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Identification of diastereomeric propranolol-*O*-glucuronides by gas chromatography-mass spectrometry

Propranolol, widely used for the treatment of hypertension, has been found by Drs. T. Walle and E. Conradi (unpublished findings) to be extensively metabolized in man by glucuronic acid conjugation. Evidence for the structure of the glucuronic acid conjugate is now presented.

The mass spectrometric analysis was carried out on an LKB 9000S instrument with an ionizing electron energy of 20 eV. The glass g.c. column (3′ × 1·5 mm, i.d.) was packed with 1% OV-1 on silanized 60/80 mesh Chromosorb W. The helium flow was 10 ml min^{-1} with a column temperature of 200°.

(±)-Propranolol was administered chronically to a patient (4 × 80 mg daily). The urine (5 ml) was adjusted to pH 11 (1M NaOH) and washed with 2 × 10 ml of diethyl ether (discarded). The remaining traces of ether were removed by evaporation at 60°. The pH was adjusted to 7 (1M HCl) and the sample was transferred to an Amberlite XAD-2 (20–50 mesh) column (25 × 1 cm) which had been washed with methanol, acetone and distilled water. The column was eluted with 150 ml of distilled water (discarded) and with methanol (10 ml fractions collected) (cf. Fujimoto & Haarstad, 1969). The major portion of the propranolol glucuronide was found in fraction 3 (determined by g.c. as trifluoroacetyl derivative after hydrolysis with Glusulase, Walle & Conradi, unpublished).

Fraction 3 was evaporated to dryness and the residue in methanol (200 μl) treated (15 min, 20°) with diazomethane in diethyl ether (prepared according to Stanley, 1966). Evaporation under nitrogen at 80° gave a residue which, mixed with ethyl acetate (400 μl) and trifluoroacetic anhydride (100 μl) was shaken for 15 min (Vortex mixer)

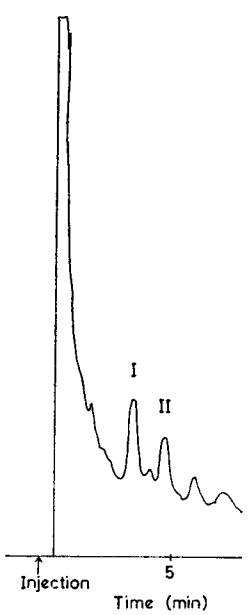

FIG. 1. Total ion current recording from human urine extract. Column: 1% OV-1. Column temperature: 200°. Helium flow: 10 ml min^{-1}.

