filtered and the filtrate was evaporated to dryness. The residue was chromatographed over a cellulose-powder column (1.8 × 33 cm) using propylene-glycol as the stationary phase. Elution was effected with cyclohexane-toluene (7:3) and 5-ml fractions were collected. Fractions 88–120 afforded 80 mg of XIII after two crystallizations from acetone–petroleum ether (mp 128.5–130.5°); $[\alpha]_D^{27}$ +52.6° (c, 1.09 in $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.73 μ.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 69.90; H, 7.91.

Added in Proof

Since this manuscript was accepted we have isolated pure 3aα-H-4α-[3′-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII) from the fermentation broth of *N. restrictus*. It has been shown to be identical in all respects (mmp and infrared spectrum) to a sample of XIII obtained by hydrogenation of the enol lactone (XIV).

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Isolation and Characterization of Human Urinary Metabolites of Aldosterone. IV. The Synthesis and Stereochemistry of Two Bicyclic Acetal Metabolites*

William G. Kelly, Lajos Bandi, and Seymour Lieberman

From the Departments of Obstetrics and Gynecology and of Biochemistry, College of Physicians and Surgeons, Columbia University, New York

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The synthesis of the urinary metabolite of aldosterone, 3α-hydroxypregnane (11β-18S)(18S-20α) dioxide, M1, reported in this paper, serves to confirm the structure previously proposed on the basis of spectroscopic and degradative evidence, and to establish the configurations at C_{18} and C_{20} as well. The synthesis of a second urinary metabolite of aldosterone, 3α,21α-dihydroxypregnane (11β-18S)(18S-20α) dioxide, M8, also reported here, establishes the configuration at C_{18} but not at C_{20}. However, spectroscopic and chemical evidence support the assignment of the 20α configuration to M8. The synthesis and manipulation of 3α,11β,20α-trihydroxypregnan-18-al (18 → 11β) hemiacetal strongly suggest that the bicyclic acetal metabolites, M1 and M8, are not artifacts of the isolation procedure.

The isolation from human urine and the characterization of two metabolites of aldosterone, 3α-hydroxypregnane-(11β,18)(18,20) dioxide, M1, and 3α,21-dihydroxypregnane-(11β,18)(18,20) dioxide, M8, bearing a bicyclic acetal structure, have been reported (Kelly *et al.*, 1962a). This report concerns the confirmation of these structures by synthesis and the elucidation of the stereochemical configurations at C_{18} and C_{20} in these metabolites.

The synthesis of the metabolites was based on the work of Beal and Pike (1960) and Schmidlin and Wettstein (1962). Recently the latter investigators have described the synthesis of both of the C_{20} epimers of 3-keto-Δ^4-pregnene (11β,18S)(18S,20) dioxide (1α and 1β) (Figure 1). These authors have shown that reduction of (18 → 11) lactone-20-ketosteroids with $LiAlH_4$ leads directly to a mixture of approximately equal amounts of the C_{20} epimers, 1α and 1β. On the other hand, treatment of (18 → 20)-lactone-11-ketosteroids with $LiAlH_4$ gives the (18 → 11β) hemiacetal-20-ol (analogous to (6) in Fig. 2) with retention of configuration at C_{20}. Each of the epimeric hemiacetal-20-ols could then be converted to the corresponding

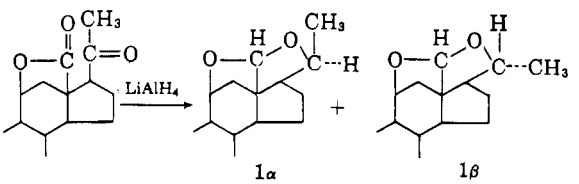

Fig. 1.—Synthesis of bicyclic acetals (Schmidlin and Wettstein, 1962).

bicyclic acetal (1α or 1β), with retention of configuration at C_{20} in each case. Beal and Pike (1960) independently prepared (1β) from 3α-acetoxypregnane-(11β,18S)(18S,20β)-dioxide ((2) in Fig. 2) which in turn was prepared from the known 20β-hydroxy derivative, 3α-acetoxy-20β-hydroxypregnan-11-one. The infrared spectra of (1β) prepared by Beal and Pike (1960) and of (1β) prepared by Schmidlin and Wettstein (1962) were identical (Beal and Pike, personal communication).

Comparison (infrared spectra, nuclear magnetic resonance [NMR][1] spectra, and melting point) of (2) with the acetate of M1 (see Figure 2) established that these two were different compounds. Oxidation of M1 acetate and of (2) with CrO_3 in acetic acid (Kelly *et al.*, 1962a) gave rise in each case to 3α-acetoxy-11β-hydroxy-20-ketopregnane-18-oic (18 → 11β) lactone

* Supported by a United States Public Health Service grant (AM-00110) and a General Research Support grant from the National Institutes of Health of the United States Public Health Service. Paper III in this series, Kelly *et al.* (1962b).

[1] Abbreviation used in this work: NMR, nuclear magnetic resonance.

