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Note

Gas chromatographic determination of 18-hydroxy-11-deoxycorticosterone

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Besides aldosterone and deoxycorticosterone, a further mineralocorticoid 18-hydroxy-11-deoxycorticosterone (18,21-dihydroxy-pregn-4-en-3,20-dione; 18-OH-DOC) is also secreted by the human adrenal cortex. Its determination is of importance for the differential diagnosis of some adrenocortical disorders and of some forms of arterial hypertension [1]. However, 18-OH-DOC determination in biological material is difficult because of its easy conversion into multipolar compounds [2], ketalic forms [3] and possibly "dimers" [4,5]. Gas chromatography represents the most usual approach to the determination of 18-OH-DOC in plasma. Rapp [6] and Palem et al [7] determined it as the γ-lactone, Mason and Fraser [8] as the γ-lactone heptafluorobutyric derivative (HFB). The formation of this derivative (Fig. 1) offers a possibility to increase the sensitivity of the method, linearity of the response and precision of the determination.

This paper describes a modification of the method for 18-OH-DOC determination by gas chromatography described by Mason and Fraser [8]. The use of canrenone as an internal standard reduced the time of the analysis, since the HFB derivative of canrenone has a shorter elution time than the HFB derivative of aldosterone γ-lactone.

EXPERIMENTAL

Reagents

The 18-OH-DOC standard was kindly supplied by the Clinical Research Institute of Montreal, Canada, and later purchased from Searle de Mexico, (Naucalpon, Mexico).

[1,2-$^3H_2$]-18-OH-DOC, specific activity 51 Ci/mmole was obtained from The Radiochemical Centre (Amersham, Great Britain). The commercial product supplied in benzene—methanol (9:1) solution should be purified before

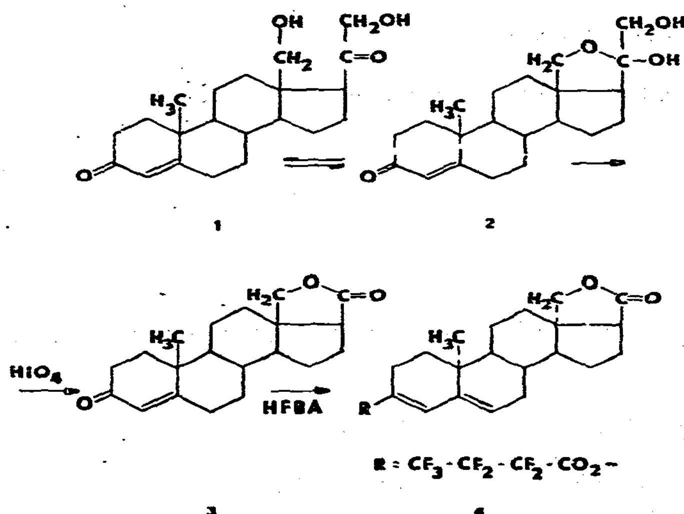

Fig.1. Formation of the γ-lactone HFB derivative of 18-OH-DOC. 1 = 18-OH-DOC; 2 = 18-20 hemiketal configuration; 3 = 18-OH-DOC γ-lactone; and 4 = 18-OH-DOC γ-lactone HFB derivative.

use by thin-layer chromatography and the eluate should be kept in a non-polar solvent [2].

Canrenone γ-lactone (3-oxo-17β-hydroxy-4,6-androstadien-17α-ylpropionic acid was from Boehringer (Mannheim, G.F.R.).

Heptafluorobutyric anhydride (HFBA) was from Serva (Heidelberg, G.F.R.).

All solvents were analytical-reagent grade and were purified by distillation before use.

Paper and thin-layer chromatography

Paper, Whatman No 1, 47 cm long, was pre-washed with methanol for two days before use and air dried. Aluminium foils coated with 0.1 to 0.14-mm silica gel layer (150 × 150 mm, Silufol, UV 254 Kavalier) were pre-washed three times with benzene—acetone (3:1) before use and air dried.

Gas chromatography

A Carlo Erba Fractovap 2200 chromatograph was used with a ^{63}Ni electron-capture detector, supplied with 25 V d.c. A 2-m glass column with an internal diameter of 4 mm was packed with Gas-Chrom Q 100—120 mesh (Serva) coated with 1% SE-30 (Applied Science Labs., State College, Pa., U.S.A.). The temperature of the column, of the evaporation area and of the detector were 222, 270 and 270°, respectively. Dried nitrogen served as a carrier gas and the flow-rate through the column was 60 ml/min. The flow-rate of the purging gas (nitrogen) was 35 ml/min.

