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Note

Gas chromatographic determination of the laxative 1,8-dihydroxyanthraquinone in urine and faeces

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1,8-Dihydroxyanthraquinone (danthron) is the basic structure of the aglycones of naturally occurring laxative glycosides, *e.g.* in the Cassia, Aloe, Rheum and Rhamnus species. Danthron itself is in use as a synthetic laxative (Istizin®, Dorbane®)[1–3].

In order to investigate the metabolic fate of danthron in the rat (to be published in full detail elsewhere), it was necessary to develop a method for analysing small amounts of danthron in faeces and urine.

The fluorescence method of Lane[4] did not meet our expectations, possibly due to other substances in faeces and urine interacting with the fluorescence of the danthron moiety. We report here a procedure consisting of an extraction followed by adsorption chromatography (to purify the faeces extract) and gas chromatographic determination of danthron (without derivatisation): it allows the detection of nanogram quantities of danthron, undisturbed by other products excreted in faeces and urine.

EXPERIMENTAL

Danthron (Istizin) was purchased from Bayer (Leverkusen, G.F.R.) and recrystallized from ethyl acetate. All solvents were of a chemically pure or analytical grade, and were redistilled before use.

Thin-layer chromatography (TLC) was performed on silica gel-covered glass plates (Kieselgel 60 F_{254}; E. Merck, Darmstadt, G.F.R.), with benzene–carbon tetrachloride–glacial acetic acid (150:50:1, by vol.) as developing solvent; plates were prerun in hexane–glacial acetic acid (100:1) and dried before use. Spots were examined under ultraviolet (UV) light.

Gas–liquid chromatography (GLC) was carried out on a Hewlett-Packard 5710A and a Becker 420, equipped with solid injection and flame ionization detectors (FID). The columns (80 cm × 1.2 mm I.D.) were U-shaped, and were packed with a mixture of 1% OV-17 and 2% OV-225 on Gas-Chrom Q (180–200 μm). Conditions: oven temperature, 235°; injection port temperature, 300°; detector temperature, 350°; nitrogen flow-rate, 5 ml/min and hydrogen flow-rate, 2 ml/min.

Danthron was administered to rats, and urine and faeces were collected in metabolic cages. Urine was added to an equal volume of acetic acid–sodium acetate

buffer (0.2 *M*, pH 5.0) and thoroughly mixed. The pH was controlled and when necessary adjusted to 5.0 in order to keep danthron in the non-ionized form ($pK_a \approx 8$). The mixture was extracted twice, each time with a two-fold volume of light petroleum (boiling range 60–80°). Phase separation was accelerated by centrifugation at 900 *g* for 5 min in a table centrifuge. Completeness of extraction was tested by TLC as described. Extracts were washed with an equal volume of 0.1 *N* hydrochloric acid and evaporated to dryness under reduced pressure.

Faeces (1 part by weight) were added to acetic acid–sodium acetate buffer (5 parts by volume) and disrupted with an Ultra-Turrax apparatus (Janke & Kürkel). The resulting suspension was extracted as described for urine. In contrast to the urine extracts, the faeces extracts had to be purified before GLC was possible. This was done by adsorption chromatography on silica gel columns (Kieselgel H; E. Merck). After evaporation of the faeces extract the residue was dissolved in a small amount of benzene–carbon tetrachloride–glacial acetic acid (150:50:1) and applied to a silica gel column (5–10 cm × 3 cm I.D.). Elution was performed with the same solvent and the fraction containing the bright yellow danthron was collected. Occasionally, extracts contained very small amounts of danthron: these were detected by UV light. Fractions obtained were evaporated to dryness under reduced pressure.

The evaporated urine extracts and evaporated purified faeces extracts were dissolved in a small (known) amount of a solution of 2-chloroanthraquinone (which served as external standard) in benzene. For routine assays, 1 ml of a solution containing 100 μg of 2-chloroanthraquinone was used. 1-μl samples were taken for GLC analysis.

RESULTS AND DISCUSSION

Furuya *et al.*[5] described an analysis of anthraquinones based on GLC of trimethylsilyl derivates. However, when many samples containing small amounts of one anthraquinone are to be measured, it should be easier to avoid derivatization.

For this purpose the packing as described in Experimental appeared to be quite satisfactory, the only disadvantage being the occurrence of considerable tailing with liquid injection on our standard columns (180 cm × 3 mm I.D.). This was overcome by using short columns (see Experimental) with solid injection, while the dead space in the injection port was reduced by filling it with a glass rod instead of glass wool[6]. Use of these short columns had no influence on the resolving power.