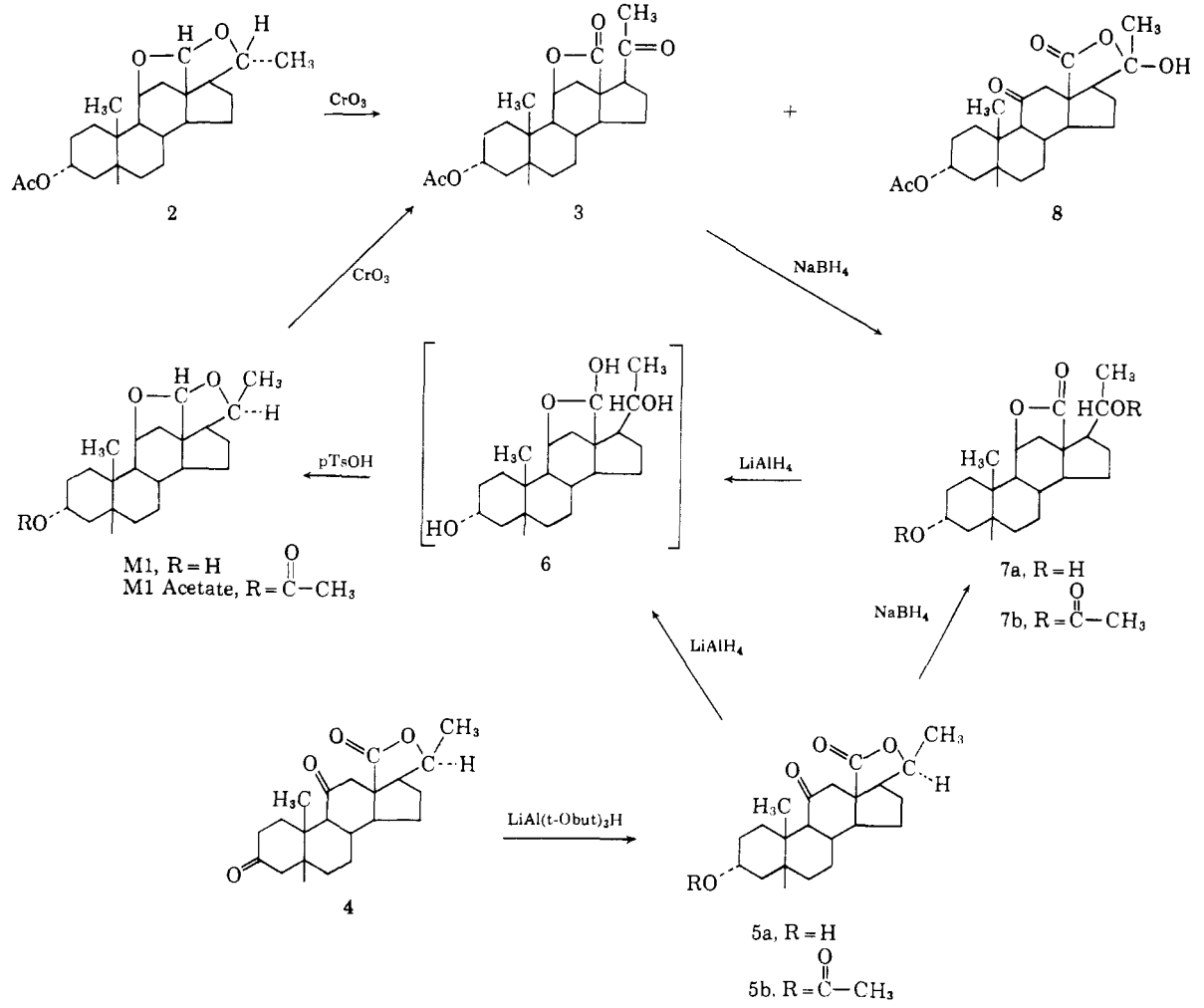

FIG. 2.—Stereospecific synthesis of the 21-deoxybicyclic acetal metabolite, M1.

(3), a compound synthesized by Wieland *et al.*, (1961). Therefore, M1 and (2) must be stereoisomeric at either C_{18} or C_{20} or both. As was pointed out to us by Beal (personal communication), the synthetic bicyclic acetals must have the 18S configuration, which is the *cis* fusion of the five-membered acetal rings, because the alternative *trans* ring fusion would be so highly strained that its formation is unlikely under conditions where the *cis* fusion is possible. However, the presence of the *trans* ring fusion in the metabolite could not be ruled out on the basis of the strain alone, since highly strained ring systems are known to occur in natural products. On the other hand, synthetic M1 and M8 would be expected to have the 18S configuration. In order to establish the stereochemistry of M1 at both C_{18} and C_{20} the synthesis of M1 from a suitable 11-keto (18 → 20) lactone of known configuration at C_{20} was undertaken and is described.

M1 Synthesis.—Reduction of 20α-hydroxy-3,11-diketopregnan-18-oic (18 → 20α)-lactone (4) (Heusler *et al.*, 1961) with either $NaBH_4$ or $LiAl(t\text{-}BuO)_3H$ gave the 3α-hydroxy derivative (5a). Reduction of (5a) with $LiAlH_4$ presumably gave the 3α,20α-dihydroxy (18 → 11β) hemiacetal (6) which was, without purification, converted to M1 by treatment with *p*-toluene sulfonic acid. Acetylation of (5a) gave the 3α-acetoxy derivative (5b) which on reduction with $NaBT_4$ in aqueous ethanol[2] yielded the 3α,20α-dihydroxy (18 → 11β) lactone (7a) of specific activity 45 cpm/μg. Acetylation with acetic anhydride-C^{14} of known specific activity gave the diacetate (7b). Reduction of (3) with $NaBT_4$ also gave exclusively (7a). Thus, whereas the reduction of (18 → 11) lactone-20-ketones with $LiAlH_4$ gives a mixture of C_{20} epimers with the 20α isomer slightly predominating, reduction with $NaBH_4$ in the same series produced exclusively the 20α isomer. Reduction of (7a) with $LiAlH_4$ yielded the uncharacterized (6) which was converted to M1, as expected. This synthesis of M1 confirms the structure previously proposed for this metabolite and in addition establishes the configuration at C_{18} as S and at C_{20} also as S (20α-O-).

