dioxaphosphorinane, oxidation with I_2–$H_2{}^{18}O$ gave rise to the pyridinium salt of 5-*tert*-butyl-2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane **14** with *ca.* 80% of the ^{18}O *cis* to the *tert*-butyl group. Clearly, the reaction is completely non-stereospecific supporting the mechanistic proposals described above. These observations are in marked contrast to our earlier observations concerning the oxidation of phosphite triesters.[2]

† 5-*tert*-Butyl-2-hydroxy-1,3,2-dioxaphosphorinane [25 mg, 140 mmol dm^{-3}; δ_P (CH_2Cl_2): +3.8 (major), +1.8 (minor)] in pyridine (2 ml) was treated with iodine (53 mg; 208 mmol dm^{-3}) in $H_2{}^{18}O$ (200 μl, *ca.* 50 atom%) and pyridine (1 ml). The reaction mixture was stirred at room temp. for 90 min and the excess of iodine discharged by addition of mercaptoethanol. The reaction mixture was evaporated and the residue partitioned between water and chloroform and the (^{18}O) 5-*tert*-butyl-2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane was isolated as its pyridinium salt from the aqueous layer [δ_P(CH_2Cl_2–CH_3OH) −3.9]. Without further purification, this material was methylated with diazomethane at 0 °C in acetonitrile–diethyl ether. The resulting *cis* and *trans* 5-*tert*-butyl-2-methoxy-2-oxo-1,3,2-dioxaphosphorinane were obtained as an oil [65% yield overall; δ_P (CH_2Cl_2) −4.58 (eq), −6.19 (ax)].

Although the reaction is non-stereospecific, it shows a high degree of stereoselectivity in giving rise to a *ca.* 4 : 1 mixture of *cis* : *trans* (^{18}O) 5-*tert*-butyl-2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane as their salts **14**. The major diastereoisomer of the cyclic phosphite diester has been assigned the *cis* configuration (P–H and Bu^t *cis*) on the basis of the relative ^{31}P NMR chemical shifts[8] and confirmed by NOE experiments. In the absence of any knowledge of the diastereoisomeric ratio in the iodophosphate intermediates (these could not be detected by ^{31}P NMR spectroscopy during the course of the reaction) it is difficult to account fully for this stereoselectivity. Assuming that the formation of the iodophosphonium salts **10** and **11** proceed stereospecifically, total lack of stereospecificity must imply that a thermodynamic ratio of the iodophosphates **12** and **13** is obtained, presumably either catalysed by pyridine[9,10] or involving nucleophilic displacement by iodide.‡ It is not possible to deduce the stereochemical course of the hydrolysis **12** and **13** and indeed this could proceed with inversion, retention or with mixed stereochemistry.

Thus, oxidation of phosphite triesters provides a convenient stereospecific route to ^{18}O or ^{17}O-labelled phosphates, whereas the corresponding oxidation of diastereoisomeric *H*-phosphonates is clearly not appropriate.

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‡ The fact that 5 : 1, 2.5 : 1 and 1 : 1 mixtures of **8** and **9** all gave the same 4 : 1 ratio of **14a** and **14b** cannot be accounted for by simply assuming that the iodophosphates **12** and **13** hydrolyse non-stereospecifically and requires us to assume that some equilibration of **12** and **13** must occur.