The radioactivity of [1,2-$^3H_2$]-18-OH-DOC was measured on a Packard Tricarb liquid scintillation spectrometer, Model 2405.

Sample preparation for gas chromatography

(1) Five ml of plasma was kept at −20°, then thawed. A known amount

of [1,2-$^3H_2$]-18-OH-DOC (65,000 dpm) was added in a manner similar to that for the 18-OH-DOC standard sample in water. The plasma was extracted three times with dichloromethane (10 ml each portion). The combined extracts were washed with 2 ml of 0.1 *M* sodium hydroxide then 2 ml of water, and evaporated to dryness.

(2) The dry residue was dissolved in dichloromethane, applied to the paper together with the standards and separated chromatographically in the system Bush 3 [9,10]: light petroleum (b.p. 30—50°)—benzene—methanol—water (667:333:800:200) for 4 h at room temperature. Depending on the location of the reference compound detected under short-wave UV irradiation (254 nm) the corresponding area was eluted three times with methanol—dichloromethane (1:1). The $R_{deoxycorticosterone}$ of the reference compounds were: deoxycorticosterone 1.0; corticosterone 0.32; 18-OH-DOC 0.25; aldosterone, 0.06; and cortisol, 0.04.

(3) The extract was evaporated in vacuum and oxidized as described in ref. 8.

(4) The evaporated oxidation products were dissolved in a small volume of methanol—dichloromethane (1:1), applied to a Silufol foil and developed in the solvent system benzene—acetone (3:1) up to the height of 14 cm. The R_F of the reference compounds were: corticosterone, 0.07; 18-OH-DOC, 0.07 and 18-OH-DOC γ-lactone, 0.29. The 18-OH-DOC γ-lactone was detected under UV irradiation (254 nm), the corresponding areas were scraped off, moistened with water and extracted three times with 1 ml benzene.One-tenth of each sample was taken for measuring tritium radioactivity to determine the losses at this stage. The residue was evaporated under nitrogen.

(5) Benzene (10 μl) and 10 μl heptafluorobutyric acid were added to the dry sample and the mixture was heated to 60° for 30 min. The reaction mixture was evaporated in a nitrogen atmosphere and the evaporate was dissolved in 10—30 μl benzene with a known amount of canrenone as internal standard. A 3- to 4-μl portion of this solution was injected into the gas chromatograph. Retention times of 18-OH-DOC and canrenone derivatives were 6 and 8.5 min, respectively. Peak areas were measured by planimetry.

RESULTS AND DISCUSSION

For typical chromatograms of plasma samples from the vena cava inferior and from the adrenal vein see Fig. 2. To estimate losses during the extraction, paper chromatography, oxidation and thin-layer chromatography, labelled 18-OH-DOC was added to six test-tubes with 5 ml of plasma, and aliquot portions were used for measuring the losses of tritium after each operation. The results are summarized in Table I. In 8 other samples the recovery of [1,2-$^3H_2$]-18-OH-DOC added to water was determined and the total recovery was found to be 54.29 ± 2.3%.

The response of the detector to the HFB derivative of the 18-OH-DOC γ-lactone as well as to the internal standard (canrenone HFB derivative) was linear within the range 0 to 10 ng. The lowest detectable amount was 0.06 ng. The precision of the method was examined by simultaneous treating of six 5-ml samples of plasma to which 100 ng of 18-OH-DOC were added.

