ISOLATION AND IDENTIFICATION OF NOVEL SULFUR-CONTAINING METABOLITES OF SPIRONOLACTONE (ALDACTONE®)

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ABSTRACT

In the urine of subjects given an oral dose of spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone], six metabolites have been detected. One of the major metabolites was found to be the previously characterized de-thioacetylated compound, 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone (canrenone). Besides this a new major sulfur-containing metabolite has been isolated and identified as 3-(3-oxo-7α-methylsulfinyl-6β,17β-dihydroxy-4-androsten-17α-yl)propionic acid γ-lactone. This structural assignment was based on detailed analysis of its IR, NMR and UV spectra as well as comparison of its physical constants and chromatographic (TLC and GLC) characteristics with a synthetic sample. The three minor metabolites were found to be very labile and were readily converted to canrenone.

INTRODUCTION

Spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, I, Fig 1] has been extensively used in the clinic as an aldosterone-antagonist diuretic. Recent studies, both in vivo (1) and in vitro (2)

in experimental animals have indicated that this drug markedly induces the activity of hepatic microsomal enzymes. The metabolism of spironolactone in humans (3,4) and in rats (5) has been reported previously, but so far only the de-thioacetylated metabolite, canrenone [aldadiene, 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone, II] has been fully characterized. In humans this metabolite accounts for only 4% of the administered dose (6) in 0-24 hour urine samples. Thus, a large portion of the administered drug remains unaccounted. Our interest in the biotransformation of spironolactone was stimulated by the recent findings of Selye (7,8) and Garg et al. (9) that pretreatment of rats with spironolactone prevented the fatal renal damage produced upon administration of mercuric chloride. It is thus of particular interest to investigate whether the metabolic elimination of the thioacetate group from the steroid skeleton of spironolactone is quantitative, or if metabolites retaining the sulfur atom of the parent drug are present.

In this communication we report isolation and structural elucidation of novel sulfur-containing metabolites of spironolactone from urine of human subjects given an oral dose of the drug.

MATERIALS AND METHODS

Thin-layer Chromatography (TLC): Analytical thin-layer chromatography was run on 5 x 20 cm glass plates coated with 250 μ of silica gel HF_{254} (E. Merck, Darmstadt, Germany). Thin-layer plates were activated for one hour at 120°C before use and developed in a solvent system composed of methanol-chloroform (10:90). Metabolites and reference compounds were visualized under long- or short-wave ultraviolet light before and after spraying the plate with a 5% solution of ceric sulfate in 10% (v/v) aqueous sulfuric acid. The sprayed plates were heated at 110° for 10 min before visualization. Preparatory thin-layer chromatography was run on 20 x 20 cm glass plates coated with 1 mm silica gel HF_{254}.

Gas-liquid Chromatography (GLC): Gas-liquid chromatographic studies were made with a Varian Aerograph model 1400 I having a flame ionization detector system with a 6-foot long 2 mm inside diameter silanized spiral glass column packed with 3% OV-1 on 80-100 mesh Chromosorb W-H.P. (Johns-Manville, Trenton, N.J.). The following operating conditions were used: column temperature, 250°C; detector temperature, 280°C; injection port temperature, 260°C; and flow rate of the carrier gas (helium), 52 ml/min.

Spectroscopic Analysis: Infrared spectra (IR) were measured in chloroform with an ultramicro cell on a Beckman IR-12 instrument. Ultraviolet absorption spectra (UV) were obtained in methanol on a Beckman DK-2A ratio recording spectrometer. Mass spectra were obtained by direct probe technique at 20 eV and 200°C on an AEI-MS 902 mass spectrometer. Nuclear magnetic resonance spectra (NMR) were obtained on a Varian Associates HA-100 spectrometer in deuterochloroform ($CDCl_3$) solution. Chemical shifts have been recorded in ppm (δ) values. Specific rotations were measured in chloroform solution (c=1) at 26°C.

Isolation of Metabolites of Spironolactone from Urine: Twenty adult male volunteers were each given 100 mg tablets of spironolactone. Their 0-6 hour urine samples were collected and stored at -20°C until analyzed.

