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SEPARATION AND CHARACTERIZATION OF THE REDUCED METABOLITES OF THE 18-HYDROXYDEOXYCORTICOSTERONE HORMONE BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

OCCURRENCE OF STEREOISOMERIC FORMS IN RAT ADRENALS AND LIVER

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SUMMARY

In order to study the metabolism of 18-OH-11-deoxycorticosterone, the mineralocorticoid hormone responsible for hypertension in rats and humans, we have synthesized the following dihydrogenated and tetrahydrogenated reference derivatives: 18,21-dihydroxy-5α-pregnane-3,20-dione, 18,21-dihydroxy-5β-pregnane-3,20-dione, 3α,18,21-trihydroxy-5α-pregnan-20-one (I), 3β,18,21-trihydroxy-5α-pregnan-20-one (II), 3α,18,21-trihydroxy-5β-pregnan-20-one (III) and 3β,18,21-trihydroxy-5β-pregnan-20-one (IV). A complete separation of these compounds from each other and from tetrahydrocorticosterone isomers has been realized only by the association of thin-layer chromatography and gas–liquid chromatography on high-efficiency glass capillary columns. Characterization by gas–liquid chromatography–mass spectrometry is described. The stereoisomer distribution in rats is: adrenals of adult males and females (compounds I and II in the ratio 4:1); adrenals of 23-day-old males and females (compounds I, II and III, 11:11:3); liver of females (compound I and traces of III); and the liver of adult male (compounds II, III and IV, 4.5:4.5:1).

INTRODUCTION

The zona fasciculata of adrenal glands secretes under the stimulation of adrenocorticotropic hormone (ACTH) 18-OH-11-deoxycorticosterone (18-OH-DOC), a mineralocorticosteroid hormone[1]. This hormone has been implicated as a hypertensive agent in rats as well as in humans[1,2]. Direct evidence of its hypertensive action in rats has been shown recently[3].

Until now, little has been known about the metabolism of 18-OH-DOC except the finding of 16α,18-dihydroxydeoxycorticosterone from human adrenal homogenates incubated with tritiated 18-OH-DOC[4] and of an elevated excretion of

18-hydroxytetrahydrodeoxycorticosterone (18-OH-TH-DOC) in the urine of hypertensive patients[2], though in the latter case no definite identification was achieved. The lower mineralocorticosteroid activity of 16α,18-dihydroxydeoxycorticosterone compared with that of 18-OH-DOC, and the hypothesis that other derivatives of 18-OH-DOC possess significant activity[2], prompted us to carry out further studies on 18-OH-DOC metabolism.

On the other hand, however, enzyme differentiation in liver[5–11] and in adrenals[12–14] linked to the reductive pathways of corticosteroids has been the subject of studies restricted to corticosterone metabolism. Therefore, further information in this field could be gained by a better knowledge of 18-OH-DOC metabolism.

The lack of reference compounds and difficulties encountered in analytical procedures are the main reasons for the slowing down of investigations into 18-OH-DOC metabolites.

The existence, in 18-hydroxycorticosteroids, of an equilibrium between the (18→20) hemiacetal and the 18-hydroxy-20-oxo forms gives rise to difficulties during the isolation procedure[15]. They were overcome by suitable derivative formation in order to obtain one peak for each compound in gas–liquid chromatographic–mass spectrometric (GLC–MS) analysis[16].

In a recent article[17], we described the identification, by GLC–MS, of 3β,18,21-trihydroxy-5α-pregnan-20-one, a tetrahydro derivative of 18-OH-DOC, as an endogenous component produced by rat adrenals and liver. During the separation procedure, search for the three other stereoisomeric forms in rat tissues was subject to interference from three of the four tetrahydrocorticosterone (THB) isomers also present in these rat tissues.

In this article, we describe (i) the synthesis of the two stereoisomers of 18-hydroxydihydrodeoxycorticosterone (18-OH-DH-DOC) and of the four stereoisomers of 18-OH-TH-DOC as reference compounds, (ii) a separation method allowing the complete resolution of the mixture of the four tetrahydro derivative isomers of corticosterone and the four tetrahydro derivative isomers of 18-OH-DOC using thin-layer chromatography (TLC) and GLC on packed and capillary columns, (iii) the characterization of 18-OH-DH-DOC and 18-OH-TH-DOC isomers by GLC–MS, (iv) the occurrence of the various stereoisomeric forms of 18-OH-TH-DOC, in rat adrenals and liver, which depend on the sex and age of the animal and on the particular organ being considered.

MATERIALS AND METHODSS

Nomenclature and reference compounds

The following trivial names and abbreviations are used: 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) = 18,21-dihydroxy-4-pregnene-3,20-dione; 18-hydroxydihydrodeoxycorticosterone (18-OH-DH-DOC) = 18,21-dihydroxy-5α/β-pregnane-3,20-dione; 18-OH-5α-DH-DOC = 18,21-dihydroxy-5α-pregnane-3,20-dione; 18-OH-5β-DH-DOC = 18,21-dihydroxy-5β-pregnane-3,20-dione; 18-hydroxytetrahydrodeoxycorticosterone (18-OH-TH-DOC) = 3α/β,18,21-trihydroxy-5α/β-pregnan-20-one; 18-OH-TH-DOC I = 3α,18,21-trihydroxy-5α-pregnan-20-one; 18-OH-TH-DOC II = 3β,18,21-trihydroxy-5α-pregnan-20-one; 18-OH-TH-DOC III = 3α,18,21-trihydroxy-5β-pregnan-20-one; 18-OH-TH-DOC IV = 3β,18,21-trihydroxy-