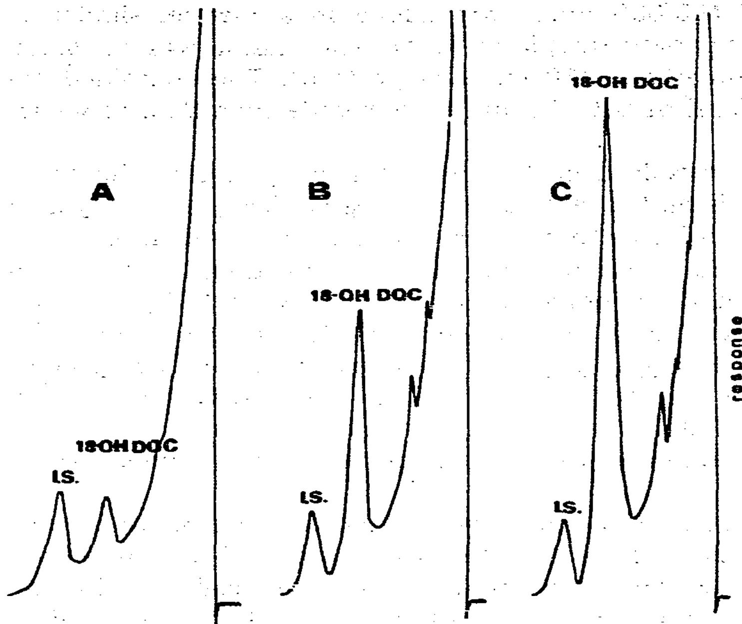

Fig.2. Gas chromatograms (for conditions see Experimental) of the plasma samples from the vena cava inferior below (A) and above (B) the orifice of the adrenal and renal veins, and from the left adrenal vein (C). I.S. = Internal standard; 18-OH-DOC = peaks corresponding to the 18-OH-DOC γ-lactone HFB derivative. The amounts found were: A, 0.55 ng; B, 2.18 ng; C, 6.15 ng.

TABLE I

PERCENTAGE OF [1,2-$^3H_2$]-18-OH-DOC RECOVERY IN INDIVIDUAL STEPS OF THE METHOD

Values are means of six samples ± standard error.

Recovery (%)			
Extraction	Paper chromatography	Oxidation + thin-layer chromatography	Total recovery (%)
70.62 ± 1.73	83.35 ± 2.29	78.06 ± 4.20	47.07 ± 3.09

In these samples the concentration of endogenous 18-OH-DOC was simultaneously determined before addition of the standard. After subtraction of the standard the average value was 98.54 ± 4.08 ng.

In a series of control experiments we found that most compounds interfering in the gas chromatography of 18-OH-DOC (cortisol, deoxycorticosterone, aldosterone) were separated by paper chromatography. However, corticosterone was separated only partially in both Bush 3 and Bush 1 systems. We chose the system Bush 3 because it does not produce tailing of the cortico-

sterone spot. Total separation is possible on thin-layer chromatography only after oxidation of 18-OH-DOC to its γ-lactone.

The basic pre-requisite is the absolute purity of all solvents and glassware used. This prevents formation of multipolar 18-OH—DOC derivatives whose mobilities are different [2]. Roy et al. [3] demonstrated the formation of C-20 ketals in alcoholic solvents, and particularly in the presence of acid impurities. This can be prevented by removing impurities or by their neutralization. Dominguez [2] previously described the existence of two interconvertible forms of 18-OH-DOC with different chromatographic mobilities.

Estimation of 18-OH-DOC in plasma from the adrenal veins in our 18 patients with low-renin hypertensions revealed a clinical importance of determining this mineralocorticoid for the differential diagnosis of some types of arterial hypertension. Together with measurement of plasma concentration of aldosterone, the estimate was able to contribute significantly to the differentiation of morphological changes of the adrenals in mineralocorticoid-dependent hypertensions [11]. In aldosterone-producing adenoma of the adrenal cortex the concentration of 18-OH-DOC in the adrenal venous blood was on average 3.6 times higher on the side of the adenoma (average value 5.681 μg/100 ml on the side of the adenoma and 1.261 μg/100 ml on the intact side). In idiopathic hyperaldosteronism with bilateral hyperplasia of the adrenals, the 18-OH-DOC concentration, similarly to that of aldosterone, was increased symmetrically on both sides (average concentrations 8.962 μg/100 ml on the left and 7.108 μg/100 ml on the right side). In low-renin essential hypertensions, 18-OH-DOC concentration was high in both adrenal veins (10.889 μg/100 ml on the left and 7.146 μg/100 ml on the right side), whereas aldosterone concentrations were low. In Cushing's syndrome, 18-OH-DOC concentrations in the adrenal venous blood were variable (0.116—6.828 μg/100 ml). For details see our previous communication [11]. Synthetic ACTH increased the 18-OH-DOC concentration several times. Our findings of 18-OH-DOC concentrations in the adrenal venous blood were in the range reported by Melby et al. [1].

The determination of 18-OH-DOC in the peripheral venous blood is important for the investigation of the possible etio-pathogenic participation of this mineralocorticoid in the initiation and in the course of certain types of arterial hypertension, particularly in patients with suppressed renin [12—14]. The 18-OH-DOC values in peripheral venous blood of our patients with hypertension varied in the range of 0.069—0.360 μg/100 ml and are somewhat higher than those presented by Mason and Fraser [8] for control persons when a similar technique was used. The administration of ACTH raised the peripheral 18-OH-DOC concentration in a similar manner to that in the adrenal veins.

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