Two liter aliquots were taken from the pooled urine and passed through an Amberlite XAD-2 resin (Rohm and Haas Co., Philadelphia, Pa.) column (1 kg, 56 x 5.0 cm) prepared as described by Bradlow (10). The column was first eluted with water (6L) followed by methanol (3L). Evaporation of the methanol eluate furnished extract A which was partitioned between chloroform and water. The chloroform layer was dried over anhydrous sodium sulfate and evaporated to dryness to yield extract B. This extract was dissolved in chloroform (10 ml) and passed through a silica gel (Davison Chemical Company, Baltimore, Md.) column (100 g, 20 x 2.0 cm) packed in chloroform. The column was first eluted with chloroform (250 ml) followed by increasing proportions of methanol in chloroform. Elution with 1% methanol (250 ml) gave eluate C, with 5% methanol (250 ml) gave eluate D and 10% methanol (250 ml) gave eluate E. The column was finally stripped with methanol (250 ml).

Thin-layer chromatographic analysis of eluate C showed the presence of three drug related compounds: metabolite A (R_f 0.79), the metabolite with R_f 0.67 (Fig. 2) and metabolite B (R_f 0.53). This eluate was streaked on a preparatory thin-layer chromatographic plate and developed in a solvent system of methanol-chloroform (10:90) mixture. The above metabolites were located as the dark bands when the plate was viewed under short-wave UV light. These bands were eluted with 20% methanol in chloroform. The eluate from each band was evaporated to dryness with a stream of nitrogen at 40-50°C. The zone with R_f 0.79 gave 5 mg of metabolite A which was indistinguishable from canrenone (II). Rechromatography on an analytical TLC plate of zones with R_f 0.67 and 0.53 (metabolite B) showed that both of these metabolites were converted to a common product with R_f 0.79. This product was identified as canrenone by its IR and UV spectral analysis. GLC analysis of eluate C showed only one major drug related peak with a retention time identical to that of canrenone.

Eluate D on TLC analysis showed the presence of two drug related compounds: the metabolite with R_f 0.46 (Fig. 2) and a major metabolite C (R_f 0.38). The former was also found to be very labile and an attempt to purify it by preparatory TLC using the conditions described above also resulted in its conversion to canrenone. GLC analysis of eluate D showed two compounds with retention times of 14.6 minutes (identical to canrenone) and 19.5 minutes (metabolite C). The latter was obtained as a crystalline homogeneous material by preparatory TLC of eluate D. Recrystallization from ether gave 6 mg of

pure metabolite C: m.p., 235-237°C, $[\alpha]_D$ = +4°.

Base Treatment of Metabolite C: A solution of metabolite C (1 mg) in 1N methanolic potassium hydroxide (10 ml) was refluxed for 10 min. An aliquot was subjected to UV spectrophotometric analysis before and after heating the solution. The reaction mixture was diluted with water (50 ml) and extracted (pH 3) with chloroform. The product was identified as 3-(3,6-dioxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone (IX) by comparison of its TLC mobility (Table I) and IR spectrum with that of an authentic sample.

SYNTHESIS OF REFERENCE COMPOUNDS AND THEIR INTERMEDIATES

3-(3-oxo-7α-methylthio-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, IV: A solution of 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone (II, 5 g) in methanol (50 ml) and piperidine (5 ml) was cooled to 5°C. Gaseous methylmercaptan was then passed in until a weight gain of 18 g had been achieved. The pressure container was sealed and held at 25°C. After 20 hours, the solution was transfered into 500 ml of ice water. The precipitate was collected, washed with water and air dried. Crystallization from methanol yielded 2.6 g of IV: $[\alpha]_D$ = 0°; λmax 240 nm (ε 15,700); νmax 1768, 1670 and 1621 cm^{-1}.

Anal. Calcd. for $C_{23}H_{32}O_3S$: C, 71.09; H, 8.30.
Found: C, 70.97; H, 8.46.