5β-pregnan-20-one; dihydrocorticosterone (DHB) = 11β,21-dihydroxy-5α/β-pregnane-3,20-dione; 5α-DHB = 11β,21-dihydroxy-5α-pregnane-3,20-dione; 5β-DHB = 11β,21-dihydroxy-5β-pregnane-3,20-dione; tetrahydrocorticosterone (THB) = 3α/β, 11β,21-trihydroxy-5α/β-pregnan-20-one; THB I = 3α,11β,21-trihydroxy-5α-pregnan-20-one; THB II = 3β,11β,21-trihydroxy-5α-pregnan-20-one; THB III = 3α,11β,21-trihydroxy-5β-pregnan-20-one, THB IV = 3β,11β,21-trihydroxy-5β-pregnan-20-one.

5α-DHB, 5β-DHB and THB isomers were purchased from Ikapharm (Ramat-Gan, Israel); 18-OH-DOC was obtained from Searle (Naucalpan, Mexico).

Syntheses of 18-OH-DH-DOC and 18-OH-TH-DOC

The 5α and 5β isomers of 18-OH-DH-DOC were prepared from 1.6 mg of 18-OH-DOC, by controlled hydrogenation, in 5 ml of ethanol with 5 mg of palladium on charcoal (5% w/w) as catalyst in a microhydrogenator (Büchi, Flawil, Switzerland). The supernatant was collected after centrifugation to remove the catalyst.

The 18-OH-TH-DOC isomers were synthesized by sodium borohydride reduction, on a 2/10 aliquot of the previous reaction products containing 5α and 5β (3:2, w/w) isomers of 18-OH-DH-DOC dissolved in ethanol. After 1 h at room temperature the reaction mixture was diluted with distilled water and the reaction products extracted with ethyl acetate.

The 18-OH-TH-DOC isomers were also obtained by enzymatic reduction of a 2/10 aliquot of the mixture of 5α and 5β isomers of 18-OH-DH-DOC using a preparation of 3α/3β-hydroxysteroid oxidoreductase from *Pseudomonas testosteroni* (Sigma, St. Louis, Mo., U.S.A.) (0.05 I.U./mg). The incubation mixture in 5 ml of 0.2 *M* Tris–hydrochloric acid buffer at pH 8.9 contained 12 μmoles of NADPH and 11 mg of enzyme preparation. After incubation at room temperature for 4 h, the products were extracted with ethyl acetate.

Derivatives

Steroids were analyzed by GLC–MS as their O-methyloxime-trimethylsilyl (MO-TMS) derivatives. The MO-TMS derivatives were prepared by the previously described method[16,17]. A 50 μg sample of the steroid was allowed to react with 50 μl of O-methyloxyamine hydrochloride (Pierce Chemical Co., Rockford, Ill., U.S.A.) in pyridine (16 mg/ml) at 65° for 3 h; under these conditions the equilibrium between the 20-oxo form and the hemiacetal form of the 18-hydroxysteroid is completely shifted towards the 20-oxo form, leading to the 20-MO derivative, so that each 18-hydroxysteroid gives only one GLC peak as an MO-TMS derivative. TMS derivatives were made to react at 65° for 16 h with bis(trimethylsilyl)acetamide–trimethylchlorosilane (80:20, v/v) in order to obtain a complete silylation of all hydroxyl groups present in the molecules.

Thin-layer chromatography

The THB and 18-OH-TH-DOC isomers were fractionated as free compounds or O-methyloxime derivatives by TLC on 0.25-mm or 0.5-mm F 251 silica gel plates from Merck (Darmstadt, G.F.R.). The following solvent systems were used: (i) for methyloxime derivatives, diisopropyl ether–acetone (70:30, v/v); (ii) for free compounds, first run, diisopropyl ether and second run, ethyl acetate–isooctane–methanol (180:15:5, v/v).

The TLC fractions were scraped off, and the free steroids were eluted with dichloromethane–methanol (1:1, v/v). After derivative formation, the steroids were analyzed by GLC–MS.

Gas–liquid chromatography and mass spectrometry

Packard (Downers Grove, Ill., U.S.A.) Model 7 400 and Packard–Becker (Delft, The Netherlands) Models 419 and 420 gas chromatographs equipped with flame ionization detectors were employed. Packed columns were 4 m and 3 m × 3 mm I.D. silanized glass tubes. The packings were 1 % OV-1, 1 % OV-17 or 1 % Dexsil-300 (Supelco, Bellefonte, Pa., U.S.A.) on 100–120 mesh, acid washed and silanized Gas-Chrom P (Applied Science Labs., State College, Pa., U.S.A.). All column packings were prepared according to Horning *et al.*[18]. The optimal efficiency of the packed columns was calculated with *n*-octacosane at 230° and with THB II at 260°. For a flow-rate of 35 ml/min with nitrogen as carrier gas, the number of theoretical plates for the 1 % OV-1 3 m × 3 mm column was 7500.

Capillary columns were 45 m × 0.25 mm I.D. capillary tubes of soda glass etched with gaseous HCl at 300° and silanized in the gas phase with hexamethyldisilazane according to Alexander *et al.*[19]. The coating was carried out by the static method with SE-30. The optimal efficiency of the capillary column was calculated with *n*-octacosane at 230°; the flow-rate was 0.5 ml/min with nitrogen as carrier gas. The capacity ratio was 7.3; the $\Delta MU_{2\sigma}$ was 0.02 and the separation number at 4σ between $C_{24}H_{50}$ and $C_{28}H_{58}$ was 110.

