Separation and Spectral Properties of Diisopropylphosphate, the Major Decomposition Product of Isoflurophate

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Abstract □ The reaction of water with isoflurophate to form diisopropylphosphate was examined and confirmed. Isolation of this decomposition product from an antiglaucoma drug formulation is described. A known reference compound was isolated from a commercial mixture also containing the monoisopropyl ester. The isolation, purification, and molecular spectroscopic and elemental confirmation of structure are described. IR, NMR, and mass spectra are included. Additionally, a GLC procedure and parameters used to identify diisopropylphosphate in a degraded peanut oil formulation of isoflurophate are reported. Reaction mixtures of this drug with water and sodium hydroxide were analyzed by GLC with the expected results.

Keyphrases □ Diisopropylphosphate—analysis, IR, NMR, mass spectra, GLC, isolation as degradation product from peanut oil isoflurophate formulation □ Isoflurophate—degradation products, diisopropylphosphate, analysis, peanut oil formulation □ GLC—analysis, diisopropylphosphate, isolation as degradation product from peanut oil isoflurophate formulation □ Antiglaucoma agents—isoflurophate, degradation products, diisopropylphosphate isolated from peanut oil formulation, GLC

Isoflurophate (I) is an important and highly potent drug in glaucoma treatment and is a well-known inhibitor of acetylcholinesterase and other enzymes (1). It reacts with moisture with concurrent loss of activity and formation of hydrofluoric acid and diisopropylphosphate (II) (1). Ion-specific electrodes were used to detect ionic fluoride resulting from this hydrolysis. Analytically, a poor correlation with the degree of hydrolysis was noted.

Several reports (2–4) indicated that the major hydrolytic decomposition product of I was II, but no confirmatory qualitative evidence was presented. The presumed chemical hydrolysis is shown in Scheme I. The present work was undertaken to obtain this necessary qualitative information. Lindemann developed an assay for intact I using GLC, which is described in USP XIX (5). This paper provides associated general analytical information for II including isolation, purification, and spectral data.

$$\underset{\text{I}}{[(CH_3)_2CHO]_2P(O)F} + H_2O \rightarrow \underset{\text{II}}{[(CH_3)_2CHO]_2P(O)OH} + 1/2H_2F_2$$

Scheme I

EXPERIMENTAL

Reagents and Chemicals—Compound II was not available commercially in pure form. A reference material was obtained by separation from a 1:1 mixture of II with the monoisopropyl ester[1]. Petroleum ether[2], used in the isolation of II, was analytical reagent grade. Compound I[3] was used in the hydrolysis experiments.

GLC—All GLC retention time data were obtained on a gas chromatograph equipped for flame-ionization detection[4]. Samples were reacted with excess diazomethane in ether, the ether was evaporated, and the residue was dissolved in carbon disulfide prior to injection. Separation was conducted on a 1.52-m long × 4-mm i.d. glass column containing 4% QF-1 on 80–100-mesh Gas Chrom Q. The column temperature was 105°, the flow rate was 40 ml/min, the inlet temperature was 225°, and the detector temperature was 295°. The chart speed was 38 cm/hr. A 1-mv recorder was used.

NMR—An NMR spectrometer equipped with a proton probe[5] and a 5-mm sample tube was used (Fig. 1).

Mass Spectrometry—An authentic II sample was derivatized with *N,O*-bis(trimethysilyl)acetamide in pyridine (for 30 min at 60°) to form the trimethylsilyl derivative. All mass spectra were obtained on a mass spectrometer[6] using a direct probe. The ionizing and accelerating potentials were 70 ev and 3.5 kv, respectively. The ion source temperature was 270°. The mass spectrum of the derivative is presented in Fig. 2.

IR Spectrophotometry—The IR spectrum of authentic II (Fig. 3) was run as a capillary film of the neat liquid between potassium bromide windows. The IR spectrophotometer[7] was set for linear frequency measurement and equipped with a wide Nernst glower. Parameters were: slits, programmed at 1000; gain, normal; scan speed, 32; attenuator speed, 1100; and source, 0.78 amp.