3-(3-oxo-7α-methylthio-6β,17β-dihydroxy-4-androsten-17α-yl)propionic acid γ-lactone, V: The mother liquor (2.8 g) from above crystallization was dissolved in benzene (100 ml) and passed through a column of silicAR CC-7 (Mallinckrodt, 200-325 mesh, 250 g) packed in benzene. Elution with ethyl acetate-benzene (10:90, 6L) gave a mixture of II and IV (1.9 g). Elution with ethyl acetate (2L) gave a mixture of polar products (100 mg). Hydroxylated methylthio V (50 mg) was isolated from this polar mixture by preparatory TLC as colorless gum: λmax 235 (ε 10,300); νmax 3615, 3450 (broad), 1772, 1675 and 1621 cm^{-1}. Larger quantities of the hydroxylated methylthio V were obtained by microbiological hydroxylation (11) of the methylthio IV.

Peracid Oxidation of the Methylthio IV: The peracid oxidation of IV was carried out in three separate runs. In the first run, the solution of IV (100 mg) in dry benzene (5 ml) was treated with 50 mg of m-chloroperbenzoic acid (85%). The reaction mixture was left at room temperature for 24 hours. TLC analysis indicated the formation of two minor products with Rf 0.67 and Rf 0.46 and a major product with Rf 0.53. Attempts to isolate these products by the previously described preparatory TLC method resulted in conversion of each product to canrenone. This experiment indicated that the three peracid oxidation products were very labile.

In the second run, the methylthio IV (500 mg) was treated with an excess (530 mg) of m-chloroperbenzoic acid (85%). The reaction mixture after 18 hours was diluted with benzene-ethyl acetate (1:1, 100 ml) mixture and then successively washed with water, 2% aqueous potassium bicarbonate (twice) and finally with water (twice). Evaporation in vacuo of the organic extract gave a crystalline residue which on crystallization from methanol yielded 380 mg of 3-(3-oxo-7α-methylsulfonyl-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone, XI: $[\alpha]_D$ = +2°; λmax 239 nm (ε 15,900); νmax 1775, 1678, 1628, 1305 and 1136 cm^{-1}.

Anal. Calcd. for $C_{23}H_{32}O_5S$: C, 65.68; H, 7.67.
Found: C, 65.51; H, 7.58.

TLC mobility of the sulfone XI corresponded to the minor product with an Rf 0.67 in the first experiment. GLC analysis of XI gave a peak with the same retention time as that of canrenone.

In the third run, the solution of methylthio IV (2.0 g) in dry benzene (60 ml) was treated with 1 g of m-chloroperbenzoic acid (85%). The reaction mixture was maintained at 2-5°C for 15 minutes, and then at room temperature for 19 hours. The work-up as described above gave 2.0 g of solid residue

which on crystallization from ethyl acetate furnished 3-(3-oxo-7α-methylsulfinyl-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, VI: $[\alpha]_D$ = +14.5°C; λmax 240 nm (ε 13,500); νmax 1777, 1680, 1628 and 1035 cm^{-1}.

Anal. Calcd. for $C_{23}H_{32}O_4S$: C, 68.28; H, 7.97.
Found: C, 67.98; H, 8.06.

TLC mobility of the sulfoxide VI corresponded to the major product with Rf 0.53 seen in the first run. GLC analysis of VI gave a single peak with identical retention time to that of canrenone. The mother liquor from the above crystallization contained the third product with Rf 0.46. This product could not be obtained pure by crystallization. An attempt to purify it by preparatory TLC resulted in its conversion to canrenone. However, on further oxidation of the mother liquor with m- chloroperbenzoic acid, this minor product was converted to the sulfone XI. From these observations, together with previous findings of Schaub and Weiss (12), the structure of this product was assigned as the sulfoxide epimer (X) of VI.

3-(3-oxo-7α-methylsulfinyl-6β,17β-dihydroxy-4-androsten-17α-yl)propionic acid γ-lactone, VII: To an ice-cooled solution of V (100 mg) in methanol (5 ml) was added an aqueous solution of sodium metaperiodate (100 mg in 5 ml). The reaction mixture was stirred at ice-bath temperature overnight and then diluted with water (100 ml) and extracted with chloroform. Evaporation of the dried (anhydrous sodium sulfate) chloroform extract yielded a crystalline residue which was shown by TLC analysis to be a mixture of two products with Rf 0.38 (major) and Rf 0.31 (minor). The major product was isolated by preparatory TLC and crystallized from ether yielding 60 mg of VII: $[\alpha]_D$ = +4°; λmax 231 nm (ε 10,400); νmax 3610, 3420 (broad), 1772, 1622 and 1010 cm^{-1}; mass spectrum m/e 404, 356, 340 and 328.