An LKB (Bromma, Sweden) 9000 GLC–MS instrument was employed. The silanized glass column (4 m × 3 mm I.D.) was packed with 1 % OV-1 on 100–120 mesh Gas-Chrom P. The temperatures were: flash heater 260°, molecular separator 290° and ion source 310°. The carrier gas was helium with a flow-rate of 30 ml/min. The accelerating voltage was 3500 V, the trap current 60 μA and the electron energy 70 eV for total mass spectrum recordings.

Mass fragmentography (MF) was carried out using the new multiple-ion detector–peak matcher from LKB. The entrance and detector slits were adjusted to 0.3 and 0.4 mm, respectively. The electron ionizing energy was 28 eV. Four fragment ions were monitored on the mass spectrometer allowing simultaneous recording of four different masses within a mass range of 25 % of the higher mass. Sensitivity, background subtraction and filtering were separately adjusted on each channel.

Tissue sample preparation

Animals used were either adult and pre-pubertal (23-day-old) Sprague-Dawley OFA rats (Merieux, Lyon, France) or adult Wistar rats, US/Commentry strain (Institut National de la Recherche Agronomique, Dijon, France). The livers and adrenals were removed after decapitation, and pooled separately for each group of rats.

Adrenal steroid extraction was carried out as described previously[17]. Separate pools of 78 glands (355 mg) from 23-day-old Wistar female rats were homogenized in 10 ml of methanol–water (70:30, v/v). After centrifugation the supernatant was kept at −20° for 24 h in order to precipitate the lipids. The methanol was evaporated in a nitrogen stream at 60° and the steroids were extracted from the aqueous solution three times with ethyl acetate and then three times with dichloromethane. Half of the

steroid extract was purified in some instances by preparative TLC, the first run being with diisopropyl ether and the second run with diisopropyl ether–acetone (60:40, v/v). The steroids of the isolated TLC fractions containing THB and 18-OH-TH-DOC or the total extract were taken up in 50 μl of reagents; aliquots of 2–7 μl were analyzed by GLC–MS as their MO-TMS derivatives.

Liver steroids were extracted as described previously[9]. Pools of 11 livers (70 g) from adult female Wistar rats, 8 livers (70 g) from adult male Wistar rats, and 24 livers (170 g) from adult female Sprague-Dawley rats were homogenized in chloroform–methanol (1:1, v/v). After centrifugation, the extract was evaporated to dryness and lipids were removed by precipitation in methanol–water (70:30, v/v) at −20° for 24 h. The steroid conjugates were then hydrolyzed using a combination of enzymatic and solvolytic methods. The free and the liberated steroids were extracted using consecutively dichloromethane and ethyl acetate.

The steroid extract was analyzed by MF on a 2/10 aliquot while the remaining material was purified by preparative TLC. In order to separate 18-OH-TH-DOC isomers from tetrahydrocorticosterone isomers, a first run with diisopropyl ether followed by a second run with ethyl acetate–isooctane–methanol (180:15:5, v/v) were carried out. The steroids of the isolated TLC fractions were converted into MO–TMS derivatives and submitted to GLC–MS analysis.

RESULTS AND DISCUSSION

Characterization by GLC and GLC–MS of di- and tetrahydrogenated derivatives of 18-OH-DOC

18-OH-DH-DOC isomers. In the analysis by GLC–MS of the mixture of 18-OH-5α-DH-DOC and 18-OH-5β-DH-DOC, their MO-TMS derivatives display good separation on OV-1, OV-17 and Dexsil-300 columns (Table I); the 5α-configuration isomer, owing to its more planar shape, is retained longer on both non-polar and polar phases, as generally observed[20].

The ratio of 5α/5β-isomer amounts in the hydrogenation mixture is 60:40, w/w. On the OV-1 stationary phase, MO-TMS derivatives of 18-OH-5α-DH-DOC are separated into two peaks corresponding to *syn/anti* isomers of the methoxime group at the 3 position; this phenomenon, described for the first time on OV-1 by Horning *et al.*[21], was also observed on Dexsil-300, while no separation was obtained on OV-17.

The mass spectrum of 18-OH-5β-DH-DOC as MO-TMS derivatives is given in Fig. 1; it shows typical ions at *m/e* 550 (M), 519 (M − 31), 447 (M − 103), 429 (M − 31 − 90), 398 (M − 2 × 31 − 90), 370 (M − 2 × 90), 325, 299 (M − 90 − α − H), 268 (M − 90 − 31 − α − H) where α corresponds to the side chain and 103 corresponds to $CH_2=^+O$-TMS as the base peak of the mass spectrum. The MO-TMS derivative of 18-OH-5α-DH-DOC exhibits essentially the same fragment ions as those of the 5β-isomer but the identification between the two forms is obtained by GLC separation.

