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Note

Gas chromatographic determination of methylthiouracil residues in meat and organs of slaughtered animals

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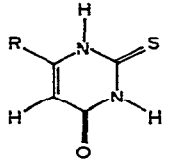
Although most of the European countries prohibit the use of thyroid-inhibiting substances in cattle breeding, this practice is still frequent. The most important thyreostatic used is a cyclic thioureide: 2,3-dihydro-2-thioxo-4-1*H*-pyrimidinone, the 6-methyl derivative of which is usually called methylthiouracil (MTU) and the 6-propyl derivative, propylthiouracil (PTU).



R = CH_3 : MTU
R = C_3H_7 : PTU

Several methods have been proposed to control the treatment of carcasses before their commercial distribution. One type of method permits the detection of organ modifications in the carcass of an animal fed with a thyreostatic drug, for example histological examination of the thyroid gland[1]. There are also procedures for the detection of residues in tissues of treated animals: colorimetry[2,3], UV detection after one-dimensional thin-layer chromatography (TLC)[4] or after high-pressure liquid chromatography[5] and fluorescence detection after two dimensional TLC[6]. The latter type of method is very sensitive but it does not allow an easy determination of the residues. Therefore we have developed a gas chromatographic (GC) analysis for the determination of MTU residues in animal tissues which makes use of the highly specific flame photometric detector.

EXPERIMENTAL

Apparatus

A Tracor Model 560 gas–liquid chromatograph was used with a flame photometric detector (394-nm filter and response linearizer) and a glass column (1.8 m × 4 in. I.D.) packed with 3% OV-1 on Chromosorb W HP (80–100 mesh). Operating conditions: carrier gas (nitrogen) flow-rate, 35 ml/min; hydrogen flow-rate, 40 ml/min;

air flow-rate, 170 ml/min; inlet temperature, 200°; oven temperature, 180°; detector temperature, 175°.

Solvents and reagents

Analytical-grade solvents and methyl iodide were purchased from Merck (Darmstadt, G.F.R.) and were redistilled in an all-glass apparatus. Reference compounds were obtained from Fluka (Buchs, Switzerland) for MTU and from Aldrich-Europe (Beerse, Belgium) for PTU. Sephadex "lipophilic" LH 20-100 was purchased from Sigma (St. Louis, Mo., U.S.A.). The Sephadex columns were glass tubes (5 mm I.D.) filled with a suspension of Sephadex in benzene–methanol (85:15) to a height of 10 cm and stored in this solvent until use.

Extraction and clean-up

Thyroid gland tissues. A 0.5-g amount of tissue was sampled and placed in an extraction tube; 20 μg of PTU were then added as internal standard (0.1 ml of an ethanolic solution containing 200 μg/ml). After homogenization in 1 ml of distilled water, the mixture was defatted by washing three times with 3 ml of light petroleum (b.p. 40–60°). MTU was then extracted by shaking the mixture three times with 3 ml of ethyl acetate. The combined ethyl acetate fractions were dried under a nitrogen stream on a water bath at 50°. The extract was then dissolved in 0.5 ml of benzene–methanol (85:15), transferred to the top of a Sephadex column and eluted with the same solvent. The first 3 ml which were eluted were discarded and the following 4 ml were collected in a reaction tube.

Other tissues (liver, kidney, muscle or fat). Due to the lower residue level present in these tissues, a 2-g amount was sampled and homogenized in 3 ml of distilled water after addition of 2 μg of PTU as internal standard (0.1 ml of an ethanolic solution containing 20 μg PTU per ml). The following extraction steps were the same as described above for thyroid glands.

Reaction

After evaporation of the 4-ml eluate under a nitrogen stream, 1 ml of a 0.1 *M* solution of potassium acetate in ethanol and 50 μl of methyl iodide were added to the residue. The reaction tube was stoppered and placed in a water bath at 55° for 70 min. Ethanol was then evaporated and the solid was redissolved in 1 ml of distilled water. The methylation derivatives were extracted three times with 1 ml of benzene. The combined benzene extracts were dried and the residue was finally dissolved in 1 ml of benzene. An amount of 2–5 μl of this solution was injected into the chromatograph. The mean retention times of methylation derivatives of MTU and PTU were 124 and 190 sec, respectively.

Determination

For calibration, the methylation reaction was performed, as described above for tissue extracts, on known quantities of standard (for thyroid gland tissue, 10,20, 40, 60 and 80 μg of MTU and 20 μg of PTU as internal standard; for other samples 0.5, 1, 2, 4 and 8 μg of MTU and 2 μg of PTU). Aliquots of 2 μl of the standard solutions were injected into the chromatograph. The calibration graph was obtained by plotting the ratio of the peak area of MTU to the peak area of PTU as a function of concentration.

