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Note

Gas–liquid chromatographic method for determining propylenthiourea in rat tissues and fluids

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Alkylenebis(dithiocarbamates), widely used as fungicides in agriculture, are degraded to several compounds which play an important toxic role[1]. Ethylenebis(dithiocarbamates) (EBDC) have been investigated in depth, in particular their main degradation product, ethylenthiourea (ETU), which has been shown to be mutagenic and carcinogenic[2–4]. This led us to examine the toxic effects of propylenebis(dithiocarbamates), the degradation of which has not been widely investigated[5,6] although these fungicides are used for agricultural purposes.

A comprehensive study of the toxic effects of these compounds has to take into account the quantitative determination of the degradation products, which can be formed *in vivo*. For this purpose, a simple analytical method had to be developed to detect nanogram amounts of degradation products in tissues and biological fluids.

At present, we have limited our study to the analysis of the main metabolite of Propineb, propylenethiourea (PLTU), previously poorly investigated[7] with regard to its toxic effects *in vivo*. Several methods[8–13] have been described for determining the ETU concentration in vegetable samples; all are based on a derivatization procedure followed by gas chromatographic analysis. The chemical structures of PLTU and ETU are so similar that we thought that the ETU derivatization procedures would also be suitable for PLTU.

A detailed examination of the methods described led us to choose two of them: the method proposed by Nash[12], which is based on a double derivatization procedure using *o*-chlorobenzyl chloride and pentafluorobenzyl chloride or trifluoroacetic anhydride, and the method proposed by King[13], who used *m*-trifluoromethylbenzyl chloride. In a preliminary step, we tried to derivatize PLTU following the Nash procedure, which was claimed to be more sensitive than that of King, but the possibility of obtaining two isomers, owing to the presence of an acyl group alternatively in the 1- or 3-position on the imidazoline ring, led us to select the method proposed by King.

EXPERIMENTAL

Standard preparation techniques

PLTU was prepared according to the method described by McKay and Hat-

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ton[14]. S-(*m*-Trifluoromethylbenzyl)propylenethiourea hydrochloride was prepared according to the method described by Boyd and Meadow[15]: m.p., 149–150°C; found, C 46.33, H 4.64, N 8.92%; calculated for $C_{12}H_{14}ClF_3N_2S$, C 46.38, H 4.54, N 9.02%; NMR (δ) (solvent $CDCl_3$), 1.2 (d, J = 3.0 Hz, CH_3), 3.0–4.4 (m, CH_2CH), 4.52 (s, CH_2), 7.0–7.63 (m, aromatic), offset (broad d, J = 8.0, NH · HCl; disappeared with D_2O).

Propineb was prepared according to reported procedure[16].

Reagent and apparatus

Trifluoromethylbenzyl chloride was supplied by EGA-Chemie. All solvents were of special grade for pesticide analysis from Merck.

A Varian 3700 gas chromatograph, equipped with a ^{63}Ni detector, a Varian CDS 111 integrator and a Varian A25 recorder was used.

Experimental procedures

The rat organs and fluids tested were fortified with a detectable amount of PLTU in order to examine the applicability of the derivatization procedures to real samples. All of the samples were derivatized following the procedure described below.

The sample was homogenized in 95% ethanol (1:5, w/v) and then centrifuged at 8000 *g* for 15 min at 5°C. Four drops of derivatization agent were added to 5 ml of the supernatant and the mixture was refluxed for 1 h in a 10-ml graduated tube. After cooling the mixture, the condenser was washed with 1–2 ml of 95% ethanol, then 1–2 drops of 6 *N* hydrochloric acid were added to the reaction tube; the ethanol was then removed by a Rotavapor apparatus (Büchi, Switzerland) at 35°C.

To the residue was added 2 ml of distilled water and the mixture washed twice with 1 ml of diethyl ether, which was subsequently removed with a Pasteur pipette; the remaining ether was removed in a water-bath at 50°C.

