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Note

Simple gas chromatographic method with flame ionization detection for the determination of aldadiene in human urine

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In order to study spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxyandrost-4-en-17α-yl)propionic acid-γ-lactone] metabolism, and the dynamic appearance of its principal dethioacetylated metabolite, aldadiene [3-(3-oxo-17β-hydroxyandrosta-4,6-dien-17α-yl)propionic acid-γ-lactone, AD], in urine, a rapid gas chromatographic method has been elaborated for the determination of urinary AD following treatment of patients with spironolactone. The procedure developed involves simple extraction and direct gas chromatography (GC) with flame ionization detection (FID).

EXPERIMENTAL

Extraction procedure

To a 10-ml aliquot of 24-h urine, 10.0 μg of Δ^1-testololactone (17-oxa-D-homo-androsta-1,4-diene-3,17-dione, TL) were added as internal standard, and the urine was extracted with 10 ml of peroxide-free diethyl ether. The extract was washed with 1 ml of 10% sodium hydroxide solution and two 1-ml volumes of water, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was transferred with small volumes of ether into a capillary tube, the solution evaporated to dryness and the residue redissolved in exactly 10.0 μl of dioxan. Quantitative measurement of 0.2–1.0-μl aliquots of this solution was made by GC–FIDwithout preliminary purification.

Gas chromatographic conditions

A Pye-Unicam Series 104 gas chromatograph, equipped with a flame ionization detector, and a glass column (1.5 m long $\times$ 4 mm I.D.) packed with 1% SE-30 on Diatomit CQ, 80–100 mesh, were employed. The column was operated at 230° (isothermal), and the detector was maintained at 260° with a sensitivity of 10^{-10} A (f.s.d.). The carrier gas was nitrogen with a flow-rate of 60 ml/min. Evaluation was made by the internal standard procedure and peak-height measurements.

RESULTS AND DISCUSSION

The results of control experiments substantiated the reliability of the method. The detector response (R_{TL}/R_{AD}) of the two steroids was linear between 0.1 and 2.0 μg,

resulting in a value for the ratio of 1.5 (1.47–1.54). The urinary estimates were corrected accordingly. The validity of using TL as internal standard was further established by the identical recoveries ($\bar{x} \pm$ standard error, %) of the two steroids, which were 70.8 $\pm$ 1.8 for AD and 71.4 $\pm$ 1.4 for TL in 12 determinations following addition of 0.1–1.0 μg/ml amounts to urine specimens. Eight duplicate determinations on

TABLE I

ALDADIENE EXCRETED IN URINE DURING THREE CONSECUTIVE DAYS FOLLOWING ADMINISTRATION OF 200 mg OF SPIRONOLACTONE AS A SINGLE DOSE

Normal subjects (age, sex)	*Aldadiene (mg per 24 h)*			*Total*	
	1st day	*2nd day*	*3rd day*	*(mg)*	*(%)**
62, f	6.20	0.45	0.11	6.76	3.38
53, f	3.10	2.40	0.60	6.10	3.05
36, m	5.10	1.86	0.36	7.32	3.66
20, m	5.97	1.79	0.23	7.99	4.00

* Per cent of dose.

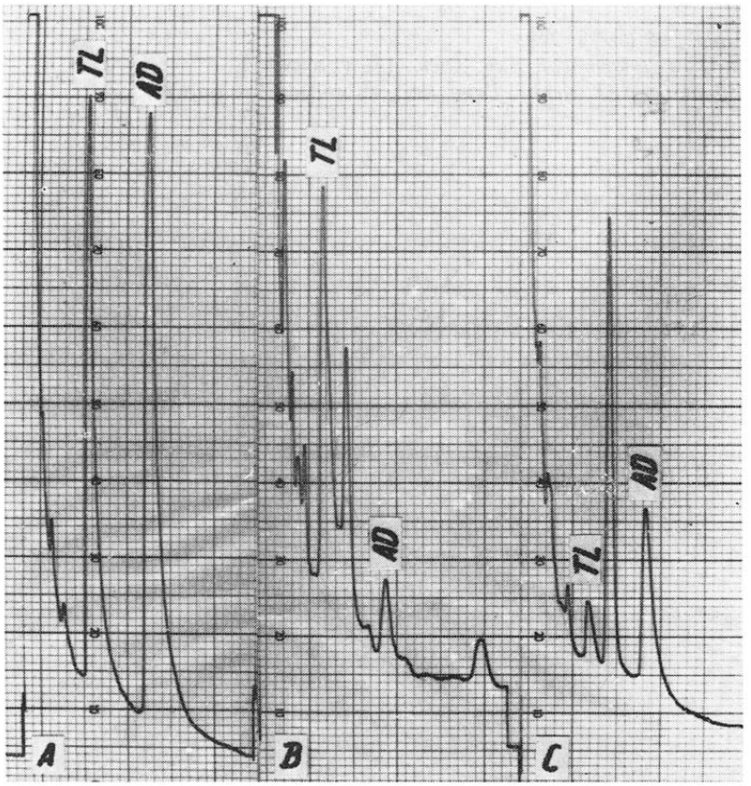

Fig. 1. Gas chromatograms of authentic Δ^1-testololactone (TL) and aldadiene (AD) (A) and of two urinary extracts (B and C) processed following administration of sprironolactone. Conditions: 1% SE-30 column at 230°; flame ionization detection at 260° with a sensitivity of 10^{-10} A; carrier gas nitrogen with a flow-rate of 60 ml/min. Δ^1-Testololactone used as internal standard.

AD-containing samples indicated satisfactory precision ($\bar{x} \pm$ standard deviation: 2.03 $\pm$ 0.25 mg per 24 h). The lower limit of flame ionization detection (f.s.d./100) was found to be 20 ng for AD. The high specificity of the method was shown by analysis of a series of control urine samples without AD and/or TL. Interfering substances whose retention behaviour is identical with that of either AD or TL were not detected when a flame ionization detector was used. This was not the case when the nickel-63 electron capture detector of the Pye-Unicam instrument was employed[1].

Table I gives representative values for the AD excreted in the urine of healthy volunteers following oral administration of a single 200-mg dose of spironolactone (Verospiron; Richter Ltd., Budapest, Hungary). Fig. 1 shows a gas chromatogram of authentic TL and AD (A) and of two urinary extracts (B and C) processed following administration of the drug. Our values seem to be slightly higher than those obtained by Gochman and Gantt[2] by their fluorimetric technique and are similar to those found by Chamberlain[1] by GC with electron capture detection.

A detailed study of spironolactone pharmacokinetics using the present simple method will be published elsewhere[3].

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