Absolute Configuration of Chiral *O*-2-Butyl Ethylphosphonothioic Acid

The absolute configuration of (−)-*O*-2-butyl (−)-ethylphosphonothioic acid, chiral at the 2-butyl carbon atom and phosphorus atom, was determined as *R* and *S*, respectively, by X-ray crystallographic analysis of its α-phenylethylammonium salt.

In a recent publication (Wustner and Fukuto, 1973) we reported on the preparation and toxicological properties of the four chiral isomers of *O*-2-butyl *S*-2-(ethylthio)ethyl ethylphosphonothioate (**1**) in which chirality resided on the 2-butyl carbon atom and the phosphorus atom. A subsequent report (Wustner and Fukuto, 1974) described a comprehensive study of the kinetic aspects of cholinesterase inhibition by a series of analogous chiral isomers of the related ester *O*-2-butyl *S*-2-(dimethylammonium)ethyl ethylphosphonothioate hydrogen oxalate (**2**). Each of the chiral isomers of these organophosphorus esters was prepared directly from the four corresponding isomers of *O*-2-butyl ethylphosphonothioic acid (**3**), resolved *via* the α-phenylethylammonium salt. In light of the profound differences in anticholinesterase and insecticidal activity observed between chiral isomers of these and related esters (Fukuto and Metcalf, 1959; Hassan and Dauterman, 1968; Hilgetag and Lehman, 1959) it was of fundamental importance to establish the absolute configuration of the asymmetric centers in the esters. Since both **1** and **2** were prepared by chemical reactions which did not affect the chiral centers, *i.e.*, the 2-butyl carbon or phosphorus atoms, it was possible to relate absolute configuration of the isomers of these compounds with those of the intermediate acid **3**. This report is concerned with establishment of the absolute configuration of (−)-*O*-2-butyl (−)-ethylphosphonothioic acid (**3a**) in the form of its α-phenylethylammonium salt by X-ray crystallographic analysis.

(−)-*O*-2-Butyl (−)-ethylphosphonothioic acid (**3a**), bp 80–81° (0.03 mm), $n^{25}D$ 1.4784, $\alpha^{26}D$ −33.967° (neat), $[\alpha]^{26}D$ −9.128° (ethanol), was treated with (−)-α-phenylethylamine to afford the corresponding ammonium salt, **4a** (Wustner and Fukuto, 1973). Appropriate crystals of **4a** were obtained as colorless needles, mp 149–149.5°, $[\alpha]^{26}D$ −13.718° (ethanol), by slow evaporation of a pentyl acetate solution. (*Anal.* Calcd for $C_{14}H_{26}NO_2PS$: C, 55.42; H, 8.64. Found: C, 55.25; H, 8.48.) Weissenberg and precession photographs showed monoclinic symmetry and space group $P2_1$. The unit cell constants, a = 11.553 (12), b = 6.700 (6), and c = 11.723 (12) Å, and β = 107.03 (2)°, were determined from a least-squares fit of 12 reflections measured on a Picker automatic diffractometer (Mo Kα, λ = 0.71069 Å). The density of the crystal measured by flotation was 1.15 g cm^{-3}, which agrees with the value of 1.161 g cm^{-3} calculated for two molecules of $C_{14}H_{26}NO_2PS$ in a unit cell.

Intensity data were collected on the above diffractor, using Mo Kα radiation. Reflections having 2θ values up to 36° (638 unique reflections) were collected by the 2θ–θ scan technique at a scan rate of 1°/min and a scan range of 1.9°. Background counts of 10 sec were made at each end of the scan.

The structure was solved by the heavy-atom method and refined by full matrix least-squares calculations, using 569 reflections which were greater than 1.5σ. The phosphorus and sulfur atoms were treated anisotropically. The refinement converged to a final residue R of 9.6%. All hydrogen atoms were observed on the difference electron-density map, but not included in the refinement. Final atomic coordinates are given in Table I.