M8 Synthesis.—The oxidation by CrO_3 in acetic acid of tetrahydroaldosterone diacetate to ketolactone diacetate M12-2 (Figure 3) and the proof of structure of M8 by means of the conversion of its diacetate to

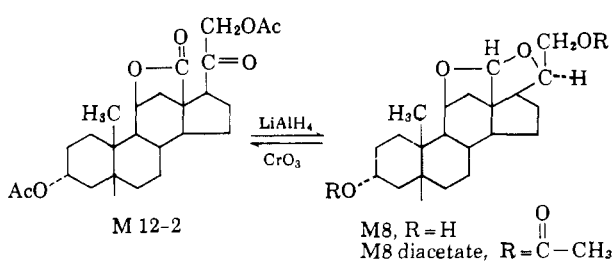

FIG. 3.—Synthesis of the bicyclic acetal M8. The characterization of M12-2 and the conversion of M8 diacetate to M12-2 have been described (Kelly *et al.*, 1962a, b).

[2] The introduction of tritium into the molecule facilitated the chromatography and manipulation of a few milligrams of M1 and the intermediates used for its synthesis.

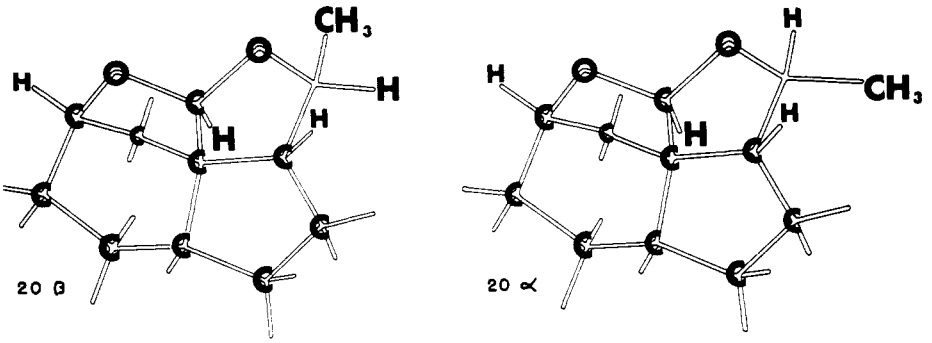

FIG. 4.—Three-dimensional representation of the C_{20} epimeric bicyclic acetals based on Drieding models.

M12-2 have been described (Kelly *et al.*, 1962a,b). We now wish to report that reduction of M12-2 with $LiAlH_4$ gave two major products in nearly equal amounts. The more abundant of these was identical with the urinary metabolite M8; the other has not yet been characterized. This synthesis of M8 established the 18S configuration in this metabolite also. However, the configuration at C_{20} was not elucidated by this synthesis.

M8 Configuration of C_{20}.—The presence of a complex of strong bands in the 1000–1100 cm^{-1} region of the infrared spectrum of M1 and M8 and of their respective acetates, and the possible correlation of these bands with the bicyclic acetal structure have already been pointed out (Kelly *et al.*, 1962a). Similar bands are present in the spectra of both isomers of (1) (Schmidlin and Wettstein, 1962) and also of (2). In these few compounds so far examined, the 20β isomers exhibit a strong band near 1075 cm^{-1} whereas the known 20α epimers display all their strong bands between 1000 and 1050 cm^{-1}. The infrared spectrum of M8 bears a marked resemblance to those of the known 20α bicyclic acetals (M1, 1α) in the 1000–1100 cm^{-1} region.

The oxidation of the 20α bicyclic acetal, M1 acetate, gave (3) in good yield (Kelly *et al.*, 1962a), and neither starting material nor by-product were detected. However, a similar oxidation of (2) gave only about 30% yield of (3) and about 30% of unreacted (2) was recovered. In addition a 30% yield of the ketolactol acetate (8), which can be regarded as an oxidation product of (3) (Kelly *et al.*, 1963), was obtained. Inspection of Dreiding models (Figure 4) of both isomeric bicyclic acetals revealed that the C_{21} methyl group affords more hindrance to attack upon the acetal system in the 20β isomer than in the 20α isomer. Therefore the difference in the rate of oxidation of the C_{20} isomeric bicyclic acetals can be rationalized as due to steric hindrance.

An extension of these considerations shows that the assignment of the 20α configuration of M8 is consistent with the behavior of its diacetate on oxidation by CrO_3 in acetic acid. The oxidation of M8 diacetate (Figure 3) proceeded smoothly and gave a good yield of M12-2. No starting material was recovered. The bulky $-CH_2-OOC-CH_3$ group at C_{20} in M8 diacetate might be expected to afford considerable hindrance of the acetal system if M8 had the 20β configuration, whereas in the 20α configuration the same group would not seem to hinder attack upon the acetal much more than does a C_{21} methyl group.

