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SYNTHESIS OF 19-HYDROXYALDOSTERONE AND THE 3β-HYDROXY-5-ENE ANALOG OF ALDOSTERONE, ACTIVE MINERALOCORTICOIDS

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Summary—19-Hydroxyaldosterone (**20**) and the 3β-hydroxy-5-ene analog of aldosterone (HAA) (**8**) were synthesized from 21-acetoxy-4-pregnene-3,20-dion-20-ethylene ketal-18,11β-lactone (**2**) as follows: the double bond was transposed from the 4,5 to the 5,6-position by enol acetylation to **3**, followed by sodium borohydride reduction. Further reduction of the resulting lactone **4a** with diisobutylaluminum hydride (DIBAH) furnished the 20-ketal of HAA **6**, from which free HAA (**8**) and the 18,21-anhydro compound **7** were obtained by acid treatment. The [^{1}H]NMR spectrum of **8** in $CDCl_3$ showed it to be a mixture of two isomeric forms. Correlation with the known aldosterone-γ-etiolactone (**10**) was established by periodate oxidation of HAA to the corresponding etiolactone **9** followed by chromic acid oxidation. The preparation of **20** was next effected in the following manner: the diacetate **4b** was converted into the 6β,19-oxido compound **13b** by addition of hypobromous acid followed by the hypoiodite reaction of the bromohydrin **11**. Mild saponification of **13b** lead to the corresponding diol **13a**, and was followed by selective oxidation to the 3-one **14**, readily dehydrobrominated to **15a**. Reductive ring opening furnished a mixture of the 19,21-diol **16a** and its 5-ene isomer **16b**, which was directly converted to the diketal **17**. Reduction with DIBAH gave the hemiacetal **18**, and hydrolysis of the latter 19-hydroxyaldosterone (**20**) as a water-soluble solid, accompanied by the 18,21-anhydro compound **19**. 19-Hydroxyaldosterone exists in $CHCl_3$ and water as a mixture of mainly two isomers. Periodate oxidation furnished the etiolactone **21**. Preliminary results indicate that HAA and 19-hydroxyaldosterone are active mineralocorticoids in the Kagawa bioassay and short-circuit current measurements.

INTRODUCTION

The recent interest in the mineralocorticoid and hypertensinogenic properties of 19-nor-, 19-hydroxy- and 19-oxo-deoxycorticosterone [1–17], and of 19-hydroxyandrostenedione [18–20] has raised the intriguing question of what would be the biological activity of the still unknown 19-hydroxyaldosterone (**20**). This paper describes a synthesis of the latter compound, as well as of the 3β-hydroxy-5-ene derivative of aldosterone **8**.

EXPERIMENTAL

Merck A. G. silica gel ("60", mesh 70–230) was used in column chromatography. TLC was performed with acetone–hexane or $CHCl_3$–ethanol mixtures and the plates (silica gel Merck F254) were sprayed with 10% H_2SO_4 in ethanol before heating. [^{1}H]NMR spectra (in $CDCl_3$ with TMS as internal standard) were obtained with a Bruker WH-90 or AM-360 spectrometer equipped with an ASPECT 3000 computer. ppm Values obtained on the latter instrument are reported with 3 digits after the decimal point. i.r. Spectra were recorded with a Perkin–Elmer 297 spectrometer. Mass spectra were recorded with a Finnigan 4021 spectrometer: ionizing conditions for EI 17–19 EV, for CI (isobutane) 70 EV; source temperature 260°C, inlet temperature 200–250°C. Melting points were determined in capillaries with the electrothermal apparatus and are uncorrected. The following abbreviations are used in the text: dichloromethane, MDC; ethyl acetate, EtOAc; sodium metaperiodate, $NaIO_4$; tetrahydrofuran, THF; triethylamine, Et_3N; diisobutylaluminum hydride, DIBAH; petroleum ether b.p. 60–80°C, PE.

