Synthesis of (Diethyl-d_{10}) Coumaphos and Related Compounds

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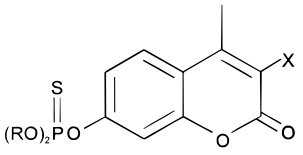
Two deuterated insecticides were prepared for use as internal standards for gas−liquid chromatographic−mass spectrometric analyses. Diethyl chlorothiophosphate-d_{10} was prepared by reaction of ethanol-d_6 with P_2S_5 to give labeled diethyldithiophosphoric acid, followed by chlorination. Treatment of the acid chloride with 3-chloro-4-methyl-7-hydroxycoumarin and potassium carbonate in acetone at reflux gave labeled coumaphos. An analogous reaction with 4-methyl-7-hydroxycoumarin gave labeled potasan, and the technique should be usable for synthesis of labeled forms of other dialkyl thiophosphate insecticides.

Keywords: *Coumaphos-d_{10}; potasan-d_{10}; deuterated pesticides; labeled pesticides*

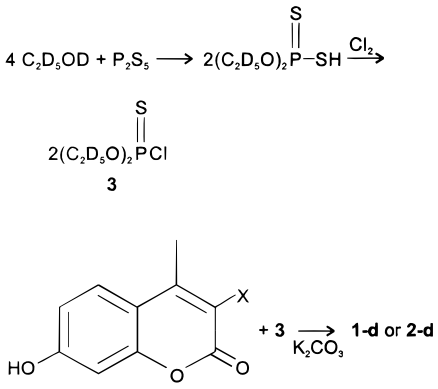
Coumaphos (**1**) was introduced as an insecticide in 1954 (Bayer 21/199; Gersdorff et al., 1954) and has been widely used since as an insecticide, particularly for the control of arthropod pests of domesticated animals. It was introduced in Germany for the control of the parasitic mite *Varroa jacobsoni* (Oudemans) infesting honey bees, *Apis mellifera* L. (Ritter, 1985), and was given an emergency registration in various of the United States in 1999 for the control of fluvalinate-resistant *Varroa* mites and small hive beetles infesting honey bees. Because of its nonpolar nature, it would be expected to partition heavily into wax in the hives.



1	X = Cl	R = C_2H_5
1-d	X = Cl	R = C_2D_5
2	X = H	R = C_2H_5
2-d	X = H	R = C_2D_5

We began a study of residues of this compound because the emergency registration allowed no tolerance for residues in hive products. Solid-phase extractions of honey gave concentrates containing interfering substances by HPLC, and we wished to avoid extensive partitions [for example, Thrasyvoulou and Pappas (1988)] or column chromatographic separations [for example, Kraus et al. (1983)] if possible. To improve accuracy in future GLC-MS analyses, we sought a good internal standard. Bacharova et al. (1988) reported the use of potasan (**2**, the dechloro analogue of coumaphos) as an internal standard, claiming that it had been reported not to be a metabolite of coumaphos [referencing Kaemmerer and Buntenkötter (1973)], but Kaemmerer and Buntenkötter gave a reference to a report (Bowman et al., 1968) of the detection of potasan after coumaphos had been fed to a cow. In any case, potasan has since been reported as a product of the bacterial degradation of coumaphos (Shelton and Karns, 1988; Karns et al., 1995) and so might be found in samples.

Scheme 1



Deuterated compounds are excellent for internal standards because they are not found naturally, and their extraction properties can be expected to be identical to those of the unlabeled analogues. For example, completely deuterated naphthalene, acenaphthene, phenanthrene, chrysene, and perylene are used as internal standards in analyses of semivolatile contaminants in environmental samples (U.S. EPA, 1996). No source of deuterated coumaphos could be found, nor could any reference to deuterated coumaphos be found in *Beilstein* or *Chemical Abstracts*, although coumaphos labeled with ^{32}P (Krueger et al., 1959) and ^{14}C [referred to in Shelton and Karns (1988)] has been reported. We prepared coumaphos containing 10 deuterium atoms/molecule by a modification of the method for the preparation of parathion reported by Fletcher et al. (1950), as summarized by Scheme 1. This involves reaction of an alcohol (4 mol) with phosphorus pentasulfide (1 mol as P_2S_5) to give diethyl phosphorodithioic acid, chlorination to a mixture of diethyl chlorothiophosphate and sulfur monochloride, hydrolysis of the S_2Cl_2 with water, and isolation of the desired acid chloride by extraction and distillation. This chloride is then converted to the desired ester. Parathion and diazinon similarly labeled are commercially available (C/D/N Isotopes Inc., Pointe-Claire, PQ, Canada), but no reference to deuterated parathion could be found in *Beilstein* or *Chemical Abstracts*.