18-OH-TH-DOC isomers. The reduction of 5α/5β-isomers of 18-OH-DH-DOC by 3-oxidoreductase or $NaBH_4$ gives rise to 18-OH-TH-DOC isomers which were analyzed by GLC and GLC–MS. The determination of the configuration of each isomer, identified first as 18-OH-TH-DOC by GLC–MS, is based on the stereospecifici-

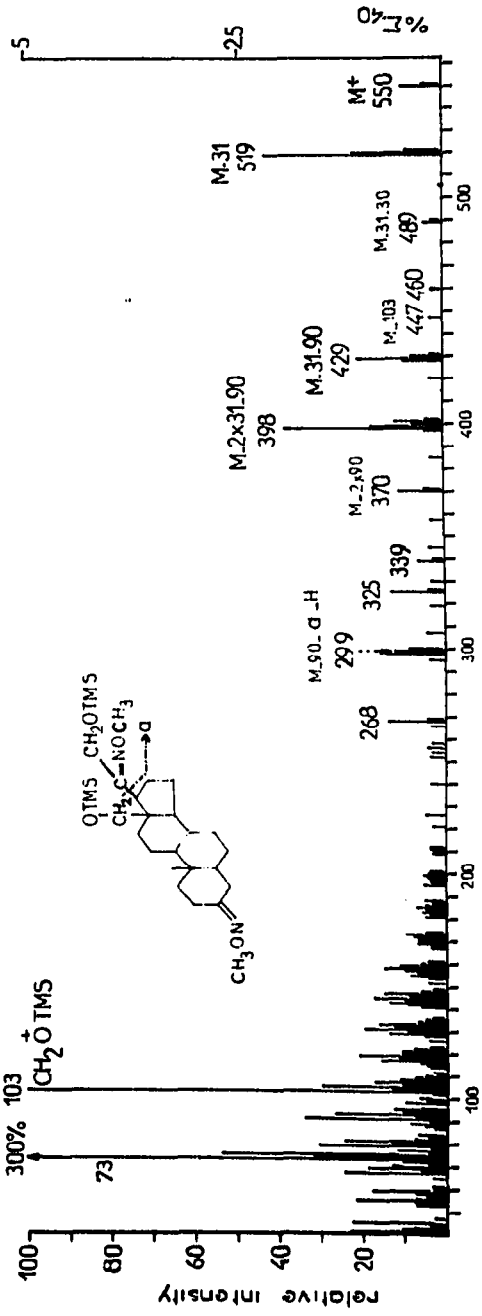

Fig. 1. Mass spectrum of the MO-TMS derivative of an authentic sample of 18,21-dihydroxy-5β-pregnane-3,20-dione. Main fragment ions of structural interest include those at *m/e* 103 ($CH_2=^+O$-TMS), 447 (M−103), those corresponding to the elimination of silanol (90 a.m.u.) from the TMS groups and/or of OCH_3 (31 a.m.u.) from the methyloxime groups, those at *m/e* 299 (M − 90 − α−H) and 268 (M − 90 − 31 − α − H) where α corresponds to the side chain.

TABLE I

METHYLENE UNIT (MU) VALUES AND RELATIVE RETENTION TIMES (RRT) OF MO-TMS DERIVATIVES OF 18-OH-DH-DOC AND 18-OH-TH-DOC

Compounds	*MU values*			*RRT on SE-30 capillary column***
	*OV-1**	*OV-17***	*Dexsil-300***	
18-OH-5β-DH-DOC	30.48	32.72	30.80	—
18-OH-5α-DH-DOC	31.12§	33.54	31.37§	—
	31.22		31.53	
18-OH-TH-DOC I	29.92§§	30.68§§§	29.58§§§	1.49
18-OH-TH-DOC II	31.11	31.90	30.88	2.01
18-OH-TH-DOC III	29.77	30.68§§§	29.58§§§	1.41
18-OH-TH-DOC IV	29.92§§	30.68§§§	29.58§§§	1.51

* MU values were obtained with a 3 m × 3 mm 1% OV-1 column by temperature programming at the rate of 1°/min from 180°.

** MU values were obtained with 4 m × 3 mm 1% OV-17 and 1% Dexsil-300 columns by temperature programming at the rate of 1°/min from 180°.

*** Retention times relative to 5α-cholestane obtained with a 45 m × 0.25 mm I.D. SE-30 glass capillary column at 260° under isothermal conditions.

§ Separation of *syn* and *anti* isomers of 18-OH-5α-DH-DOC was obtained on OV-1 and Dexsil-300 columns.

§§ Isomers I and IV are unresolved on OV-1 column.

§§§ Isomers I, III and IV are unresolved on OV-17 and Dexsil-300 columns.

ty of the reactions, giving in both cases a 3-hydroxyl group orientated mainly in the equatorial position, on the relative retention behavior in GLC and on the comparison of peak-area ratios of the isomers with those of 18-OH-DH-DOC isomers. The gas chromatogram in Fig. 2 shows the two main isomers of 18-OH-TH-DOC with an area ratio of 62:38. The last eluted peak is the most abundant; consequently it comes from 18-OH-5α-DH-DOC with a 3-hydroxyl group in the equatorial position. It is identified as 18-OH-TH-DOC II of 3β,5α configuration, which agrees well with its longer retention time, indicating a more planar molecular shape given by the combination of the A/B ring in the *trans* position and equatorial 3-hydroxyl group. The first eluted peak comes from 18-OH-5β-DH-DOC and is identified as 18-OH-TH-DOC III of 3α,5β configuration.

The two minor isomers of 3α,5α and 3β,5β configuration, 18-OH-TH-DOC I and 18-OH-TH-DOC IV, respectively, also present in the reduction mixture are not resolved by GLC on a polar or apolar packed column or even on a capillary column of 50,000 theoretical plates. Their separation, shown in Fig. 3, was obtained with an SE-30 glass capillary column of high efficiency, *viz.* 110,000 theoretical plates.

