and NADPH. While the results of others cited above indicate that one pathway for the enzymatic removal of carbon atom 32 of 14α-alkyl and 14α-hydroxymethyl sterols requires molecular oxygen, our results clearly demonstrate that the enzymatic removal of a steroidal 14α-hydroxymethyl group can also proceed via oxygen-independent processes. Moreover, our findings provide a logical explanation for the occurrence of $\Delta^{8(14)}$ sterols in nature[30–34] and for the existence of enzymes in liver which catalyze the conversion of $\Delta^{8(14)}$ sterols to cholesterol.[11,25,26,35,36]

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Robert A. Pascal, Jr., Peter Chang
George J. Schroepfer, Jr.*
Departments of Biochemistry and Chemistry
Rice University, Houston, Texas 77001
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Determination of the Absolute Configuration of [^{16}O,^{17}O,^{18}O]Phosphate Monoesters by Using ^{31}P NMR

Sir:

We recently reported a general synthetic approach to chiral [^{16}O,^{17}O,^{18}O]phosphate monoesters and a mass spectrometric method that allows the quantitative determination of the absolute configuration at phosphorus in such molecules.[1] These techniques have subsequently been used to determine the stereochemical course of enzymes that catalyze phosphoryl group transfer reactions, namely, phosphatases,[2] phosphokinases,[3] and phosphomutases.[4] Our original method for the determination of the absolute configuration involves linked-scan metastable ion mass spectrometry, and we have been concerned to devise a less circuitous approach to solve this problem. We report here an alternative solution that is simpler both conceptually and practically, and exploits the sensitivity of ^{31}P NMR signals to the nature of the attached oxygen isotopes.

To illustrate the method, we use the key compound of our earlier approach, 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol (**1**). When this material, *R* at phosphorus, is treated with (diphenylphosphoryl)imidazole,[1,5] three isomeric cyclic diesters (the 2,2-dioxo-1,3,2-dioxaphospholanes) are produced in equimolar ratios by "in-line" ring closure, as shown in Figure 1. Methylation of these species with diazomethane[1] results in the formation of the six cyclic triesters, comprising three "syn" isomers (**2**, **3**, and **4**) and three "anti" isomers (**5**, **6**, and **7**) (see Figure 1). While these two sets of diastereoisomers can be separated,[1] there is no need to do this in the present case, since the ^{31}P NMR signals for the syn and anti materials (for the all-^{16}O mixture) are separated by about 0.1 ppm. It has recently been shown that the quadrupolar

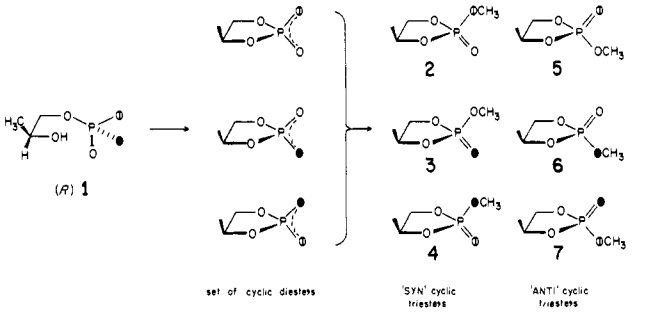

Figure 1. The three cyclic diesters and six cyclic triesters that are derived from 1-(*R*)-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol by "in-line" ring closure and methylation.[15]

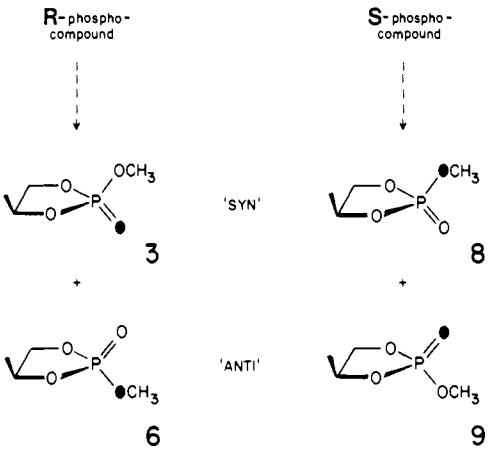

Figure 2. The two ^{18}O-labeled species that are derived from the "in-line" ring closure and methylation of labeled 1-phospho-(*S*)-propane-1,2-diols that are *R* or *S* at phosphorus.[15]