Further evidence concerning the configuration of M8 was obtained from the NMR spectra of M1, M8, and (2). The hydrogen at C_{20} of M1 gave rise to a signal at δ = 4.6 ppm (Kelly *et al.*, 1962a) whereas in the 20β epimer it gave rise to a pair of quartets near 3.6 ppm (Beal and Pike, 1960). These chemical shifts are similar to those reported for equatorial and axial protons, respectively, of the steroid alcohols (Shoolery and Rogers, 1958). The difference in chemical shift between the axial and equatorial protons of the steroid alcohols has been ascribed to the greater shielding of the axial proton by the magnetic properties of C—C bonds comprising the cyclohexane ring (Jackman, 1959). Inspection of Dreiding models (Figure 4) of the isomeric bicyclic acetals, M1 and (2), reveals that, by virtue of its proximity and geometric relationship to ring D, the proton on C_{20} in the 20β isomer might be expected to be shielded by ring D in a manner analogous to the shielding of the axial protons of the steroid nucleus. On the other hand the proton of the 20α isomer is probably not near enough to ring D to experience this type of shielding and its signal might therefore be expected to occur at the lower field strength than does that of the proton of the 20β epimer. The NMR spectrum of M8 (Figure 5) displays a quartet centered at 4.55 ppm, whose intensity indicates that it arises from a single proton. This frequency is very close to that assigned to the proton at C_{20} in M1 (4.6 ppm). That a quartet is observed in place of a pair of triplets indicates that the C_{20} proton is coupled equally to the C_{21} methylene protons and to the C_{17}-α proton. The coupling constant, J, is about 6–7 cps, which indicates that the dihedral angle between the C_{17}—C_{20}—H bond and the C_{20}—C_{17}—H bond must be either about 10–30° or about 150° (Karplus, 1959). Inspection of the Dreiding models indicates that this angle is about 10° in the 20α isomer and about 110° in the 20β isomer. Hence the 20β isomer would be expected to give rise to a pair of triplets because the coupling between the C_{20} proton and the C_{21} protons would not be the same as the coupling between the C_{20} proton and the C_{17} proton. The presence of the quartet is then convincing evidence for the 20α configuration in M8.

The signal near 3.7 ppm is assigned to the resonance of the protons on C_{21} and is split by spin-spin coupling to the C_{20} proton. Superimposed on this doublet is the resonance of the proton at C_3. The proton at C_{11} gives rise to the doublet centered at 4.70 ppm and the sharp peak at 5.20 ppm is assigned to the resonance of the proton at C_{18}.

On the basis of the chemical and spectroscopic evidence presented above, the 20α configuration can be assigned to M8. This configuration is also consistent with the known course of metabolism of 17-deoxy-20-keto pregnanes, which invariably are predominantly catabolized to the 20α alcohols.

The synthesis of dihydroxy hemiacetal (6) and its manipulation through several laboratory procedures including extraction, partition chromatography, and evaporation to dryness under vacuum without spontaneous dehydration to M1 strongly support the contention that M1 is not an artifact of (6). If (6) were

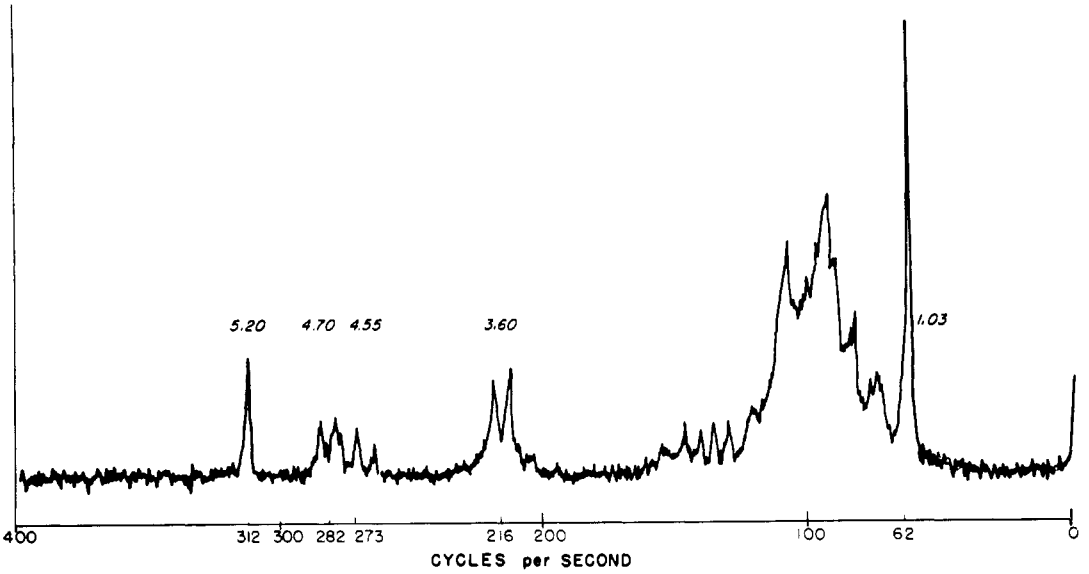

FIG. 5.—Nuclear magnetic resonance spectrum of M8 in $CDCl_3$ at 60 mc using tetramethylsilane as internal standard. The chemical shifts are given in both cycles per second and field independent units (δ). In both these systems increasing frequency corresponds to a decrease in the applied magnetic field strength.

excreted into urine in substantial amounts, most of it would surely have been isolated as (6). Since substantial amounts of M1 and no (6) were isolated, it is apparent that M1 is a true metabolite of aldosterone. This argument may also be applied to support the *in vivo* formation of M8.