Anal. Calcd. for $C_{23}H_{32}O_5S$: C, 65.68; H, 7.67.
Found: C, 65.20, H, 7.66.

TABLE I. Melting points and chromatographic (TLC and GLC) properties of the synthetic reference compounds described in the text.

Compound	Melting points[a], °C	TLC, Rf	GLC, Retention Time (min)
I; 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone	205-206[b]	0.83	14.6[c]
II; 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propionic acid γ-lactone	160-161	0.79	14.6
III; 3-(3-oxo-7α-thio-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone	195-198[b]	0.82	14.6[c]
IV; 3-(3-oxo-7α-methylthio-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone	212-213[b]	0.82	14.6[c],28.4
VI; 3-(3-oxo-7α-methylsulfinyl-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone	173-177[b]	0.53	14.6[c]
VII; 3-(3-oxo-7α-methylsulfinyl-6β,17β-dihydroxy-4-androsten-17α-yl)propionic acid γ-lactone	235-237[b]	0.38	19.5
IX; 3-(3,6-dioxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone	228-230	0.79	19.5
X; Sulphoxy epimer of VI		0.47	
XI; 3-(3-oxo-7α-methylsulfonyl-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone	165-167[b]	0.67	14.6[c]

a. Melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus.
b. Melts with decomposition.
c. Suggests conversion of these compounds to canrenone (II) on GLC analysis.

TABLE II. Nuclear magnetic resonance[a] data for spironolactone, its proposed metabolites and their postulated intermediates.

Compound	C-4	C-6	C-7	C-19	C-18	C-7 substituent
I	5.73 brs		3.98 brs ($w^1/_2$ = 8)	1.23 s	0.99 s	2.33 (3H,s) -S-COMe
II	5.71 s	6.12 s	6.12 s	1.13 s	1.04 s	
III	5.80 brs		3.27 brs	1.20 s	0.99 s	
IV	5.77 brs		2.93 brs ($w^1/_2$ = 8)	1.19 s	0.95 s	2.00 (3H,s) -SMe
V	5.89 s	4.35 d (J = 3)	2.82 t (J = 3)	1.35 s	0.99 s	2.07 (3H,s) -SMe
VI	5.62 brs		2.95 brs	1.28 s	1.00 s	2.62 (3H,s) -SOMe
VII	5.73 s	4.23 d (J = 3)	3.13 t (J = 3)	1.42 s	1.00 s	2.62 (3H,s) -SOMe
XI	5.82 brs		3.30 brs ($w^1/_2$ = 8)	1.26 s	0.98 s	2.88 (3H,s) $-SO_2Me$

a. Spectra were determined on a Varian HA-100 MHz spectrometer in deuterochloroform solution. Values are in ppm (δ) units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; $w^1/_2$, width at half height in hertz; J, coupling constants in hertz.