21-Acetoxy-4-pregnene-3,20-dion-20-ethylene ketal-18,11β-lactone (2)

The previously described [21] procedure was modified as follows. A solution of 19.3 g of 21-acetoxy-5-pregnene-3,20-dione-di-(ethylene ketal)-18,11β-lactone (**1**) in 300 ml of dioxan was treated with 7 ml of 0.5 N HCl and allowed to stand at 20°C for 3 days with occasional TLC monitoring (acetone–hexane, 1:2). Addition of 500 ml of water and 100 ml of saturated aq. $NaHCO_3$ was followed by three extractions with MDC, drying of the extracts with Na_2SO_4 and evaporation *in vacuo* at 40°C. The gum was chromatographed on 800 *g* of silica gel

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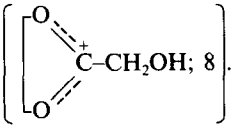
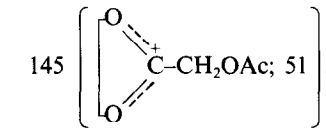
using PE–acetone 4:1 containing a trace of Et_3N, collecting 250 ml fractions. Flasks 25–38 gave 3.8 g of starting diketal **1**, while fractions 54–84 furnished 10.6 g of the desired 3-one **2**, m.p. 155–160°C (reported 160–162°C [21]). Elution with a 1:1 solvent mixture yielded varying amounts of 21-hydroxy-4-pregnene-3,20-dione-18,11β-lactone.

3,21-Diacetoxy-3,5-pregnadien-20-on-20-ethylene ketal-18,11β-lactone (**3**) *and 3β,21-dihydroxy-5-pregnen-20-on-20-ethylene ketal-18,11β-lactone* (**4a**)

An ice-cold solution of 4.3 g of **2** in 50 ml of acetic anhydride was treated with 5 ml of trimethylchlorosilane followed by 7.5 g of NaI (weighed out under N_2) and swirled with occasional ice-cooling, with precipitation of NaCl. The mixture was kept in the dark at room temperature for 1 h, then poured in a thin stream into a mechanically stirred suspension of 250 g of ice in 150 ml of aq. saturated $NaHCO_3$ solution and 200 ml of MDC. Ten ml of 5% sodium bisulfite solution was added, the aq. phase was re-extracted with 2 × 150 ml portions of MDC, and the combined extracts were treated with 1 ml of pyridine and dried with Na_2SO_4. The solvents were removed *in vacuo*, finally with the aid of an oil pump at 55°C (bath temperature). The residual oil was treated with 2 drops of pyridine and 20 g of ice to decompose any residual acetic anhydride and initiate crystallization of the enol acetate **3**, exhibiting λ_{max}^{KBr} 5.66 and 5.74 (sh) μ. It was washed with water by decantation, directly treated with 130 ml of ethanol and 6.7 g of sodium borohydride, and the suspension was refluxed for 30 min. Most of ethanol was then removed on the steam bath and 200 ml of water was added. The clear solution was heated for additional 30 min, cooled in ice, poured into a 1 l separatory funnel containing 400 ml of MDC, acidified with 15 ml of acetic acid, and the lactone was extracted with a total of 700 ml of MDC. The combined extracts were washed with aq. $NaHCO_3$, dried with Na_2SO_4 and evaporated *in vacuo*. The residue was treated with a little ether, the diol **4a** collected and washed with ether: 3.37 g, m.p. 206–212°C. The pure sample had m.p. 214–216°C (acetone with a trace of Et_3N); λ_{max}^{KBr} 2.96 and 5.66 μ; δ 1.123 (s, 19-CH_3), 2.93 (dd, J = 11.6, 12-H), 3.41 (m, 3α-H), 3.46, 3.54 (ABq, J = 7, 21-H_2), 3.98 (m, 20-dioxolane), 4.73 (d, J = 6, 11α-H) and 5.28 (brd, J = 3.5, 6-H) ppm; EI: *m/z* 386 (M^+–H_2O; 5%), 374 (M^+–CHOH; 100) [usual fragmentation in 21-ol-20-dioxolanes], 356 (M^+–CHOH–H_2O; 16), 312 (M^+–CHOH–CO_2; 9) and

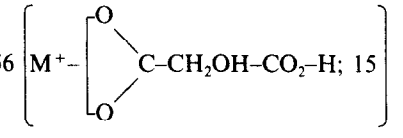


The *3,21-diacetate* ***4b***, prepared in the usual manner with acetic anhydride and pyridine, had m.p. 161–164°C (ether-PE); λ_{max}^{KBr} 5.68 and 5.76 μ; δ 1.136 (s, 19-CH_3), 2.034, 2.082 (s, s, 3-OAc, 21-OAc), 2.98 (dd, J = 11; 6, 12-H), 3.97 (m, 20-dioxolane), 3.97, 4.08 (d, d, ABq, J = 12, 21-H_2), 4.59 (m, 3α-H), 4.74 (d, J = 6, 11α-H) and 5.39 (brd, J = 3.5, 6-H) ppm; EI: *m/z* 416 (M^+–HCOAc; 100%), 356 (M^+–CHOAc–AcOH; 93), 312 (M^+–CHOAc–AcOH–CO_2; 16) and