Isolation of Major Decomposition Product of I—A 10-year-old sample of 1% I in mineral oil was selected for isolation of the major decomposition product. The sample had been stored in a wide-mouth bottle enclosed in a metal can at ambient conditions near 24° during this period. No other protection against moisture uptake was in evidence. The sample assayed 0% I by GLC (5). An immiscible phase in the mineral oil was removed by centrifugation and subjected to IR analysis.

Isolation of Authentic II—Pure II was isolated utilizing the low solubility of the monoisopropyl compound in petroleum ether. Fifty grams of the 1:1 mixture in a 500-ml erlenmeyer flask was diluted to 300 ml with petroleum ether. After thorough shaking, the mixture was allowed to separate and the petroleum ether phase was decanted[8] into a large calibrated beaker. Second and third elutions were made in this manner, and the petroleum ether phases were combined by decantation[8] and evaporated without heat; decantations were performed when 450-, 350-, and 200-ml volumes were reached. The solution was allowed to stand until clear. Materials not remaining in the petroleum ether were discarded.

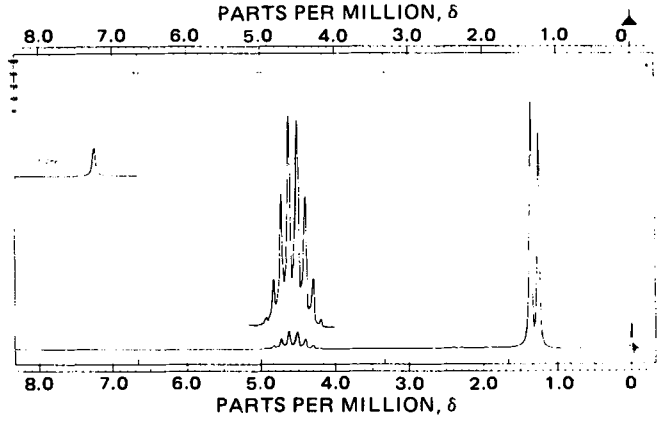

Figure 1—*PMR spectrum of diisopropylphosphate (I).*

[1] Lot 50989X, ICN, K & K Labs.
[2] Lot WCML, Mallinckrodt.
[3] Lot 030-127-1, Aldrich.
[4] Micro Tek MT-220.
[5] Varian A-60D.
[6] LKB model 9000S.
[7] Perkin-Elmer PE-521.
[8] Concentration of the petroleum ether was performed in open beakers. An isolable oil (mostly monoisopropyl ester) separated and was easily removed by decantation of the more mobile hydrocarbon phase into a clean beaker. The evaporation was continued.

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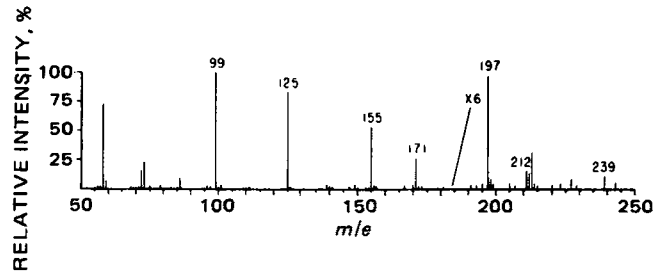

Figure 2—*Mass spectrum of diisopropylphosphate (I) as the trimethyl silyl derivative.*

The petroleum ether was warmed to ~30°. Caution was exercised to prevent sample loss through ebullition. Evaporation was continued using an air stream. The top phase was decanted[8] when the 150-, 125-, 100-, and 85-ml volumes had been reached. In this series of decantations[8], the insoluble oil phases were eluted in the same order that they were obtained with a single 35-ml portion of fresh petroleum. This eluate was combined with the 85 ml remaining from the evaporation step, placed in a separator, and extracted with 8 and then with 4 ml of absolute methanol. The petroleum ether phase was separated and evaporated using an air stream, with the last traces of solvents being removed at room temperature under vacuum. The product was dried over sulfuric acid. The yield was 6 g or 24% based on the claimed 1:1 mixed ester weight ratio.