The steric course of the reduction of 20-ketolactone acetate (3) with $NaBH_4$ is worthy of comment. The normal course of reduction of 20-ketopregnanes by metal hydrides generally favors the formation of 20β alcohols. However, the reduction of an (11β → 18) lactone-20-ketone pregnane with $LiAlH_4$ gave a mixture of the C_{20} epimeric bicyclic acetals with a slight preponderance of the 20α isomer (Heusler and Wettstein, 1962). This was attributed to the influence of the C_{18} oxygen function upon the conformation of the intermediary steroid—metal hydride complex, such that the formation of substantial amounts of the 20α hydroxy isomer was favored. In this paper, the reduction of an (18 → 11β) lactone-20-ketopregnane with $NaBH_4$ was shown to yield the 20α alcohol alone. Hence it is clear that the presence of a lactone carbonyl group on C_{18} influences the steric course of the reduction of a C_{20}-carbonyl by $NaBH_4$ as well as by $LiAlH_4$, and that $NaBH_4$ is more stereo-selective than $LiAlH_4$ under these circumstances.

EXPERIMENTAL

Melting points were determined using a Kofler block and are corrected. Infrared spectra were obtained on a Perkin-Elmer Model 221 spectrometer. Spectra of crystalline solids dispersed in KBr were determined using a sample-beam-condensing unit. About 20 μg of steroid was dispersed in about 2 mg of dry KBr. Spectra of solutions were determined with compensation for solvent. The NMR spectrum was obtained through the courtesy of LeRoy F. Johnson of Varian Associates, Palo Alto, California, at 60 mc using $CDCl_3$ as solvent and tetramethylsilane as internal reference.

Tetrahydrofuran was purified by refluxing over KOH followed by distillation. The distillate was refluxed with metallic potassium and then distilled with precautions against atmospheric moisture. The pure dry tetrahydrofuran was stored over $LiAlH_4$ and redistilled immediately before use. Ethylene dichloride saturated with *p*-toluene sulfonic acid, was refluxed for 4 hours and then distilled. All polar solvents packaged in metal containers were distilled regardless of grade. All other solvents were either analytical grade or were distilled before use.

Sodium borotritide was purchased from the New England Nuclear Corp., Boston, and diluted with sodium borohydride obtained from Metal Hydrides, Inc., to a suitable specific activity.

Radioactivity was determined using a Packard Tri-Carb liquid scintillation spectrometer as described previously (Kelly *et al.*, 1962a).

Tritium and C^{14} were simultaneously determined according to the discriminator ratio method of Okita *et al.* (1957).

The specific activity of the $NaBT_4$ was determined by the reduction of a steroid monoketone which gave a steroid alcohol of specific activity of 15,900 cpm/μmole. Similarly, the specific activity of the C^{14}-acetate anhydride was determined by acetylation of deoxycorticosterone which yielded a monoacetate with a specific activity of 2360 cpm/μmole. The number of acetate groups in steroid acetates was determined from their specific activities according to the method previously described (Kelly *et al.*, 1962a,b).

Chromatography on Celite was carried out as described previously (Kelly *et al.*, 1962a) and the partition systems used are given in Table I. One ml of the stationary phase was mixed with every 2 g of Celite. All columns contained 10–15 g of Celite.

Oxidation of 3α-Acetoxypregnane (11β,18S)(18S,20β) Dioxide (2).—Nine mg of bicyclic acetal (2)[3] was treated with 0.5 ml of 2% CrO_3 in 90% acetic acid overnight at room temperature. The reaction mixture was poured into 100 ml of water and the product was extracted three times with an equal volume of methylene chloride. The combined organic extracts were washed with water until free of acid, dried over Na_2SO_4, and finally evaporated to dryness under vacuum. The

[3] Kindly supplied by Dr. Philip Beal, Upjohn Co., Kalamazoo, Mich.

TABLE I

PARTITION SYSTEMS EMPLOYED IN COLUMN CHROMATOGRAPHY

System	Components
B	Benzene 4, methanol 2, water 1
G	Ethyl acetate 1, hexane 1, methanol 0.7, water 0.3
I	Ethyl acetate 0.33, hexane 0.67, methanol 0.35, water 0.15
S	Ligroin C 1, methanol 0.85, water 0.05
E-4[a]	Isooctane 0.5, *t*-butyl alcohol 0.225, water 0.05, methanol 0.225

[a] Eberlein and Bongiovanni (1955).

oily residue was chromatographed in the E-4 system. Four substances were eluted and three of these were crystalline. About 3 mg of (2) and 3 mg of ketolactone acetate (3) were recovered in 1.5 and 6.0 hold-back volumes, respectively. Comparison of the infrared spectra of (3) with that of authentic 3α-acetoxy-11β-hydroxy-20-ketopregnan-18-oic (18 → 11) lactone[4] indicated that these two substances were identical. The conversion of M1 acetate to (3) has been described (Kelly *et al.*, 1962a). About 3 mg of ketolactol acetate (8) was eluted as an oil in the third hold-back volume. The characterization of this substance is reported in the following paper (Kelly *et al.*, 1963). The most polar substance eluted in approximately 8 hold-back volumes was a semi-crystalline ketolactone acetate which was not purified further because of the small amount present. The unidentified ketolactone acetate exhibited an infrared spectrum not identical to that of the 17α-isomer of (3).