3β,21-Dihydroxy-4-pregnen-20-on-20-ethylene ketal-18,11β-lactone (**5**)

Reduction of **2** with sodium borohydride furnished compound **5**, m.p. 214–217°C (EtOAc with a trace of Et_3N), in TLC slightly less polar than the 5-ene isomer **4a**; λ_{max}^{KBr} 2.92 and 5.65 μ; δ 1.160 (s, CH_3), 2.88 (dd, J = 11; 6, 12-H), 3.49 (d, J = 7, 21-H_2), 3.97 (m, 20-dioxolane), 4.03 (m, 3α-H), 4.716 (d, J = 6, 11α-H) and 5.32 (brs, 4-H) ppm; EI: *m/z* 373 (M^+–CH_2OH; 32%),



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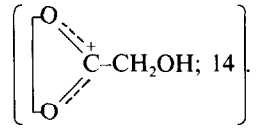


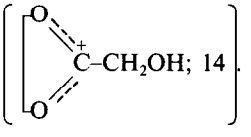
Conversion of **5** *into* **2**

An ice-cold suspension of 600 mg of chromic trioxide in 7 ml of pyridine was treated with 382 mg of the diol **5**. After stirring for 18 h at room temperature the product was shaken with 70 ml of cold water and 50 ml of MDC, the mixture was filtered with suction through a pad of celite, the aq. phase was reextracted with MDC, the extracts were dried and evaporated *in vacuo*. The dark residue was kept in 2 ml each of acetic anhydride and pyridine for 3 h, the solvents were removed with a stream of N_2, the resulting gum was treated with ice, and the soft solid collected and washed with water. Chromatography on 4 silica plates 2 mm thick using PE–acetone 2:1 and elution of the relevant zone with methanol gave material which on contact with ether furnished 88 mg of crystals, identical (i.r., TLC) with authentic lactone 2.

11β,18-Epoxy-3β,18,21-trihydroxy-5-pregnen-20-on-20-ethylene ketal (**6**)

A mechanically stirred solution of 1.23 g of lactone **4a** in 50 ml of dry MDC was treated in a N_2 atmosphere at −45°C with 25 ml of 1 M DIBAH solution in cyclohexane from a syringe through a rubber septum over a 5 min period. After stirring for

80 min at −30°C the reaction mixture was again cooled to −45°C, further 30 ml of DIBAH solution was added, the solution stirred at −30°C for additional 50 min, and then treated at −60°C with 45 ml of 2 M isopropanol solution in toluene, followed by 4 ml of water. Celite (24 g) and Na_2SO_4 (36 g) were added at 0°C, the mixture was stirred for 10 min, filtered with suction and the solid washed with 100 ml of THF to furnish, after evaporation *in vacuo* of the combined filtrates, a gelatinous mass which on contact with ether gave 745 mg of solid ketal **6** still contaminated with inorganic material, but suitable for hydrolysis. Further washing with a total of 500 ml of THF furnished additional 224 mg of **6**. A small sample was purified by TLC using $CHCl_3$–ethanol, 40:2. The product was eluted from the appropriate zone with methanol and after removal of solvent crystallized on contact with ether to afford material melting over a wide range above 163°C; λ_{max}^{KBr} 2.96 μ; EI: *m/z* 370 (M^+–$2H_2O$; 4%), 359 (M^+–CHO–H_2O; 72), 341 (M^+–CHO–$2H_2O$; 4), 331 (M^+–CH_2OH–CO_2; 95), 313 (M^+–CH_2OH–CO_2–H_2O; 100) and



CI = 406 (M^+; 6%).