RESULTS AND DISCUSSION

Although the starting mixture used to identify the isolated decomposition product was marketed as containing II, supportive analytical data were obtained to confirm this structure because previously reported IR spectral frequencies (6) of a II sample gave questionable agreement with the spectrum shown in Fig. 3. Close IR spectral checks, however, were obtained between the decomposition product isolate and the authentic II. The IR spectrum of II was sufficiently complex to provide a positive identification.

As indicated in Fig. 2, no molecular ion was observed in the mass spectrum of the trimethylsilyl derivative of authentic II; however, an M − 15 ion was observed at *m/e* 239 (characteristic methyl loss in trimethyl silyl derivative). Other characteristic ions were detected at the following *m/e* values: 212 (M − 42, loss of propylene due to McLafferty rearrangement), 211 (M − 43, homolytic cleavage of isopropyl group), 197 (loss of methyl and propylene), 171 (loss of two propylene moieties *via* McLafferty rearrangement mechanism), 155 [from $(HO)_2P(=O)\text{-}O{=}Si(CH_3)_2^+$ ion], and 99 [base peak, $^+HO{=}P(OH)_3$ ion]. The extremely low intensity ion at *m/e* 243 resulted from the ditetramethylsilyl phosphoric acid derivative.

Elemental analysis was obtained on "authentic" II.

Anal.—Calc. for $C_6H_{15}PO_4$: C, 39.56; H, 8.30. Found: C, 39.57; H, 8.32.

The PMR spectrum of authentic II (Fig. 1) was in accord with the structure both qualitatively and quantitatively within experimental error. Assignments are: 1.30-ppm methyl hydrogens on isopropyl groups, 4.58-ppm multiplet due to methine hydrogens on two isopropyl groups;

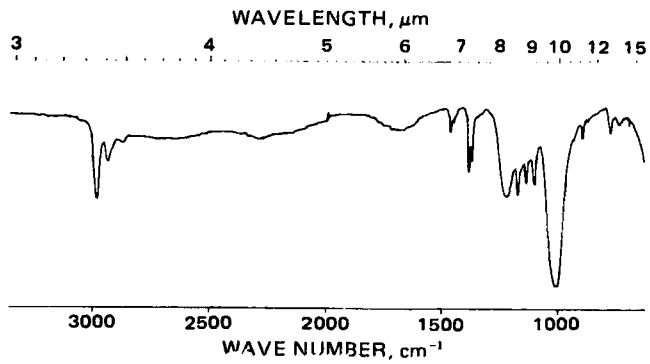

Figure 3—*Diisopropylphosphate IR spectrum.*

J = 7 Hz, with coupling of about the same J value to ^{31}P; and 12.35-ppm acidic exchangeable proton on P–O–H.

Assignments for the IR spectrum from Fig. 3 were reported in a study of band origins on analogous phosphoric acid derivatives (6).

GLC retention time data[9] were obtained for the decomposition product from mineral oil and authentic II. These retention times were matched by peaks obtained for decomposition caused by the action of water and by the action of 0.1 *N* NaOH (aqueous) on I. An old lot of a product formulation of 0.1% I in peanut oil chromatographed in the same system also showed the peak.

The GLC retention time of authentic II (225 sec) showed excellent agreement with the times for the following samples: (*a*) major product formed on reaction of I and water, 227 sec; (*b*) major product formed on reaction of I and aqueous 0.1 *N* NaOH, 227 sec; (*c*) 1:1 I–monoester mixture (starting material), 227 sec; and (*d*) aged 0.1% I in peanut oil, 226 sec.

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[9] All retention time data refer to the methylated products.