Results of gas chromatographic analysis are shown in Fig. 1. Fig. 1A illustrates a standard chromatogram of a solution of danthron and 2-chloroanthraquinone; Fig. 1B shows the chromatogram of an extract of urine collected in the period 0 to 24 h after oral administration of danthron; Fig. 1C shows the chromatogram of extracted and purified faeces, collected in the same period. The danthron peaks represent 22 and 65 ng/μl, respectively (which means therefore 22 and 65 μg danthron in the total extracts).

Fig. 2 shows the calibration graph constructed from GLC analysis of solutions containing different ratios of danthron and 2-chloroanthraquinone. There exists a good relationship between the peak ratios of chromatograms and the amount ratios in different mixtures covering the range from 0–2000 μg danthron per millilitre. The reproducibility is better than 2%. The lower limit of danthron which can easily be

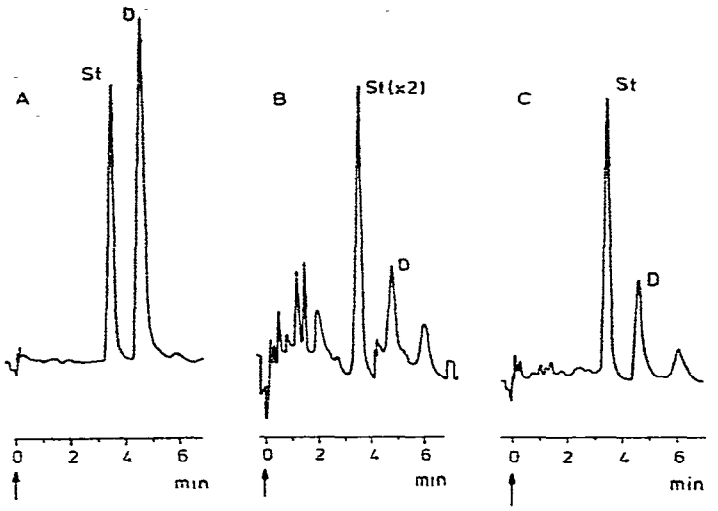

Fig. 1. (A) Gas chromatogram of 1 μl of a solution of 200 ng/μl danthron (D) and 100 ng/μl 2-chloroanthraquinone standard (St) in benzene. (B) and (C) Gas chromatograms of an extract from rat urine (B) and faeces (C), collected in the period 0 to 24 h after administration of danthron. Arrows indicate injection points.

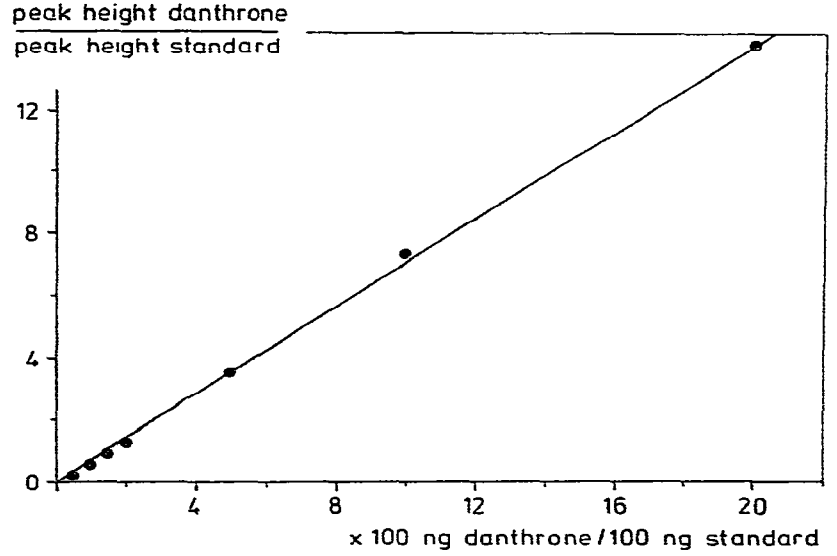

Fig. 2. Calibration graph of standard mixtures of danthron and 2-chloroanthraquinone.

detected is 3 ng/μl. Differences in results when measuring peak heights or peak surfaces were not observed.

The described assay allowed an easy determination of nanogram amounts of danthron in rat faeces and urine, while disturbances from other components of faeces or urine could not be detected. However, the method is not applicable to the quali-

tative determination of different anthraquinones because many of them have retention times —on these columns— very close to that of danthron.

Nevertheless, for quantitative purposes, the procedure seems to be very useful for one or for a few anthraquinones, as in the case of our study of danthron metabolism. The lack of a derivatization routine and the differences in results between standard and short columns are remarkable features of this method.

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