Methylene unit (MU) values on the different phases and relative retention times on capillary columns are given in Table I. The mass spectra of the two isomers 18-OH-TH-DOC II and 18-OH-TH-DOC III reported in Fig. 4 are very similar; they are characterized by a base-peak at *m/e* 103 and the ratio 595/564 a.m.u. of 0.2. These

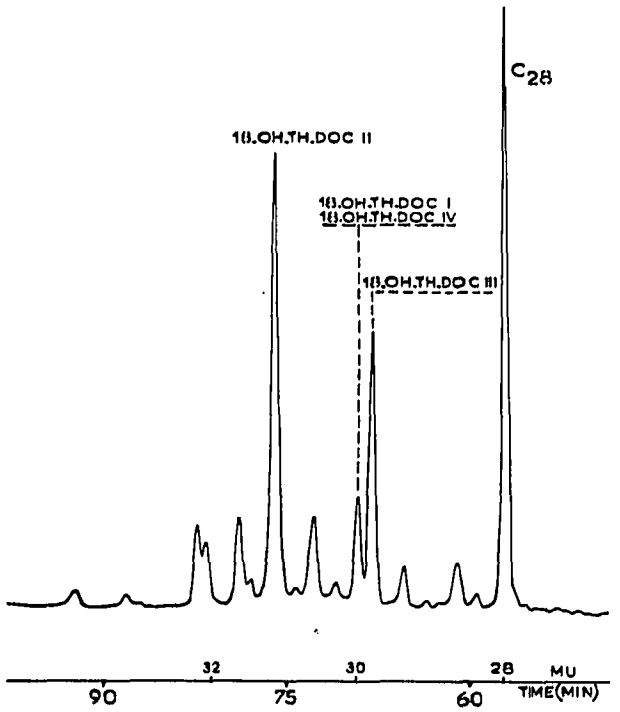

Fig. 2. GLC separation of MO-per-TMS derivatives of 18-OH-TH-DOC isomers synthesized by chemical reductions of 18-OH-DH-DOC isomers. 18-OH-TH-DOC I = 3α,18,21-Trihydroxy-5α-pregnan-20-one; isomer II = 3β,18,21-trihydroxy-5α-pregnan-20-one; isomer III = 3α,18,21-trihydroxy-5β-pregnan-20-one; and isomer IV = 3β,18,21-trihydroxy-5β-pregnan-20-one; *n*-octacosane (C_{28}) was used as internal standard; other minor peaks correspond mainly to hexahydro derivatives obtained in the course of the chemial reduction. Chromatographic conditions: 10 ft., 1% OV-1 column; temperature programmed at 1°/min from 180°.

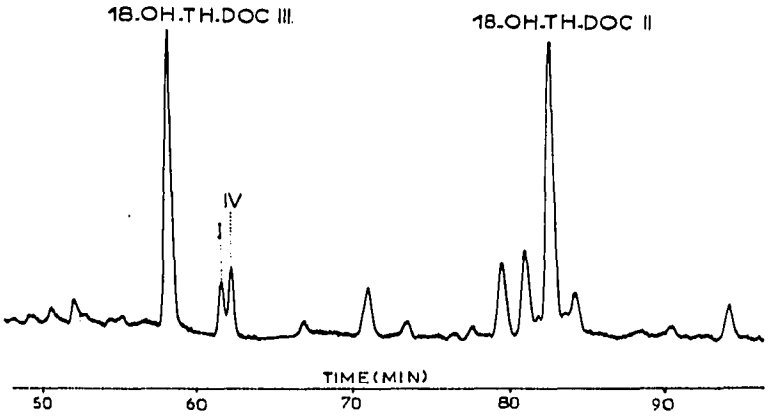

Fig. 3. Separation of 18-OH-TH-DOC isomers using a glass capillary column (45 m × 0.25 mm I.D.) coated with SE-30. Column temperature was kept constant at 260°. Temperatures: flash heater, 240°; detector, 280°. Same injected sample and abbreviations as in Fig. 2.