11β,18-Epoxy-3β,18,21-trihydroxy-5-pregnen-20-one (HAA) (**8**) *and 11β,18;18,21-diepoxy-3β-hydroxy-5-pregnen-20-on-20-ethylene ketal* (**7**)

The crude ketal **6** (335 mg) was almost completely dissolved in 70 ml of dioxan, treated at room temperature with 1.5 ml of 0.5 N HCl, and the progress of hydrolysis was monitored by TLC ($CHCl_3$–ethanol, 40:4). After 4.5 h, when only traces of the starting material were still present, the solution was diluted with 140 ml of saturated aq. $NaHCO_3$, and extracted with 280 ml and then 2 × 70 ml of MDC. The combined extracts were dried with Na_2SO_4 and evaporated *in vacuo* to a gum which was dissolved in 2 ml of methanol, treated with 1 mg of cortisol as a marker and chromatographed on 4 silica plates 2 mm thick using $CHCl_3$–ethanol, 60:4. Location of the bands was determined by cutting off narrow strips on both sides of each plate and visualizing with ethanolic H_2SO_4 in the usual manner. Since cortisol ran only a little more slowly than a troublesome contaminant and just in front of **8**, the latter could be conveniently separated from the impurity by elution with methanol of the appropriate zone. Evaporation *in vacuo* and scratching with a few drops of water caused crystallization of **8** which was collected, washed with water and dried in desiccator: 87 mg, m.p. ranging from 114–125°C to 138–145°C; λ_{max}^{KBr} 2.98 μ (Fig. 3); δ 1.096, 1.135 (s,s, 19-CH_3, ratio 4:5), 3.40 (m, 3α-H), 3.52 (m, 21-H_2), 4.57, 4.81 (d;d, J = 6;6, 11α-H), 4.34, 4.99 (s,s, 18-H) and 5.34 (m, 6-H) ppm; MS—see Fig. 4.

Elution with methanol of the much less polar zone and evaporation *in vacuo* furnished the cyclic ketal **7** which was washed with EtOAc: 48 mg, m.p. 230–233°C (dec.). Recrystallization from acetone raised the m.p. to 249–250°C; λ_{max}^{KBr} 2.81 μ; δ 1.127 (s, 19-CH_3), 2.68 (dd, J = 11.5;6.7, 12-H), 3.42 (m, 3α-H), 3.44 (s, 21-H_2), 3.96 (m, 20-dioxolane), 4.60 (d, J = 6, 11α-H), 4.698 (s, 18-H) and 5.32 (brd, J ~ 4, 6-H); EI: *m/z* 358 (M^+–CHO; 58%), 341 (M^+–CHO–H_2O; 1), 330 (M^+–OCHOCH (C_{18} + C_{21}); 100) and 312 (M^+–OCHOCH–H_2O; 71).

In another experiment, the gum obtained by hydrolysis of 970 mg of crude ketal **6** was chromatographed on 110 g of silica gel. Elution with $CHCl_3$-ethanol 96:4 and scratching of the appropriate fractions with water furnished a total of 234 mg of **8** over 95% pure.

11β,18-Epoxy-3β-hydroxy-5-pregnene-17β,18-carbolactone (**9**)

A solution of 20 mg of **8** in 2 ml of methanol was treated with a solution of 150 mg of $NaIO_4$ in 2 ml of water. After 1 h the solution was concentrated *in vacuo* at room temperature, the crystals were collected and air-dried: 17 mg, m.p. 240–245°C. Recrystallization from EtOAc raised the m.p. to 245–250°C; λ_{max}^{KBr} 2.86 and 5.67 μ (Fig. 3); δ 1.157 (s, 19-CH_3), 2.90 (dd, J = 11;6, 12-H), 3.46 (m, 3α-H), 4.87 (d, J = 6, 11α-H), 5.328 (brd, J ~ 4, 6-H) and 5.48 (s, 18-H) ppm; EI: *m/z* 330 (M^+; 15%), 312 (M^+–H_2O; 23), 286 (M^+–CO_2; 35), 257 (M^+–CO_2–CHO; 15) and 256 (M^+–OCHOCO (C_{18} + C_{20})–H; 32).

Aldosterone-γ-etiolactone (**10**)

To a cold solution of 12 mg of **9** in 3 ml of acetone was added 1 drop of the Jones reagent, followed after 10 min by a drop of isopropanol and 2 drops of water. Concentration *in vacuo* furnished a solid which was collected, washed with water and air-dried, 8 mg. Isomerization of the double bond into conjugation was effected by refluxing with 45 mg of sodium acetate in 6 ml of ethanol for 15 min. The product was recrystallized from EtOAc (3.5 mg) and found to be identical in all respects with authentic etiolactone **10** [22].