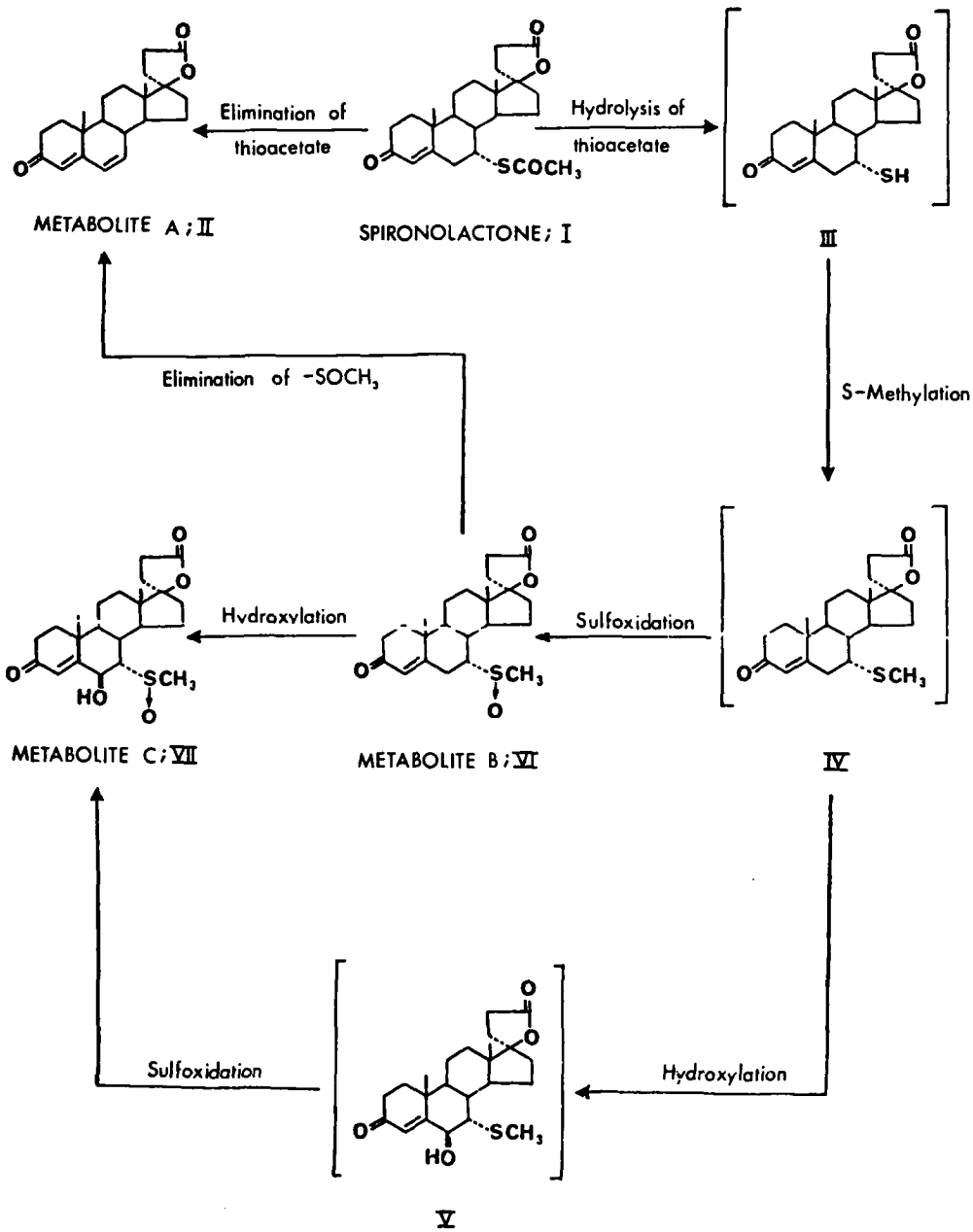

FIG. 1. Proposed structures of metabolites A, B and C isolated from human urine after an oral dose of spironolactone. Compounds III, IV and V given in parentheses represent postulated logical precursors leading to sulfur-containing metabolites B and C.

RESULTS

Figure 2 shows the thin-layer chromatogram of urinary chloroform extract B before and after ingestion of the drug. After drug treatment, six metabolites with R_f values of 0.79, 0.67, 0.53, 0.46, 0.38 and 0.31 were detected. Three of these, R_f 0.79, 0.53 and 0.38 were present in major amounts and were designated metabolites A, B and C, respectively. Metabolites A and B and those with R_f 0.67 and 0.46 stained light brown with ceric sulfate-sulfuric acid spray and exhibited yellow fluorescence under long-wave UV light; metabolite C stained deep red and gave purple-blue fluorescence. Production of pronounced fluorescence by the above method greatly facilitated detection of these metabolites.