RESULTS AND DISCUSSION

Methylation reaction

This reaction was performed in order to transform MTU in a volatile compound. As shown in Table I and II, under the conditions described, mass spectrometry, elementary analysis and proton magnetic resonance spectroscopy were in agreement wih the introduction of two methyl groups into the molecule of MTU. The IR spectrum showed that the carbonyl group remained unchanged. All these data are in agreement with the formulation of the derivative as 3,6-dimethyl-2-(methylthio)-uracil. The same reaction was observed when thiouracil was treated with dimethylformamide dimethylacetal in acetonitrile[7]. We found that the above derivative was also the main product formed on treatment of MTU with dimethyl sulphate in 0.1 *N* aqueous sodium hydroxide, with dimethylformamide dimethylacetal in pyridine (Methyl 8; Pierce, Rockford, Ill., U.S.A.) or with methyl iodide in 1 *N* aqueous sodium hydroxide. However, under all these conditions, side reactions were always observed and other derivatives formed in variable amounts obscured the final chromatogram.

Fig. 1 represents the course of the methylation reaction of 20 μg of MTU and 20 μg of PTU under our conditions. The two compounds were found to react at practically the same rate.

Clean-up

The use of gel chromatography in an organic medium was found to be very useful as a final clean-up step. This procedure allowed us to reduce the number of purification steps. Extracts purified in this way were very clean and interferences were not detected, whatever the tissue analysed. Fig. 2 shows the elution profile of MTU and PTU from a Sephadex column. This column may be reused after careful washing with the elution solvent.

TABLE I

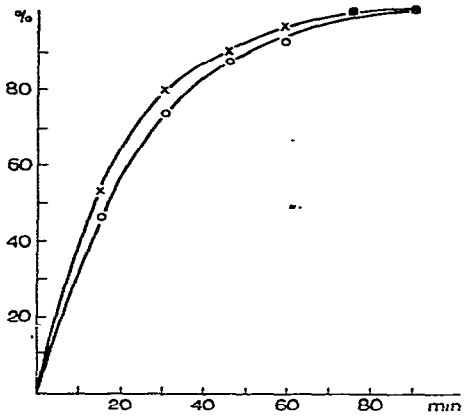
ELEMENTARY ANALYSIS (%) OF THE METHYLATION DERIVATIVE OF MTU

	Calculated	*Found*
C	49.41	49.4
H	5.88	6.0
N	16.47	16.5

TABLE II

PROTON MAGNETIC RESONANCE SPECTRUM IN $CDCl_3$–HEXAMETHYLDISILOXANE
Molecular mass, 170; IR (KBr disc), ν_{CO} at 1667 cm^{-1}.

Chemical shift (ppm)	*Signal*	*Coupling constant (Hz)*
2.15	3H (*d*) 6-CH_3	0.6
2.50	3H (*s*) 2-SCH_3	—
3.41	3H (*s*) 3-NCH_3	—
5.96	1H (*q*) 5-H	0.6



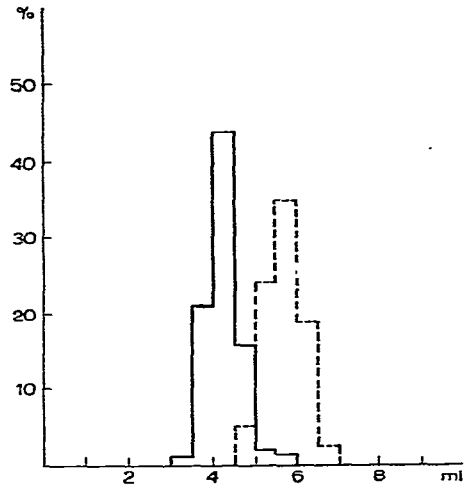


Fig. 1. Course of reaction of MTU (○) and PTU (×) with methyl iodide.
Fig. 2. Elution profile of PTU (full line) and of MTU (broken line) from a Sephadex column.

Determination

Determinations performed on samples of different tissues to which MTU and PTU had been added showed that the recovery of these two compounds is of the order of 40–60% depending on the nature of the tissue. The determination of MTU in 10 muscle samples to which 2 μg of MTU had been added gave a mean value of 2.05 μg (S.D. 0.21). The detection limit for MTU was found to be 0.5 ng. For samples containing very low levels of residue, the extract of 2 g of tissue may be reduced to a final volume of 0.2 ml and 5 μl of this solution may be injected into the chromatograph without notable interferences. With a mean recovery value of 50%, the detection limit is thus of the order of 10 ppb.

CONCLUSION

Our method presents a very simplified clean-up procedure and gives quantitative results very easily. It is also of the same order of sensitivity as the official method used in our country for the detection of thyreostatic residues in meat and organs of cattle[6].

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