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MATERIALS AND METHODS

Phosphorus pentasulfide and all organic starting materials were obtained from Aldrich Chemical Co. All calculations are based on phosphorus pentasulfide as P_2S_5 rather than P_4S_{10}. Benzene was dried by azeotropic distillation (Dean−Stark trap). Chlorine was obtained in a lecture bottle from Air Products. Methyl *tert*-butyl ether (MTBE, high-purity grade) was obtained from Arco Chemical Co. and used without further purification. Glassware was dried overnight or longer at 120 °C.

Gas chromatography was carried out on a Hewlett-Packard 5890 series II instrument with a 15 m × 0.53 mm DB-1 column (J&W Scientific, Folsom, CA). The column was held at 50 °C for 2 min, programmed at 15 °C/min to 300 °C, and held at 300 °C for 20 min, although the program was frequently terminated manually after the compounds of interest eluted. GLC-MS was performed on a Finnigan MAT GLCQ instrument with a Restek Rtx-5MS column (30 m, 0.25 mm i.d., 0.25 μm df, column head pressure = 0.9 bar, Restek Corp., Bellefonte, PA). The initial column temperature of 60 °C was held for 1 min, and then the temperature was raised 20 °C/min to 270 °C and held at 270 °C for 14.5 min (total run time = 26 min).

Proton and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker QE 300 instrument with a Mac NMR v5 data system. All proton and ^{13}C spectra were consistent with expected structures.

Synthesis of Diethyl Chlorothiophosphate-d_{10} (3). A 100 mL pear-shaped flask was fitted with a Claisen adapter equipped with a septum inlet and a condenser with a drying tube (Drierite). To the flask were added phosphorus pentasulfide (10.6 g, 48 mmol) and benzene (15 mL). The slurry was stirred magnetically and heated to reflux. To the slowly refluxing solution was added ethanol-d_6 (99+ atom % D, 9.948 g, 182 mmol, net contents of the ampules were somewhat below label amount) dropwise from a syringe over 30 min. A steady release of H_2S occurred during the addition, and most of the solid P_2S_5 dissolved. When all of the ethanol was added, the solution was heated at reflux for a further 2 h (most of the remaining solid dissolved, and the solution turned a dull green) and then cooled to room temperature.

Chlorine (5.5 mL, 122 mmol) was distilled from the cylinder and condensed into a 15 mL graduated centrifuge tube, which was cooled in a cold (∼−100°, Cryo-Cool CC-100 II, Neslab Inc, Portsmouth, NH) methanol bath. The flask containing the diethyldithiophosphoric acid was placed in a water bath, and ice was added occasionally to the bath to maintain it at ∼15 °C. The tube containing chlorine was fitted with a distillation head consisting of a right-angle adapter, a short piece of Tygon tubing, and a disposable Pasteur pipet. The chlorine was then distilled into the solution over 15−20 min, regulating the rate of distillation by cooling the tube as necessary with chilled methanol from the cold bath. The green color disappeared during the addition, and the solution became a clear orange-yellow. After an additional 20 min of stirring, the solution of diethyl chlorothiophosphate-d_{10} and sulfur monochloride was added over 30 min to water (40 mL) magnetically stirred in a 125 mL Erlenmeyer flask. After the addition was complete, the suspension was stirred for an additional 15 min and filtered through a pad of Celite to remove the gummy precipitate of sulfur. The organic layer was removed, and the aqueous phase was extracted once with MTBE. The organic phases were combined, washed once with water, dried ($MgSO_4$), and concentrated on a rotary evaporator. The resulting brownish oil (16.4 g) was distilled [bp 100−101.5 °C/35 mm, lit. (undeuterated compound bp 94−96 °C/20 mm; Fletcher et al., 1950)] to give a colorless oil, 14.065 g (78%), GLC 4.320 min (unlabeled 4.393 min). The pot residue seemed to be mostly sulfur.