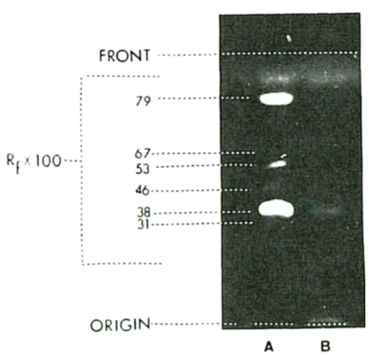

FIG. 2. Thin-layer chromatogram of urinary chloroform extract B. A - extract of 0-6 hour urine sample after an oral dose of spironolactone. B - similar extract prior to the drug administration. Thin-layer plate was developed in solvent system of methanol-chloroform (10:90), sprayed with ceric sulfate-sulfuric acid spray, heated at 110°C for 5-10 min, and then viewed under long-wave UV light.

Structural Elucidation of Metabolites of Spironolactone: The identity of metabolite A (R_f 0.79, Fig. 1) as the previously characterized de-thioacetylated compound II (canrenone), was established by comparing the IR, NMR and UV spectra of the isolated material with those of an authentic sample. Their GLC and TLC characteristics (Table I) were also identical.

Metabolite C: Besides canrenone, this was the second most abundant drug related compound in the urine. Its IR spectrum is shown in Fig. 3. A strong absorption band at 1772 cm^{-1} indicated that the γ-lactone group of spironolactone was still intact. Bands at 3610 and 3420 cm^{-1} showed that the compound was hydroxylated. The UV spectrum of this metabolite gave a maximum at 231 nm (ε 10,400). There was no change in the position of this maximum on addition of base; heating the basic solution, however, resulted in a bathochromic shift to 340 nm. After acidification, the product was identified as 3-(3,6-dioxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, IX (Fig. 4). These spectral properties of metabolite C, as well as its conversion to compound IX, were consistent with the reaction sequence shown in Fig. 4. The bathochromic shift from 231 nm to 340 nm on treatment with base can be rationalized by production of the enolate ion VIII: acidification of this enol would then result in formation of Δ^4-3,6-dioxo steroid IX. The NMR spectrum of metabolite C was very useful in assigning the

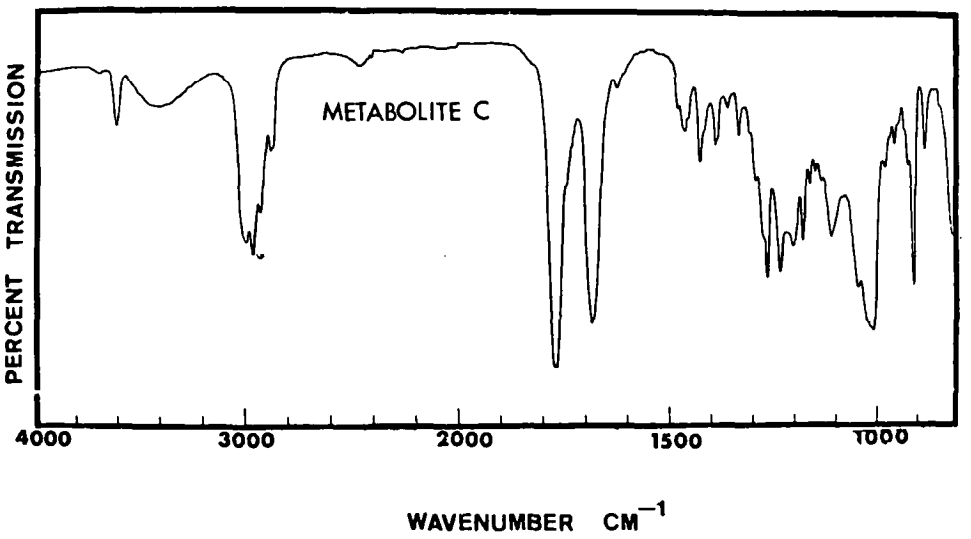

FIG. 3. Infrared spectrum ($CHCl_3$) of metabolite C.