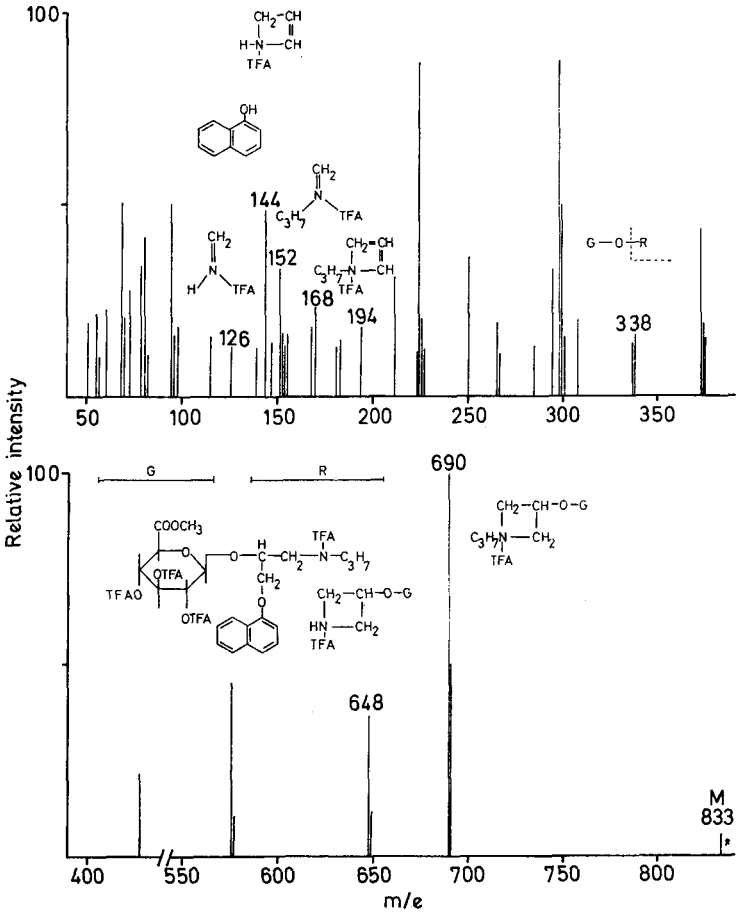

FIG. 2. Mass spectra of methyl-trifluoroacetyl derivative of propranolol-*O*-glucuronide (peak I, Fig. 1). Fragments with relative intensity <10% of the base peak are excluded.

at 20° (Ehrsson, Walle & Wikström, 1974). 2–5 μl was taken for analysis by g.c.-m.s. The 24 h urine output of propranolol glucuronide was 40 μg ml^{-1}.

Fig. 1 shows a typical g.c. recording of an extract of human urine (monitored by the total ion current of the mass spectrometer). The mass spectrum of peak I is given in Fig. 2 interpreted by analogy with the work of Garteiz & Walle (1972). Peak II gave a spectrum almost identical with that of peak I suggesting that they were isomers both consistent with the structure of propranolol-*O*-glucuronide. Peaks I and II thus represent diastereomers formed by conjugation with the optically active β-D-glucuronic acid with the racemic propranolol, their separation is in agreement with previous reports on other diastereomers (Underwood & Frye, 1972; Nahrstedt, 1970). Administration of the enantiomeric (+)- and (−)-propranolol separately to two dogs (3 × 15 mg for 2 days) allowed the isolation of the diastereomers singly, each individual peak having retention times and mass spectra identical with those of peaks I and II respectively. The propranolol glucuronide in the 24 h urine of the dog given the (+)-isomer was 8 μg ml^{-1} and that of the dog given the (−)-isomer was 133 μg ml^{-1} as measured on Day 2. The clear separation (α = 1·32) must be attributed to the facts that the chiral carbon atoms of propranolol and the glucuronic acid are separated by only one atom and that both molecules are very bulky (Gil-Av & Nurok, 1974).

(—)- and (+)-propranolol were kindly provided by ICI, U.K.
This work was supported by grant GM-20387-03.

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April 8, 1975

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A procedure for the micro-phase extraction of lipophilic drugs from biological fluids with low-density organic solvents

The use of small volumes of organic solvents for the extraction of lipophilic drugs from biological fluids in which the ratio (v/v), between the organic solvent and the aqueous phase is of the order of 1:50 to 1:100 ("micro-phase" technique) has important advantages over conventional extraction procedures in the case of many drugs (Ramsay & Campbell, 1971; Aggarwal, Bath & Sunshine, 1974). These include elimination of the time-consuming and destructive concentration of extracts by means of distillation techniques and the fact that "cleaner" extracts with less back-ground interference in chromatographic separations are obtained. Although the method has many other advantages in the extraction of a variety of drugs, it has not yet gained wide acceptance in drug analysis schemes. This is perhaps due to the inherent limitation of the method due to the necessity of using only solvents that are heavier than water, such as chloroform, carbon disulphide and carbon tetrachloride with relatively high polarities limiting their selectiveness in extraction.

To overcome this problem we have used successfully the simple and easy-to-construct apparatus depicted in Fig. 1 for the extraction of aqueous solutions with small volumes of low-density organic solvents. It may be constructed from an ordinary 10 ml glass-stoppered test tube by drawing out the bottom portion into a thick walled capillary tube (capacity approximately 100 μl). A thick walled side arm (3 mm internal diameter) is then attached as shown.

The capillary end is closed with a small plug (polythene tube) and the aqueous solution to be extracted (e.g. 5 ml of urine suitably buffered and saturated with respect to sodium chloride) is poured into the tube. The glass stopper is then replaced and the tube inverted as shown in Fig. 1. The required volume (usually 50–100 μl) of the organic phase is then injected through the open capillary end with the aid of a syringe and the unit shaken on a Vortex mixer for 3–5 min with the open capillary end pointing upwards. After centrifugation to separate the phases, a syringe filled with saturated aqueous sodium chloride solution is attached to the side