A structural view of **4a** is shown in Figure 1. The quaternary ammonium protons are hydrogen bonded to three neighboring ions, one on the sulfur and two on the oxygen atoms of two different organophosphorus molecules. Hydrogen bonding angles and distances were not thought relevant to the resulting inhibitor molecules and were not calculated. One of the N-H···O bonds is shown in Figure 1. The absolute configuration of the (−)-*O*-2-butyl (−)-ethylphosphonothioate moiety was determined as R_CS_P, *i.e.* *R* for carbon and *S* for phosphorus, by relating to the known configurations of the (*S*)-(−)-α-phenylethylammonium ion and (*R*)-(−)-2-butyl alcohol (Cahn *et al.*, 1956).

Based on the assignment R_CS_P for **4a**, the absolute con-

Table I. Final Positional Parameters[a] in (*S*)-(−)-α-Phenylethylammonium Salt of (*R*)-(−)-*O*-2-Butyl (*S*)-(−)-Ethylphosphonothioate

Atom	*x*	*y*	*z*
S	−0.0053 (7)	1.0000 (0)	0.2046 (6)
P	−0.0377 (7)	0.7145 (15)	0.2328 (6)
O-1	−0.0119 (12)	0.6464 (24)	0.3627 (13)
O-2	−0.1762 (14)	0.6592 (28)	0.1758 (14)
N	−0.0861 (15)	0.2702 (27)	0.4025 (14)
C-1	−0.2956 (24)	0.3879 (46)	0.3961 (23)
C-2	−0.2583 (19)	0.4577 (42)	0.5092 (20)
C-3	−0.3315 (24)	0.6013 (44)	0.5534 (23)
C-4	−0.4418 (23)	0.6542 (49)	0.4694 (25)
C-5	−0.4777 (24)	0.5858 (47)	0.3551 (25)
C-6	−0.3991 (23)	0.4527 (43)	0.3181 (21)
C-7	−0.2209 (20)	0.2308 (46)	0.3519 (19)
C-8	−0.2501 (20)	0.0208 (46)	0.3938 (19)
C-9	−0.3424 (24)	0.8630 (60)	0.0557 (25)
C-10	−0.2401 (22)	0.7090 (52)	0.0471 (23)
C-11	−0.2955 (28)	0.4787 (63)	0.0003 (30)
C-12	−0.3516 (38)	0.5146 (59)	−0.1156 (41)
C-13	0.0394 (23)	0.5439 (46)	0.1524 (21)
C-14	0.1765 (22)	0.5628 (44)	0.2092 (21)
H-C-7	−0.243	0.238	0.263
H-C-10	−0.195	0.769	−0.005

[a] Standard deviations given in parentheses are in least significant digits.

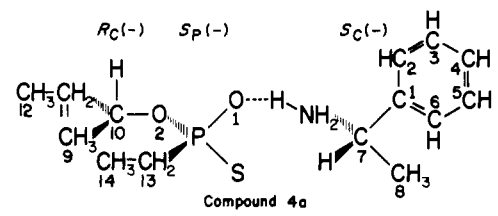

Figure 1. Absolute configuration of (*S*)-(−)-α-phenylethylammonium (*R*)-(−)-*O*-2-butyl (*S*)-(−)-ethylphosphonothioate (**4a**). The numbering of the atoms corresponds to the numbering of the atomic coordinates given in Table I.

figurations of the 2-butyl carbon atom and phosphorus atom in (−)-*O*-2-butyl *S*-2-(ethylthio)ethyl (−)-ethylphosphonothioate (1a) and (−)-*O*-2-butyl *S*-2-(dimethylammonium)ethyl (−)-ethylphosphonothioate hydrogen oxalate (2a) also are assigned the configurations R_CS_P. The configurations of the remaining chiral isomers may be deduced from their respective optical rotations.