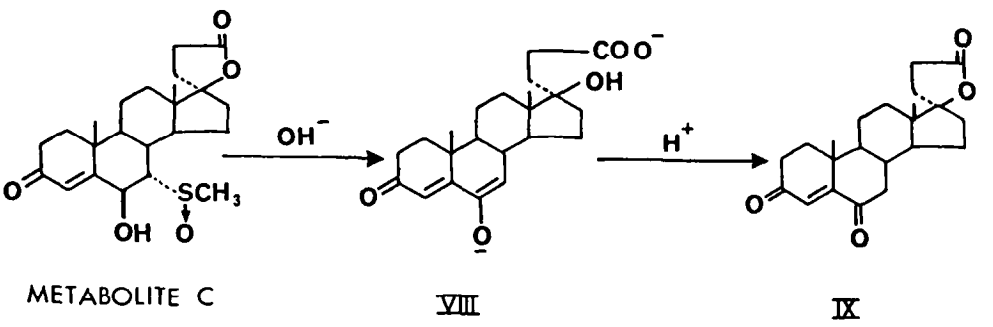

FIG. 4. Reactions described in the text for conversion of metabolite C to Δ^4-3,6-dioxo steroid IX.

stereochemistry of the substituents at C-6 and C-7. The chemical shifts of characteristic signals of this metabolite and the related reference compounds are summarized in Table II. The C-19 methyl signal in metabolite C was shifted downfield by 15 Hz as compared to this signal in reference compounds III, IV and VI. This deshielding effect can be attributed to the 1,3-diaxial interaction with the 6β-hydroxy group (13). The doublet at 4.23δ (J=3 Hz) was assigned to the C-6 proton while the triplet at 3.13δ (J=3 Hz) to the C-7 proton. These assignments were supported by decoupling experiments. Irradiation at 3.13δ resulted in collapse of the doublet at 4.23δ to a singlet, while irradiation at 4.23δ resulted in conversion of the triplet at 3.13δ to a doublet. The small coupling constant suggested a β-configuration of the hydroxyl group at C-6 and a α-configuration of the substituent at C-7 (14). The 3-proton singlet at 2.62δ was assigned to the methyl protons of the methylsulfinyl group at C-7. This assignment was supported by the strong IR absorption band at 1000-1050 cm^{-1} (Fig. 4) which was in good agreement with the value reported for sulfoxides (15).

The above spectral data strongly suggested structure VII for metabolite C. For further confirmation, this compound was synthesized as follows: treatment of canrenone (II) with methylmercaptan gave 7α-methylthio compound (IV) as the major product, and the corresponding 6β-hydroxy-7α-methylthio com-

pound (V) as a very minor product (16). Further quantities of V were obtained by microbiological hydroxylation (11) of the methylthio IV. Oxidation of V with sodium metaperiodate (17) gave two products. The major product VII (R_f 0.38) was indistinguishable from the isolated metabolite C by IR, NMR and mass spectra, melting point and mixed melting point, as well as TLC and GLC behaviors. The minor oxidation product (R_f 0.31), possibly an epimer (12) of the sulfoxide VII, was not investigated further.

Metabolite B and the Minor Metabolites with Rf 0.67 and 0.48: These metabolites possessed a common characteristic in that they were very labile. Attempts to obtain pure specimens by preparatory TLC resulted in their conversion to canrenone (II). A very informative reaction which suggested their possible structures was the peracid oxidation of the methylthio IV. This reaction gave three products with R_f values identical to those of metabolite B, and the minor metabolites with R_f 0.67 and 0.48. Furthermore, the three peracid oxidation products were also converted to canrenone when these products were subjected to the isolation procedure used for the separation of the above metabolites. The R_f value of the sulfoxide VI coincided with that of metabolite B while the mobilities of the epimeric sulfoxide X and the sulfone XI (Fig. 5) were identical to those of metabolites with R_f 0.48 and 0.67, respectively. From these observations the structures VI (meta-

bolite B), X and XI (Fig. 5) were proposed for the above minor urinary metabolites. It should, however, be emphasized that these structural assignments were only tentative. Further studies to obtain pure samples of these labile metabolites for their spectroscopic analysis are presently under investigation.