3α,20α-Dihydroxy-11-ketopregnan-18-oxy-(18 → 20α) Lactone (5a) and Its 3α-Acetate (5b).—(a) Prepared from (4) by Reduction with $LiAl(t\text{-}BuO)_3H$.—Twenty mg of 3,11-diketo-20α-hydroxypregnen-18-oic (18 → 20) lactone[5] (4) was dissolved in 5 ml of dry freshly distilled tetrahydrofuran, and 100 mg of $LiAl(t\text{-}BuO)_3H$ was added slowly. The reaction mixture was allowed to stand overnight at room temperature and was then poured into 100 ml of water. The product was extracted from the water three times with equal volumes of methylene chloride. The combined methylene chloride extracts were washed with water, dried over Na_2SO_4, and evaporated to dryness under vacuum. The residue gave crystals, mp 210–211°, from methanol-ether. The infrared spectrum (Figure 6) displayed bands at 1760 cm^{-1} (γ-lactone) and 1712 cm^{-1} (ketone) and was different in the fingerprint region from the spectrum of the precursor (4).

(b) Prepared from (4) by Reduction with $NaBT_4$.—A solution containing 35 mg of (4) and 10 mg of $NaBT_4$ in 8 ml of ethanol was allowed to stand overnight at room temperature. The reaction mixture was poured into 100 ml of water which was then made slightly acid with acetic acid. A crude product, mp 203–205°, was isolated as described under (a). Recrystallization from methanol-ether gave 20 mg of lactone melting at 209–210°. The infrared spectrum of this sample was identical with that of (5a) prepared as in (a) above.

Acetylation of (5a).—Three mg of (5a) was dissolved in 0.06 ml of dry pyridine and 0.03 ml of acetic anhydride was added. The reaction mixture was allowed to stand overnight in a tightly stoppered tube. Then

[4] Obtained through the generosity of Dr. A. Wettstein, CIBA AG., Basle, Switzerland.

[5] Samples of the compound were provided by Dr. A. Wettstein and by Prof. Leon Velluz, UCLAF, Paris, France.

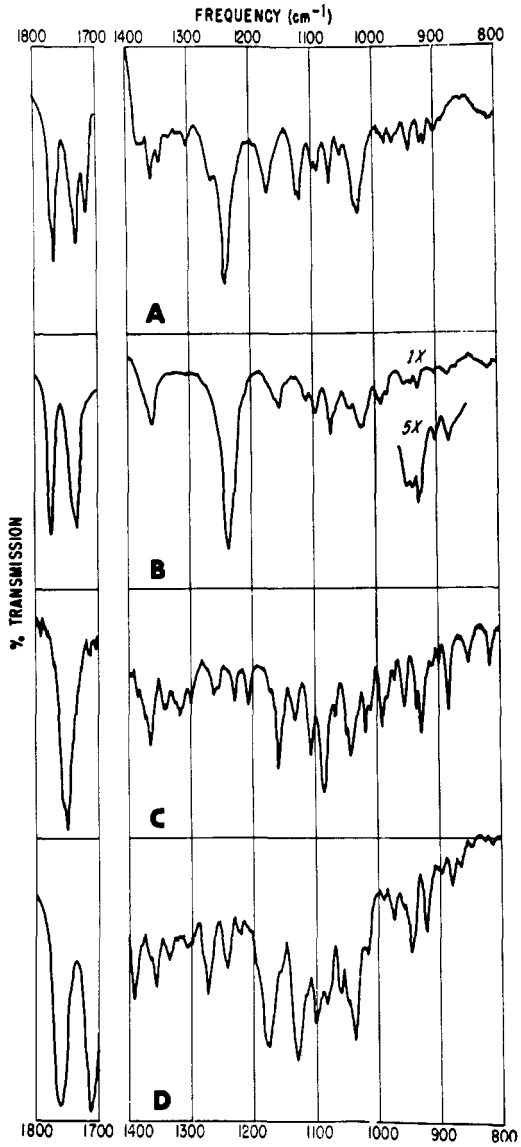

FIG. 6.—Infrared spectra. (A) (5b),3α-Acetoxy-11-keto-20α-hydroxy-pregnan-18-oic acid (18 → 20α) lactone in CS_2 solution. (B) (7b),3α,20α-Diacetoxy-11β-hydroxy-pregnan-18-oic acid (18 → 11β) lactone in CS_2 solution (1×); (7b) in CS_2 solution, expanded 5 times (5×). (C) (7a), 3α,11β,20α-Trihydroxypregnan-18-oic acid (18 → 11β) lactone in KBr dispersion. (D) (5a),3α,20α-Dihydroxy-11-ketopregnan-18-oic acid (18 → 20α) lactone 1800–1700 cm^{-1}, in CH_2Cl_2 solution; 1400–800 cm^{-1}, in KBr dispersion.

5 ml of benzene was added and the solution was taken to dryness under a stream of nitrogen. The residue gave the crystalline acetate (5b), mp 208–210°, from methanol-ether. The infrared spectrum (Figure 6) displayed bands characteristic of a γ-lactone (1770 cm^{-1}), equatorial 3-acetoxy group (1735 and 1240 cm^{-1}), and a ketone (1718 cm^{-1}).