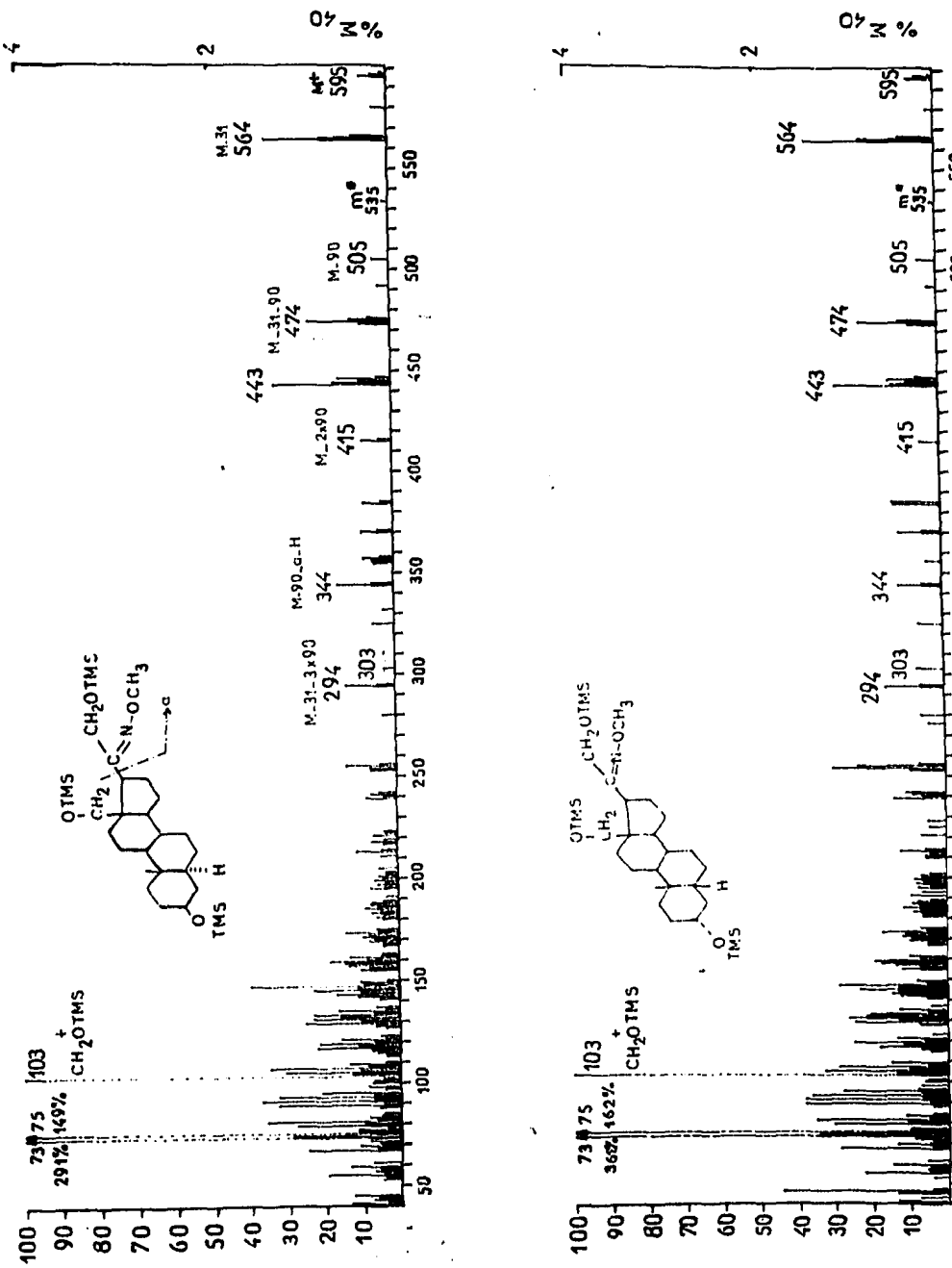

Fig. 4. Mass spectra of the MO-TMS derivative of an authentic sample of 18-OH-TH-DOC II (upper panel) and 18-OH-TH-DOC III (lower panel). A metastable ion was observed at $m/e = 535.0$. It corresponds to the transition $595 \xrightarrow{-31} 564$ (m^* calculated = 534.6). Notice the base peak at m/e 103 and the intensity ratio of ions at m/e 595 and 564, which allow 18-OH-TH-DOC and THB to be distinguished[9].

mass spectral features allow 18-OH-TH-DOC to be distinguished from THB. The mass spectra of the latter have been reported previously[9].

Separation by TLC and GLC of isomeric tetrahydrogenated derivatives of 18-OH-DOC and of corticosterone

The isolation of the four isomers of 18-OH-DOC from biological samples (adrenals, liver, urine) is hampered by the presence of tetrahydrocorticosterone isomers. In GLC with an OV-1 packed column or a glass capillary column of 50,000 theoretical plates, 18-OH-TH-DOC I and 18-OH-TH-DOC IV, which are not separated from each other, interfere with the $3\alpha,5\beta$ isomer of tetrahydrocorticosterone (THB III). The five other isomers have been separated. In GLC on a glass capillary column with high efficiency (110,000 theoretical plates), THB III and 18-OH-TH-DOC IV are still unresolved, but are separated from 18-OH,TH-DOC I.

The previous TLC separation of 18-OH-TH-DOC isomers from THB isomers has been carried out with three solvent systems (Fig. 5). The solvent system S_1 was used to separate free compounds, and the solvent system S_2 for methyloxime derivatives. They both allow the separation of THB III from 18-OH-TH-DOC I and IV. Consequently, the further analysis by GLC–MS on a packed column of the steroids from the three different zones of the thin layer does permit the identification of the four isomers of THB and two isomers of 18-OH-TH-DOC, *i.e.* II and III. The analysis, on a high-efficiency capillary column, of fraction 3 from system S_1 and of fraction 2 from system S_2 allows the separation and the identification of 18-OH-TH-DOC I and IV.

The use of solvent system S_3 for TLC leads to the complete separation of THB isomers from 18-OH-TH-DOC isomers. This previous separation is satisfactory for the detection and determination by GLC and GLC–MF when the relative concen-