3β,21-Diacetoxy-5-bromo-6β-hydroxy-5α-pregnan-20-on-20-ethylene ketal-18,11β-lactone (**11**) *and 3β,21-diacetoxy-5,6β-epoxy-5β-pregnan-20-on-20-ethylene ketal-18,11β-lactone* (**12**)

An ice-cooled solution of 3.0 g of **4b** in 60 ml of dioxan was treated with 7.6 ml of 4.6% aq. perchloric acid, followed in portions over a 5 min period by 3.3 g of *N*-bromoacetamide. After 30 min at room temperature in the dark the solution was shaken with a mixture of 150 ml of saturated aq. $NaHCO_3$, 100 ml of water, 40 ml of 5% aq. sodium metabisulfite solution and 200 ml of MDC, and then reextracted with 2 × 50 ml portions of MDC. The combined extracts

were dried with Na_2SO_4 and evaporated *in vacuo* to an oil containing **11**, which was best directly converted to **13b** without further purification. A 100 mg sample was chromatographed on 10 g of silica gel: elution with PE–acetone 4:1 gave at first a trace of the starting **4b** followed by the bromohydrin **11**, 41 mg, m.p. 156–160°C (dec.) The pure sample had m.p. 166–167°C (methanol); λ_{max}^{KBr} 2.91, 5.65, 5.72 and 5.85 μ; δ 1.415 (s, 19-CH_3), 2.033, 2.079 (s,s, 3-OAc, 21-OAc), 2.921 (dd, J = 11.3;6.3, 12-H), 3.99 (m, 20-dioxolane), 3.99 (m, 21-H), 4.093 (d, J = 11.8, 21-H), 4.188 (brs, 6α-H), 4.67 (d, J = 6.2, 11α-H) and 5.45 (m, 3α-H) ppm.

Dehydrobromination of **11** with Et_3N or sodium acetate in ethanol furnished the epoxide **12**, m.p. 165–167°C (MDC-PE); λ_{max}^{KBr} 5.65.and 5.75 μ; 1.145 (s, 19-CH_3), 2.033, 2.075 (s,s, 3-OAc, 21-OAc), 2.90 (dd, J = 11.4;6.3, 12-H), 3.09 (d, J = 2, 6α-H), 3.96 (m, 21-H), 4.06 (d, J = 11.8, 21-H′), 3.96 (m, 20-dioxolane), 4.57 (d, J = 6.2, 11α-H) and 4.75 (m, 3α-H) ppm.

3β,21-Diacetoxy-5-bromo-6β,19-epoxy-5α-pregnan-20-on-20-ethylene ketal-18,11β-lactone (**13b**)

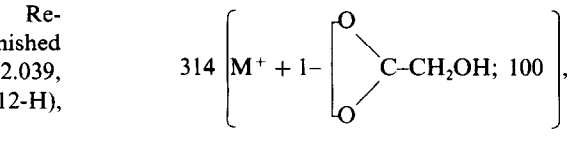
A suspension of 20 g of lead tetraacetate (washed with acetic acid, pressed and weighed moist) and 10 g of $CaCO_3$ in 600 ml of cyclohexane was refluxed with stirring for 30 min. Five and three tenths g of I_2 was added, followed by the crude bromohydrin **11** (prepared the same day from 3.0 g of **4b**) dissolved in 25 ml of MDC. The mixture was refluxed with stirring and illumination with a 150 w spotlight bulb until the purple color disappeared: in 8 runs the time varied from 18 to 30 min without affecting the yield. The mixture was cooled briefly in ice, while hot filtered with suction, the precipitate was washed with a total of 100 ml of MDC and the combined filtrates were shaken for a few seconds with 400 ml of 2.5% aq. sodium thiosulfate. The aq. layer was quickly separated before a voluminous lead salt started to precipitate, and the organic phase was washed with additional 200 ml of thiosulfate solution. The combined aq. solutions were back-washed with 50 ml of MDC, and the combined organic phases were washed with 100 ml of saturated aq. $NaHCO_3$, dried with Na_2SO_4 and distilled *in vacuo* to a volume of about 100 ml. Precipitation was completed by addition of 70 ml of PE and ice-cooling. The crude **13b** was collected and washed with PE, 3.6–3.9 g (a small additional amount could be obtained by chromatography of the filtrate). For purification, 13.5 g of the solid, absorbed on 60 g of silica gel, was chromatographed on a column of 700 g of silica gel. Elution with PE–acetone 4:1 containing a trace of Et_3N gave in fractions 32–44 (250 ml each) a total of 6.5 g of pure **13b**, m.p. 142–145°C. Rechromatography of the borderline fractions furnished additional 0.7 g; λ_{max}^{KBr} 5.63, 5.72 and 5.76 μ; δ 2.039, 2.080 (s,s, 3-OAc, 21-OAc), 2.89 (dd, J = 11;6, 12-H), 3.97 (m, 21-H), 4.087 (d, J = 11.7, 21-H′), 3.97 (m, 20-dioxolane), 3.99, 4.04 (d,d, J = 9;9, 19-H_2), 4.08 (brs, 6α-H) and 5.18 (m, 3α-H) ppm.