After cooling the mixture, 0.5–1.0 ml of benzene was added, followed by 0.3–0.5 ml of 10% sodium hydroxide solution. The mixture was immediately shaken and centrifuged and 1–5 μl of the organic layer were injected in the gas chromatograph using a 2 m × 2 mm I.D. glass column packed with 3% OV-275 on 80–100-mesh Chromosorb G. The operating conditions were as follows: column temperature, 195°C; detector temperature, 300°C; injector temperature, 200°C; nitrogen flow-rate, 30–35 ml/min.

RESULTS AND DISCUSSION

The above method was used to determine PLTU in liver, brain, kidney, heart, spleen, thyroid gland, muscle, adipose tissue, ovary, uterus, placenta, foetus, serum and urine. No interfering peaks were observed, as can be seen in Fig. 1.

A recovery test was performed on rat urine, adding PLTU to a test sample in order to obtain a final concentration of 5 ppm. This fortified sample was then derivatized. The recovery was 93.2 ± 8.7% (mean ± S.D., n = 5). The linearity range was tested from 2 to 20 ng injected.

The PLTU concentration in the urine of rats that had been given different amounts of Propineb was determined in order to obtain some preliminary information on Propineb metabolism. The method proposed by Cullen[17] was applied to the

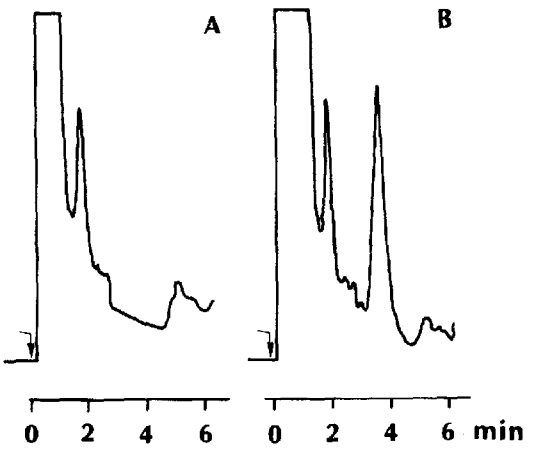

Fig. 1. Chromatograms of (A) urine sample derivatized using King's method[13]; (B) urine sample fortified with 1.5 ppm of PLTU and then derivatized.

same urine samples, to reveal the unmodified Propineb and all those metabolites, such as propylenethiourea disulphide, which can give carbon disulphide after acid hydrolysis. The results in Table I indicate that both the derivatization procedure and the analysis of biological fluids and tissues were successful.

As pointed out by King[13], the use of the S-(*m*-trifluoromethylbenzyl) derivative avoids a double reaction sequence and possibilities for loss of the sample; moreover, the gas–liquid chromatographic analysis is very rapid for the reduced retention time, and the sensitivity (0.1 ppm) is satisfactory for our purpose. Therefore, we can conclude that King's derivatization method is suitable for the determination of PLTU in biological materials. Our future studies will be extended to the development of methods for the analysis of all the propylenebis(dithiocarbamates) metabolites to provide detailed information on the toxicity of these pesticides.

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TABLE I

URINE EXCRETION (mg) OF PLTU AND CS_2 IN THE RAT AFTER ADMINISTRATION OF PROPINEB p.o.

*Propineb (g/kg, p.o.)**	*24 h*		*48 h*		*72 h*	
	PLTU	*CS_2*	*PLTU*	*CS_2*	*PLTU*	*CS_2*
1.063	5.3	0.08	5.2	N.D.**	1.50	N.D.
2.125	6.8	0.20	6.5	N.D.	0.95	N.D.
4.250	11.0	0.20	11.6	N.D.	2.50	N.D.

* The doses listed are 1/8, 1/4 and 1/2 of the LD_{50}, respectively; each dose was given to three rats and the determinations were performed on the pooled urines.

** N.D. = Not detected.

carbon disulphide determination and V. Migliaro for the physico-chemical characterization of standards. This work was supported by the CNR (Consiglio Nazionale delle Ricerche) grant RN-18350/A.

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