effect of ^{17}O causes such a broadening of the ^{31}P resonances of compounds containing ^{31}P–^{17}O bonds,[6,7] that the ^{31}P NMR spectrum of the mixture of compounds **2**–**7** should contain sharp peaks only for those species *not* containing any ^{17}O, i.e., **3** and **6**. If, therefore, the spectrum of a mixture of **3** and **6** (deriving from a phosphoryl group that was *R*) can be distinguished from the spectrum of a mixture of **8** and **9** (deriving from an *S* phosphoryl group), the absolute configuration at phosphorus can be determined (see Figure 2). The distinction between **3** + **6** and **8** + **9** is readily made, and depends upon the heavy oxygen isotope shift on the ^{31}P NMR signal.[8] The magnitude of the upfield chemical shift caused by ^{18}O directly bonded to ^{31}P depends on the nature of the ^{31}P–^{18}O bond,[7,9] the greater the double-bond character, the greater the shift. For the problem at hand, therefore, we can expect that the ^{31}P line[10] of the syn isomer will be at higher field for **3** than for **8**, and the resonance for the anti isomer will be further upfield for **9** than for **6**.

The solution is not quite so simple, however, since the isotopic content of the $H_2{}^{17}O$ used in the synthesis of **1** is no better than approximately 1:2:1 for ^{16}O/^{17}O/^{18}O.[11] This means that the ^{31}P NMR spectra will contain signals from the unlabeled ^{16}O,^{16}O compounds, the "incorrect" ^{16}O,^{18}O species, and the doubly labeled ^{18}O,^{18}O isomers, in addition to the "correct" ^{16}O,^{18}O materials. The relative proportions of all these species can easily be determined, of course, from the known isotopic content of **1** and the known isotopic composition of the $H_2{}^{17}O$ and $H_2{}^{18}O$ samples used

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(10) Broad-band proton decoupling is used in all these experiments.
(11) The actual isotopic content of the peripheral oxygens of the *R* phospho compound is the following: ^{16}O, 44.1%; ^{17}O, 16.1%; ^{18}O, 39.8%. *S* phospho compound: ^{16}O, 44.7%; ^{17}O, 14.6%; ^{18}O, 40.7%.

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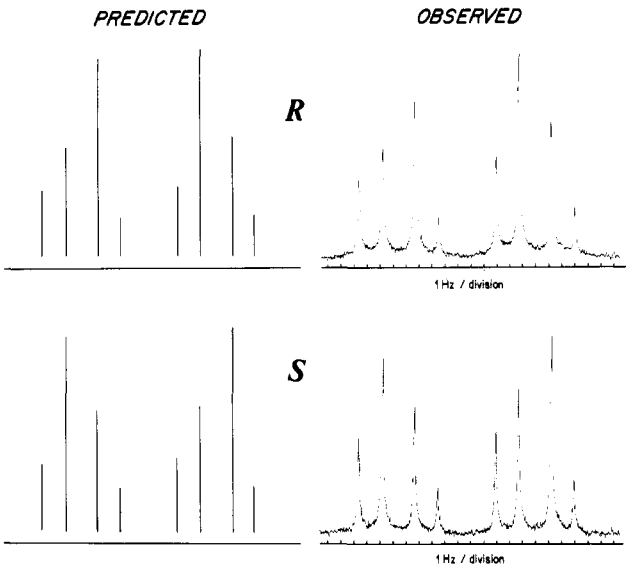

Figure 3. Predicted and observed ^{31}P NMR spectra of the mixtures of syn and anti cyclic triesters derived from labeled samples of 1-phospho-(*S*)-propane-1,2-diols that are *R* or *S* at phosphorus. (Spectrum of the *S* epimer, 200 transients; *R* epimer, 291 transients.)

in the synthesis. This knowledge permits one to predict the intensities of the eight lines in the ^{31}P NMR spectrum, four of which derive from the syn diastereoisomer and four from the anti diastereoisomer. The predicted spectra[12] are shown in Figure 3, along with the actual spectra[14] for the cyclic triesters derived from *R* and *S* 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol, synthesized independently.[15] The observed ^{18}O isotopic shifts (upfield from the ^{16}O,^{16}O compound, which gives the downfield signal of each set of four) are 0.018 ppm for the compounds containing one singly bonded ^{18}O (**6** and **8**), 0.043 ppm for the compounds containing one doubly-bonded ^{18}O (**3** and **9**), and 0.060 ppm for the materials containing two exocyclic ^{18}O atoms.