3α-Hydroxypregnane(11β,18S)(18S,20α) Dioxide, M1.—Prepared from (5a).—To 20 mg of tritium-labeled (5a) in 3 ml of dry freshly distilled tetrahydrofuran was slowly added 50 mg of $LiAlH_4$. The reaction mixture was allowed to stand overnight at room temperature and then 1 ml of ethyl acetate was added carefully to destroy the excess $LiAlH_4$. This mixture was then poured into 100 ml of water and acidified with tartaric acid. The product was extracted with three equal volumes of methylene chloride and the combined extracts were washed with water, dried over Na_2SO_4,

and finally evaporated to dryness under vacuum. A yellowish white, crystalline substance was obtained by the addition of hexane to an acetone solution of the residue. This substance, presumably (6), did not absorb infrared radiation in the region characteristic for carbonyl groups. It was, without further characterization, refluxed in 30 ml of ethylene dichloride for one-half hour in the presence of a few crystals of *p*-toluenesulfonic acid. To the cooled reaction mixture was added 100 ml of methylene chloride, and this solution was washed, first with dilute $NaHCO_3$ solution and then with water until free of base, dried over Na_2SO_4 and evaporated to dryness under vacuum. The yellow, oily residue was chromatographed in the E-4 system and 12 mg of a colorless oil was located in the fourth hold-back volume by assaying the fractions for radioactivity. The oil gave crystals, mp 142–143°, after standing in heptane-methylene chloride for several days. The urinary metabolite melted at 143–144° after crystallization from heptane-ether (Kelly *et al.*, 1962a). The infrared spectra of the synthetic product and the metabolite, each determined in CS_2 and KBr, were identical.

Prepared from (7a).—To 5 mg of (7a) in 0.5 ml of anhydrous freshly distilled tetrahydrofuran was added a suspension of 20 mg of $LiAlH_4$ in 0.5 ml of the same quality tetrahydrofuran. The reaction mixture was allowed to stand overnight, after which the excess $LiAlH_4$ was destroyed by the addition of 0.5 ml of ethyl acetate. The resultant mixture was poured into 100 ml of water, which was first acidified with acetic acid, and then extracted three times with equal volumes of methylene chloride. The combined methylene chloride extracts were washed with water until free of acid, then dried over Na_2SO_4 and finally evaporated to dryness. The yellow oily residue was chromatographed on system G. The main product, presumably (6), was eluted in 1.8 hold-back volumes. Its infrared spectrum was similar to that of (6) prepared from (5a) as described. A small amount of an oil, identified as M1, was eluted at 0.9 hold-back volumes.

A solution of the crude hemiacetal (6) in 30 ml of ethylene chloride was refluxed in the presence of *p*-toluene sulfonic acid and 3.5 mg of product was isolated and crystallized, mp 142–143°, as described. The product was identified as M1 by comparison of its infrared spectrum with those of M1 prepared from (5a) and of M1 isolated from urine.

3α,11β.20α-Trihydroxypregnan-18-oic (18 → 11β) Lactone (7a).—Prepared from (5b).—To the 11-keto-lactone (5b) dissolved in a mixture of 1 ml ethanol and 1 ml of tetrahydrofuran was added 20 mg of KBH_4 in water. The mixture was allowed to stand at room temperature for three days. The reaction mixture was poured into water and the product was isolated by extraction with methylene chloride. The methylene chloride was washed with water, then dried over Na_2SO_4, and evaporated to dryness under vacuum. The residue gave from methanol-methylene dichloride crystals which sublimed between 300° and 320°.[6]

[6] Velluz *et al.* (1960) report a m.p. of 310° for this compound. Although these authors assigned the 20β configuration to this compound it is evident in the light of recent work (Heusler *et al.*, 1961) and from this paper that the 20α configuration is correct. A comparison of the infrared spectrum of a sample of (4) prepared by Heusler *et al.* (1961), who assigned the 20α configuration, with that of a sample of (4) prepared by Velluz *et al.* (1960) without assignment of configuration at C_{20}, was carried out in this laboratory and thereby it was established that the two substances were identical. Since Velluz *et al.* (1960) prepared (7a) by reduction of (4), (7a) must have the 20α hydroxy configuration.

The infrared spectrum (Figure 6) displayed a band characteristic of a γ-lactone (1750 cm^{-1}).

Prepared from (3).—To a solution of 40 mg of 20-ketone of (3) in 10 ml methanol was added a solution of 10 mg $NaBT_4$ in 2 ml water. The reaction mixture was allowed to stand overnight at room temperature, and was then poured into 100 ml water, which was extracted three times with equal volumes of methylene chloride. The combined extracts were washed with water until free of base, dried over Na_2SO_4, and evaporated to dryness under vacuum. The crude residue weighing 35 mg was chromatographed in system I. Three radioactive peaks were eluted in 1.0, 1.5, and 4.8 hold-back volumes, respectively. The last was the major product. The infrared spectra of the two minor substances indicated the presence of acetoxy groups in both. The infrared spectrum of the major component was identical to that of (7a) prepared from (5b) as described above. The major product gave 20 mg of cubic crystals when recrystallized from methanol–methylene chloride.

(7b), the Diacetate of (7a).—Four mg of crystalline dihydroxylactone (7a) prepared from (3) was dissolved in 0.10 ml of dry pyridine and 0.05 ml of C^{14}-acetic anhydride of known specific activity. The reaction mixture was allowed to stand overnight at room temperature, then was diluted with 5 ml of benzene and taken to dryness under vacuum. On chromatography in system S the dry residue gave 3 mg of a single substance, which had a H^3/C^{14} ratio of 3.24 (calculated for a diacetate, 3.37); it melted at 275° on crystallization from acetone-ligroin and its infrared spectrum (Figure 6) indicated the absence of hydroxyl functions and the presence of γ-lactone (1775 cm^{-1}) and acetoxy groups (1745, 1238 cm^{-1}).