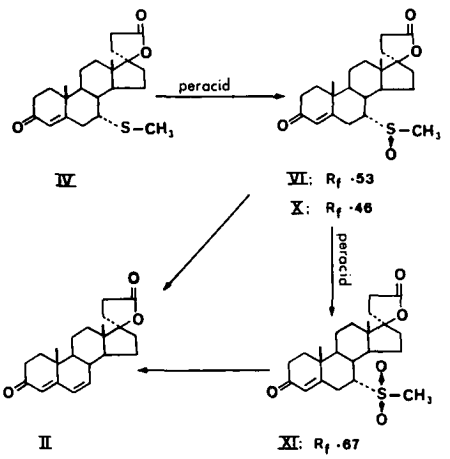

FIG. 5. Structures of the m-chloroperbenzoic acid oxidation products (VI, X and XI) of the methylthio IV and their conversion to canrenone (II) under mild conditions.

DISCUSSION

The results of the present investigation show that *in vivo* elimination of the thioacetate group of spironolactone is not quantitative. In addition to the previously characterized de-thioacetylated metabolite, canrenone, a major sulfur-containing metabolite, designated metabolite C, was also present in the urine of subjects given an oral dose of the drug. By comparison of the spot intensities in the chromatogram shown in Fig. 2, it can be seen that the amounts of canrenone and this new metabolite in 0-6 hour urine samples are approximately equal. Its structure was elucidated as 3-(3-oxo-7α-methylsulfinyl-6β,17β-dihydroxy-4-androsten-17α-yl)propionic acid γ-lactone by detailed spectroscopic analysis of the isolated material and also by comparison of its physical constants with a synthetic sample. On treatment with base, the methylsulfinyl group of metabolite C was eliminated to furnish 3-(3,6-dioxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, IX. It is noteworthy that the retention time of metabolite C (Table I) was identical to that of IX. Thus, it is possible that the conversion of metabolite C → IX also takes place on GLC analysis of the former.

The most logical biotransformation pathway leading to metabolite C is indicated in Fig. 1. The first step in this hypothetical pathway would involve the hydrolysis of the

thioacetate group of spironolactone to give the thio steroid III. Enzymatic S-methylation of III could then furnish the key intermediate, methylthio IV. To the authors' knowledge, the transformation of III → IV would represent the first example of *in vivo* S-methylation in steroid metabolism. The methylthio IV could then follow two separate pathways: it could be oxidized to give the sulfoxide VI or it could undergo 6β-hydroxylation to furnish compound V. Metabolite C could then result either by 6β-hydroxylation of the sulfoxide VI or by sulfoxidation of the methylthio V. The sulfoxide VI (metabolite B) together with its epimer X and their corresponding sulfone XI (Fig. 5) were apparently detected as very minor metabolites. Their identities, however, were only tentative. These metabolites were very labile and readily converted to canrenone (II). Elimination under mild conditions of the 7α-methylsulfinyl and the 7α-methylsulfonyl group of Δ^4-3-oxo steroids has been reported previously (12,18). The facile conversion to canrenone of these metabolites would suggest that the published analytical methods (3,6) for estimation of canrenone in the biological fluids may not be specific for this compound but also would measure canrenone derived from these labile metabolites.

Isolation of sulfur-containing metabolites described in the present article may be useful in understanding some of the observed pharmacological effects of spironolactone.

Thus, prevention of mercury toxicity in rats treated with spironolactone (8,9) may be attributed to chelation of mercury by sulfur-containing metabolites or their precursors (shown in Fig. 1) rather than to the microsomal enzyme inducing properties of the drug. Preliminary studies have shown that the thio steroid III was also effective in preventing mercury toxicity (19) in rats. It is also possible that these sulfur-containing metabolites and their precursors may chelate with other heavy metals and thus increase their excretion. These effects are presently under investigation.

Potassium canrenoate (SC-14266), the potassium salt of the hydroxy acid derived by opening the γ-lactone ring of metabolite A is a water soluble steroid which is also clinically used as an anti-aldosterone agent. Suggestions have been made that potassium canrenoate may be regarded as an injectable form of spironolactone (5,20,21). This suggestion was predominantly based on the assumption that the metabolic elimination of the thioacetate group of spironolactone to give metabolite A was quantitative. The results of the present investigation, however, show that insofar as the metabolic fate of these two anti-aldosterone agents is concerned, they should not be regarded as equivalent compounds.

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