The spectra in Figure 3 show that a complementary pattern of eight peaks is obtained from the cyclic triesters obtained by ring closure and methylation of the two chiral phosphopropanediols. The agreement between the predicted and observed spectra makes the assignment of the absolute configuration at phosphorus unambiguous. Signal integration suggests that the *S* epimer contains about 82% of the *S* material and the *R* epimer contains about 76% of the *R* compound, though the imprecision of such integration makes these only approximate values.

It is therefore clear that high-resolution ^{31}P NMR offers a straightforward and experimentally simple method for the determination of the absolute configuration of [^{16}O,^{17}O,^{18}O]phosphate monoesters. The method is general, since we have already demonstrated the practicality of transferring a phosphoryl group from any location to (*S*)-propane-1,2-diol with retention of configuration.[2,3,4] Although less quantitative than our earlier approach,[1] the NMR method avoids both the need to separate the syn and anti diastereoisomers of the cyclic triesters and the need for linked-scan metastable ion mass spectrometry.

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(17) National Science Foundation Predoctoral Fellow.

Stephen L. Buchwald,[17] Jeremy R. Knowles*

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

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Bioorganic Synthesis and Absolute Configuration of Faranal

Sir:

Faranal, a trail pheromone of the pharaoh ant, has a unique structure which is reminiscent of a juvenoid and is interesting from a biosynthetic point of view. The structure was first reported to be **6a** or its antipode (**6b**),[1] but later was revised as **7a** or its antipode (**7b**).[2] Since the pheromone is obtainable only in such a small quantity that no optical rotation datum is available, asymmetric synthesis of **7a** and **7b** by an unambiguous route and the comparison of their biological activity would be desirable to determine the absolute configuration. Farnesyl pyrophosphate synthetase is a promising candidate for an agent of central importance for this purpose, because we have shown that this enzyme can synthesize stereospecifically the *S* or *R* enantiomer of 4-methylfarnesyl pyrophosphate, depending on whether it is supplied with geranyl pyrophosphate and (*E*)-**1a** or (*Z*)-3-methylpent-3-enyl pyrophosphate (**1b**) as substrate.[3,4] This paper reports an application of biochemical systems in the asymmetric synthesis of the stereoisomers of faranal, leading to the conclusion that the absolute configuration of the natural faranal is 3*S*,4*R*.

Enzymatic condensation of **1a** or **1b** with homogeranyl pyrophosphate (**2**) obtained by the phosphorylation of 2(*E*),6(*Z*)-3,7-dimethylnona-2,6-dien-1-ol[5] was carried out on a large scale

(12) The predicted spectra are calculated on the basis that the (*S*)-2-benzylpropane-1,2-diol used in the synthesis[1] had 82.8% enantiomeric excess, as determined by the separation and quantitation (A_{254}) of the diastereoisomeric esters of (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543), that the isotopic composition of the peripheral oxygen atoms of the *R* and *S* phospho compounds was as in ref 11, and that the ratio of syn to anti cyclic triesters was 0.95 ± 0.05 (from integration of the two sets of four lines of the ^{31}P NMR spectra[13]).

(13) No attempt was made to correct for any NOE differences between the syn and anti isomers.

(14) Fourier transform ^{31}P NMR spectra were obtained through the kindness of P. Ziegler of Bruker Instruments, Inc. A Bruker WM-250 instrument was used[10] at 101.27 MHz with a deuterium field lock. A spectral width of 1000 Hz was used with a pulse width of 22 μs and an acquisition time of 8.2 s. The sample [approximately 140 μmol of the bis(tri-*N*-octylammonium) salt of *R* or *S* 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol] was cyclized by using diphenylphosphorylimidazole[1,15] (125 μmol) in CH_2Cl_2. After workup,[1] the cyclic diesters were dissolved in CD_3CN/CH_3CN (3:7, v/v) and esterified with excess ethereal diazomethane. The resulting solution of cyclic triesters was concentrated to 2 mL and filtered through glass wool into a precision 10-mm NMR tube under dry argon.

(15) The method described in ref 1 was used, the configuration at phosphorus being determined simply by the order of introduction of ^{17}O and ^{18}O.

(16) These figures are drawn on the hypothetical basis that the oxygen isotopic enrichment at the indicated positions is 100% (see text).

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