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Rapid Determination of Maleic Hydrazide in Cigarette Smoke Condensate and Particulate Matter

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione), a systemic plant growth regulator, is used extensively as a tobacco sucker inhibitor. A rapid method has been developed to determine quantities of maleic hydrazide (MH) in tobacco smoke condensates. The method involves microcolumn chromatography on alumina, derivatization to form the bis(trimethylsilyl) derivative, and quantitation by gas chromatography. This method has been applied to both cigarette smoke condensate and total particulate matter of cigarette smoke. Various aspects of methodology as well as MH transfer rates from cigarettes into smoke are discussed. Significantly, more than 99% of the MH content of cigarette tobacco was destroyed during smoking.

Maleic hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione), a systemic plant growth regulator, is used by tobacco farmers throughout the world to inhibit growth of suckers on tobacco plants (Tso, 1972). It is usually applied to the upper half or third of the tobacco plant within 24 hr after topping. Subsequently, due to absorption and translocation, MH is found throughout the entire plant. Interest in MH contents of tobacco and tobacco smoke has arisen due to future export restrictions on agricultural chemical residues on tobacco and possible tumorigenic properties of MH in animal tests (Epstein *et al.*, 1967; Epstein and Mantel, 1968).

In a recent paper (Haeberer *et al.*, 1974), a new gas chromatographic method for the analysis of MH residues in tobacco was reported. This method was a marked improvement over earlier optical methods (Wood, 1953; Anglin and Mahon, 1958; Lane *et al.*, 1958; Hoffman, 1961). Although this method (Haeberer *et al.*, 1974) produced excellent results for MH levels in tobacco, its application to cigarette smoke condensate (CSC) or total particulate matter (TPM) was complicated by interfering compounds, which could not be resolved by gas chromatography.

This report details a rapid, quantitative method for determination of maleic hydrazide in both CSC and TPM. The procedure involves microcolumn chromatography of the crude CSC or TPM, derivatization of partially purified MH, and final separation and quantitation by gas chromatography using a flame-ionization detector. Concentrations as low as 0.1 μg of MH in 1 g of CSC have been determined. MH transfer rates from cigarettes to smoke were studied for fortified and original cigarettes.

EXPERIMENTAL SECTION

Reagents. Ethyl acetate was purchased as the analytical reagent grade (Mallinckrodt Chemical Works), dimethylformamide as the spectrophotometric grade (J. T. Baker Chemical Co.), *N,O*-bis(trimethylsilyl)acetamide as the specially purified grade (Pierce Chemical Co.), and 100/120 mesh AG-7 alumina in the fully activated form (Bio-Rad Laboratories). Maleic hydrazide was purchased in the practical grade (Eastman Kodak Co.) and recrystallized twice from distilled water.

Apparatus. A Varian Aerograph gas chromatograph Model 2800, with flame ionization detectors and glass injector liners, was used for the analysis. The separation was performed on 20% OV-11 on Chromosorb W-HP (100–120 mesh). Gas chromatograms were integrated with an Infotronic Model CRS-204 electronic digital integrator.

Cigarettes and Smoke Condensate Preparation. Cigarette smoke condensate and University of Kentucky experimental reference cigarettes (85-mm, nonfilter, type 1R1) (Atkinson, 1970; Benner, 1970) were used for the development of the analytical method and for the determination of the MH transfer rates, respectively. CSC was prepared at the Roswell Park Memorial Institute from commercial 85-mm nonfilter cigarettes. Cigarettes were fortified with 0.1, 0.2, 0.4, 0.8, and 1.6 mg of MH by uniform syringe injection (Lakritz, 1973) of MH solutions. Cigarettes were conditioned at 21° and 60% relative humidity for 62 hr, then smoked on a Mason 24-port smoking machine at a standard rate (one puff/min, 2-sec duration, 35-ml puff volume) to a butt length of 23 mm. The mainstream smoke was drawn through a Cambridge-filter assembly and the smoke particulate matter trapped on a tared filter pad. Immediately after smoking, the pads were weighed and extracted with dimethylformamide (DMF) in a micro Soxhlet apparatus for 4 hr. About 25 ml of DMF was used per g of TPM. After extraction, the concentrations of the DMF solutions were adjusted to 30% TPM based on original TPM weight, by evaporation of DMF on a hot plate (100°), and diluted to volume with DMF.