3α,21-Dihydroxypregnane (11β,18S)(18S,20) Dioxide, M8 Prepared from M12-2.—To a solution of 5 mg of the (18 → 11β) lactone 3α,21-diacetate, M12-2 (Kelly *et al.*, 1962b), in 2 ml of ether and 1 ml of anhydrous peroxide-free tetrahydrofuran, was added 20 mg of $LiAlH_4$, and the resulting suspension was allowed to stand overnight. The reaction mixture was then poured into 100 ml of water and acidified with tartaric acid, and the product was extracted with three 100-ml portions of methylene chloride. The combined extracts were washed free of acid with water, dried over Na_2SO_4, and evaporated to dryness under vacuum. The residue was recrystallized from acetone-ether. However, the products from successive crystallizations did not have the same infrared spectrum and therefore they were combined and chromatographed on system B. In this way the mixture was resolved into three components eluted at 0.4, 1.4, and 2.0 hold-back volumes, respectively. The most polar compound had the same chromatographic properties and the same infrared spectrum as the metabolite, M8, isolated from urine (Kelly *et al.* 1962a). The over-all yield of M8 was 0.5 mg or 10%. However, because of large manipulative losses, this represents a minimum yield. The 0.5 mg of M8 represented 55% of the material eluted from the column.

The substance eluted at 1.4 hold-back volumes comprised 35% of the total eluted from the column and its infrared spectrum displayed bands between 1000 and 1100 cm^{-1}, with a strong band near 1075 cm^{-1}, which suggested that it might be the 20β isomer of M8. Moreover, on acetylation with C^{14}-labeled acetic anhydride it gave rise to a diacetate whose infrared spectrum was devoid of absorption bands indicative of hydroxyl groups. The substance eluted at 0.4 hold-back volumes was not investigated further.

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Isolation and Characterization of Human Urinary Metabolites of Aldosterone. V. Dihydroaldosterone and 21-deoxytetrahydroaldosterone*

WILLIAM G. KELLY, LAJOS BANDI, AND SEYMOUR LIEBERMAN

From the Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York

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The nature of the metabolites of aldosterone excreted in the urine following the oral administration of a large quantity of the tritium-labeled hormone to normal human subjects is the subject of this and preceding papers. This paper reports the isolation and characterization of two metabolites, 4,5β-dihydroaldosterone (11β,21-dihydroxy-18-oxopregnane-3,20-dione) and 21-deoxytetrahydroaldosterone (3α,11β-dihydroxy-18-oxopregnan-20-one), thus bringing to eight the number of aldosterone metabolites identified in this study. In addition a description of the partial characterization of four unidentified metabolites is given.

Previous reports (Kelly *et al.*, 1962a,b, 1963) from this laboratory have described the isolation from human urine of a number of metabolites of administered aldosterone, as well as the characterization of these metabolites by degradation, and in some cases synthesis. This report concerns the isolation and proof of structure of two additional metabolites, namely, dihydroaldosterone (11β,21-dihydroxy-18-oxopregnane-3,20-dione), M15, and 21-deoxytetrahydroaldosterone (3α,11β-dihydroxy-18-oxopregnane-20-one), M6. In addition, the isolation of several previously unknown metabolites is reported; however, because of the small amounts isolated, it was not possible to obtain sufficient data for the determination of the structure of these metabolites. The method of isolation of the metabolites is schematically illustrated in Figure 1. The proofs of structure of M15 and M6 are presented in Figures 2 and 3, respectively. M15 was characterized by oxidizing it with periodic acid to the known 3-ketolactone, M12-5, which had been prepared from tetrahydroaldosterone (Kelly *et al.*, 1962b). The structure of M6 was established by means of its conversion to the diacetate M6-1, which was in turn characterized by its spectroscopic properties and by its oxidation with chromic acid to the ketolactol monoacetate M6-2.

EXPERIMENTAL

All melting points were obtained using a Kofler block and are corrected. Infrared spectra were determined on a Perkin Elmer Model 221 spectrometer. Radioactivity as tritium and carbon-14 was counted as described previously and the partition chromatography was carried out as in previous studies (Kelly *et al.*, 1962a,b, 1963; Okita *et al.*, 1957). The partition systems used are given in Table I.

Isolation of Metabolites

A flow sheet summarizing the isolation of the metabolites is presented in Figure 1. The preparation of a neutral extract from the urine of human subjects to whom several hundred mg of tritium-labeled *d*-aldosterone-21-monoacetate had been orally administered, and the chromatographic separation of this extract into zones I, II, and III have been described (Kelly *et al.*, 1962a,b).

The separation of M6 from M7, M8, M9, M10, M11, and M12 by chromatographic analysis of zone III in system B has been described in preceding papers of this series (Kelly *et al.*, 1962a,b). Zone II from the chromatogram on system A was chromatographed in system B and a radioactive substance was eluted in 1.5 hold-back volumes. This substance, presumed to be M6, was combined with M6 from zone III. Rechromatography of the combined M6 in system B gave a single radioactive peak which was eluted in 1.5 hold-back volumes. However, M6 was separated into four components upon chromatography in system M. These substances were eluted at 2.4, 3.2, 5.2, and 8.0 hold-back volumes and were designated M13, M14, M15, and M6, respectively. Although M14 and M15 were poorly separated in this system, they were later separated easily, as described. M6 was rechromatographed in system D from which it was eluted in 2.0 hold-back volumes, and then in system W from which it was eluted in 4.0 hold-back volumes. No other

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