Synthesis of Coumaphos-d_{10} (1-d). The deuterated acid chloride (5.04 g, 25.4 mmol) was added to a slurry of 3-chloro-4-methyl-7-hydroxycoumarin (5.34 g, 25.4 mmol) and powdered anhydrous potassium carbonate (6.35 g, 46 mmol) in acetone (75 mL). The mixture was heated at reflux with magnetic stirring. A sample taken after 4 h indicated the disappearance of starting materials by GLC. The mixture was filtered, and the filtrate was concentrated on a rotary evaporator to give a brownish yellow oil, which promptly solidified. The solid was dissolved in MTBE (125 mL, heating was required; a yellow gum remained undissolved, but dissolved on the addition of sodium carbonate solution) washed three times with saturated sodium carbonate solution (∼60 mL each) and once with water. The combined aqueous phases were extracted once with MTBE, and the combined organic phases were dried with $MgSO_4$ and a little activated charcoal. After filtration, solvent removal left 9.6 g of an off-white powder still containing a little MTBE. The crude product was recrystallized from MTBE (50 mL total volume) to give a white powder (the outside of the filter cake was pale yellow): 8.29 g (93.8%), mp 92.5−93.5 °C, >99% pure by GLC, 15.185 min; MS 374 (39), 373 (17), 372 (100), 342 (16), 340 (40), 308 (15), 229 (34), 227 (92), 211 (48). Concentration of the mother liquor gave a second crop of a pale yellow powder: 264 mg (3%), mp 90−91.8 °C (∼97% pure by GLC). A coumaphos analytical standard (Chemagro, now Bayer Animal Health, Shawnee Mission, KS), nominally 99.7% pure, melted at 92.5−94 °C (lit., 95 °C; Schrader, 1954); GLC 15.213 min; MS 364 (38.5), 363 (16), 362 (100), 336 (17), 334 (44), 306 (22), 228 (26), 226 (64), 210 (36.5).

Synthesis of Potasan-d_{10} (2-d). This material was synthesized according to essentially the same method as was coumaphos-d_{10}, starting with 7-hydroxy-4-methylcoumarin (3.52 g, 20 mmol), potassium carbonate (5 g, 36 mmol), and diethyl chlorothiophosphate-d_{10} (3.97 g, 20 mmol) in acetone (75 mL), heated under reflux for 4.5 h. After filtration, removal of acetone, partitioning between sodium carbonate solution and MTBE, drying, and solvent removal, the resulting pale yellow oil (7.63 g) was crystallized from pentane/MTBE (10 mL each) to give white platelets (5.29 g, 78%): mp 35−36.5 °C; GLC 14.306 min; MS 339 (9), 338 (58.5), 306 (26), 274 (13.5), 194 (15), 193 (100), 191 (13), 177 (29), 165 (11), 149 (21). Unlabeled potasan, prepared similarly, had mp 36−38 °C (lit., 38 °C; Schrader, 1952); GLC 14.345 min; MS 329 (19), 328 (91.6), 300 (36), 272 (24), 193 (13), 192 (100), 187 (25.5), 176 (37), 164 (13), 148 (25).

RESULTS AND DISCUSSION

The yield in the preparation of the labeled diethyl chlorothiophosphate (78%) and the yield of a practice run using unlabeled ethanol (80.3%) were similar and superior to the yields of 50−65% obtained by Fletcher et al. (1950), and these authors report that the reaction is general. Yields of crude coumaphos and potasan were essentially quantitative; most losses occurred during recrystallization of the product insecticides. Both labeled pesticides crystallized well and had good GLC properties. In all cases, however, the deuterated material eluted from GLC slightly (0.03−0.04 min for the thiophosphates, 0.07 min for the acid chloride) before the unlabeled material, at least on the relatively nonpolar columns we used.

This sequence and analogous reactions starting with other deuterated alcohols, particularly methanol-d_4, could be used for the synthesis of other deuterium-labeled dialkyl thiophosphates such as parathion, parathion-methyl, and azinphos-methyl, etc. The number of deuterium atoms is sufficient to raise the molecular weight well above the isotope cluster for the unlabeled compound, making independent analysis of the internal standard and the undeuterated analyte possible and the use of such deuterated compounds as internal standards for mass spectrometric analysis quite attractive. Other deuteration patterns such as CD_3CH_2- are also possible, but the starting alcohols are more expensive.

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