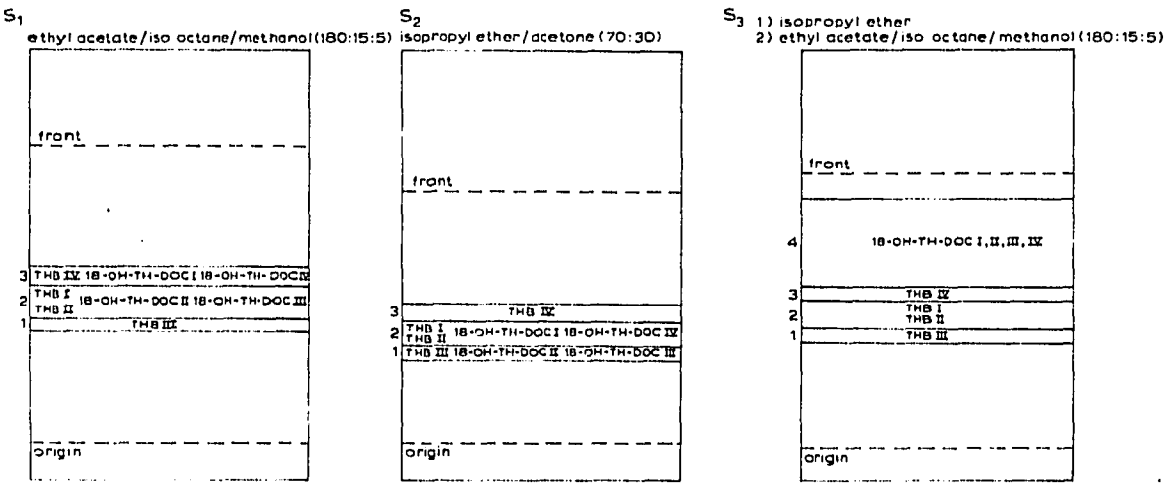

Fig. 5. Thin-layer chromatograms of isomeric 18-OH-TH-DOC and THB on 0.25 mm silica gel G plates. Free steroids were separated with solvent systems S_1 and S_3 and MO-derivatives were separated with S_2. TLC fractions were scraped off and analyzed by GLC and GLC–MS.

trations of the two groups are very different, as occurs in the liver extracts. Furthermore, the use of diisopropyl ether in a first run allows the elimination of residual lipids which migrate to the solvent front.

Occurrence in rat liver and adrenals

Fig. 6 summarizes the origins of the different 18-OH-TH-DOC isomers in the rat.

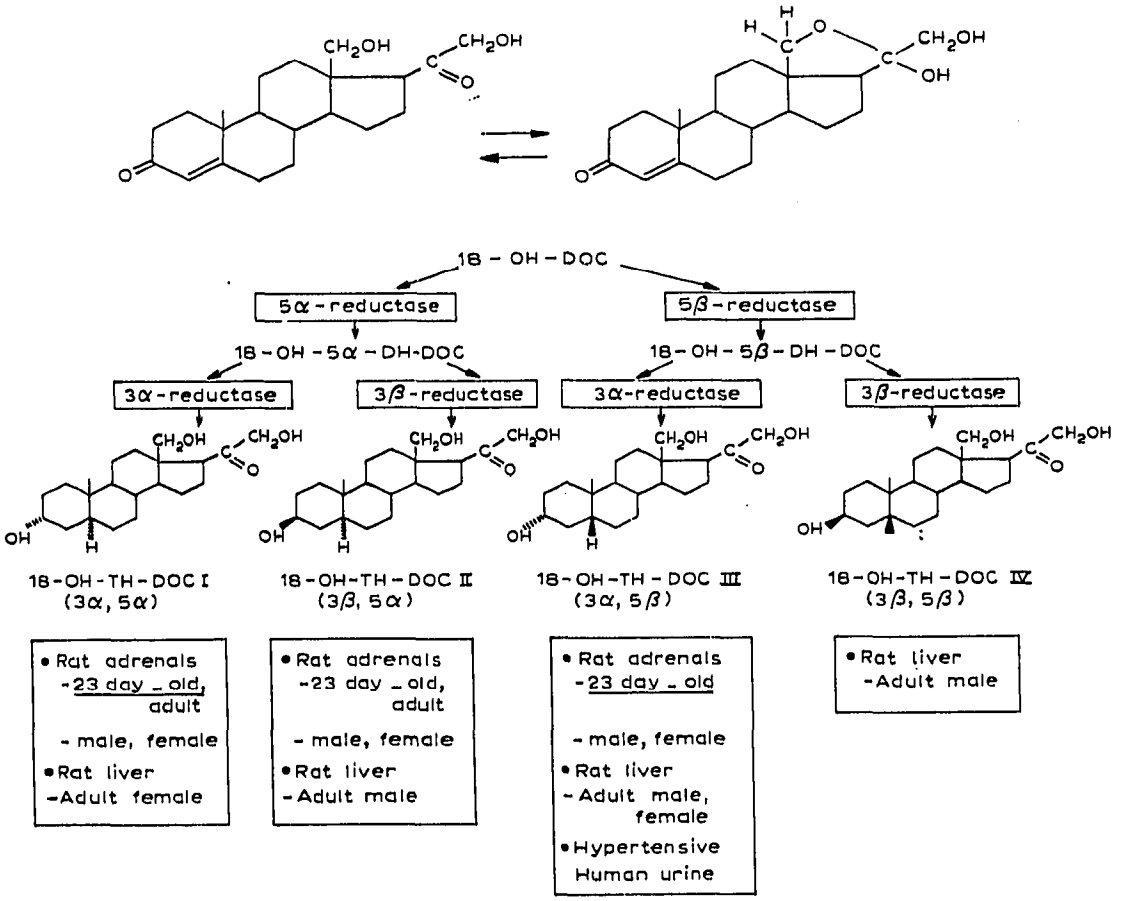

Fig. 6. Reductive metabolic pathway of 18-OH-DOC in rat liver and adrenals. The stereochemistry of the reaction is dependent on sex, age of the animal and on the organ under consideration.

The identification of 18-OH-TH-DOC isomers in adrenals shows that 18-OH-DOC is already metabolized before leaving the adrenal cortex[17]; the metabolic transformations are carried out by reductases which lead to 3β,5α (80%) and 3α,5α (20%) isomers in adults and to 3α,5α (44%), 3β,5α (44%) and 3α,5β (12%) isomers in the pre-pubertal 23-day-old animals of both sexes. This shows the high activity of the 5α-reductase together with the weak activity of 5β-reductase only before puberty. The percentage of 18-OH-TH-DOC isomers is 1% 18-OH-DOC in the adult, while it is

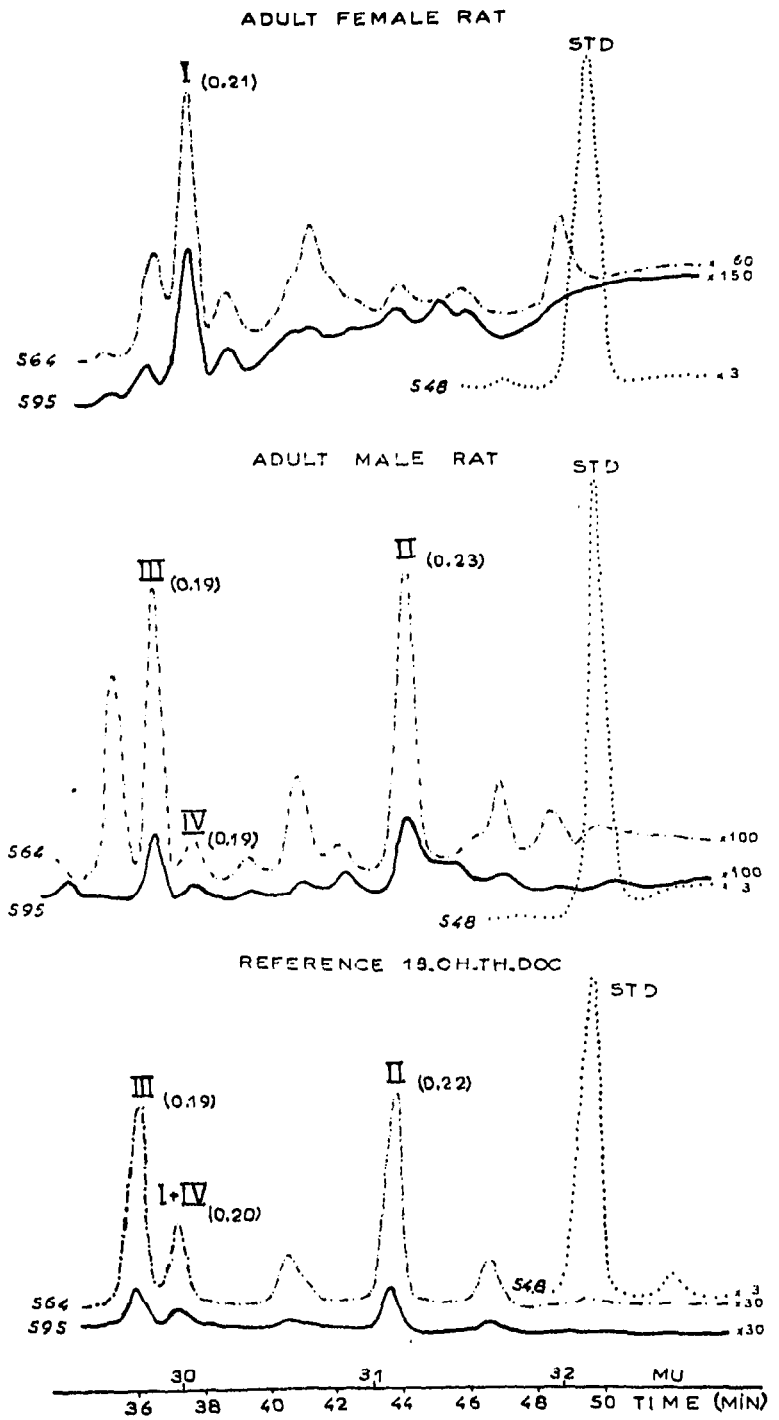

Fig. 7. Mass fragmentograms of isomeric 18-OH-TH-DOC from liver extract of adult female rat (upper panel), adult male rat (middle panel) and from a mixture of references (lower panel). Configurations of isomers I–IV are given in the legend of Fig. 2. Ions at *m/e* 564, 595 are used to record 18-OH-TH-DOC and ion at *m/e* 548 to record corticosterone as internal standard (STD). Ratios of 595/564 a.m.u. are given in parentheses. Chromatographic conditions: 12 ft., 1% OV-1 column; temperature programmed at 1°/min from 220°. MS conditions, electron energy 28 eV; trap current 60 μA; temperature 280°.

6–9% in the 23-day-old animal. This corresponds to a higher reductase activity on 18-OH-DOC than on corticosterone[17].

Several studies have indicated the presence of sexual differences in the hepatic metabolism of corticosterone in rats[9]. Sexual differences exist also in the patterns of 18-OH-DOC metabolites found in the liver tissue of adult rats, indicating that they are mainly the result of hepatic metabolism. No 18-OH-TH-DOC was found in the liver of the pre-pubertal rat while the presence of THB has been demonstrated at this age[9,10].

Fig. 7 shows mass fragmentograms recorded with the ions at *m/e* 564 and 595, demonstrating the presence of 18-OH-TH-DOC I (3α,5α-isomer) and traces of isomer III (3α,5β) in the adult female as compared to the finding of 18-OH-TH-DOC III (3α,5β) (45%), IV (3β,5β) (10%) and II (3β,5α) (45%) in the adult male rat. This sex-specific pattern is similar to that observed with corticosterone as a substrate of the reductase systems.

The compound isolated from hypertensive human urine and described by Melby *et al.*[2] as an 18-OH-TH-DOC is most likely the isomer III (3α,5β) since the 5β-reduction of corticosteroids is prominent in humans; conversely 5α-reduction is the major pathway in the female rat, while 5α and 5β-reductions are of equal level in the male rat.

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