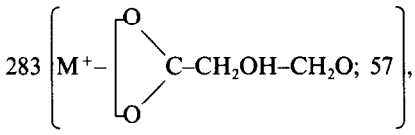
5-Bromo-6β,19-epoxy-3β,21-dihydroxy-5α-pregnan-20-on-20-ethylene ketal-18,11β-lactone (**13a**)

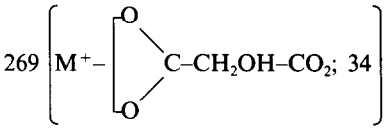
A solution of 4.0 g of the diacetate **13b** in 120 ml of hot methanol was treated with a solution of 3.2 g of $KHCO_3$ in 40 ml of water, refluxed for 1 h and concentrated *in vacuo* at 35°C to a low volume. Water (100 ml) and acetic acid (4 ml) were added and the mixture was extracted with a total of 350 ml of MDC. The combined extracts were washed with aq. $NaHCO_3$, dried with Na_2SO_4, filtered and divided into two halves, each of which was distilled *in vacuo* at 40°C until the diol **13a** was obtained as a foam free of methanol.

6β,19-Epoxy-21-hydroxy-4-pregnene-3,20-dion-20-ethylene ketal-18,11β-lactone (**15a**)

Each portion of **13a** (*vide supra*) was dissolved in 300 ml of hot acetone in a 1 l round bottom flask, cooled in an ice-bath with swirling for 5 min and treated with 3 ml of the Jones reagent. After swirling in ice for additional 5 min 3 ml of isopropanol was added, followed by 5 g of $NaHCO_3$ and 80 ml of water. The mixture was distilled *in vacuo* to remove most of acetone until a volume of about 70 ml was reached, and then cooled in ice while the second half of **13a** was processed in a similar manner. Both batches were combined and filtered through a large sintered glass funnel. The sucked-dry green precipitate was washed with a total of 150 ml of MDC, the aq. phase was extracted with a total of 200 ml of MDC, the combined extracts were dried with Na_2SO_4 and evaporated *in vacuo*. The residual gum (2.7–2.9 g) containing *5-bromo-6β,19-epoxy-21-hydroxy-5α-pregnane-3,20-dion-20-ethylene ketal-18,11β-lactone* (**14**) was refluxed in 100 ml of ethanol containing 5 g of sodium acetate for 1 h, the solvent was evaporated to dryness at 40°C and the residue worked up with water and MDC. The dried extracts were evaporated to furnish 2.3–2.7 g of a crude gum which was chromatographed on 270 g of silica gel using $CHCl_3$–ethanol, 98:2 containing a trace of Et_3N. Concentration of the early fractions yielded the desired ketal **15a** which was collected with the aid of PE and washed with EtOAc: 1.23 g, m.p. 188–190°C; λ_{max}^{KBr} 2.96, 5.60 and 5.99 μ; δ 2.987 (dd, J = 11.5;6.2, 12-H), 3.995 (m, 20-dioxolane), 3.499 (ABq, J = 11.4, 21-H_2), 3.50, 4.485 (d,d, J = 8.4, 19-H_2), 4.715 (d, J = 4.7, 6α-H), 4.776 (d, J = 6.1, 11α-H) and 5.868 (s, 4-H) ppm. On acetylation the AB system appears at 3.97 and 4.10 (d,d, J = 11;11, 21-H_2) ppm; EI: *m/z* 372 (M^+–CO_2; 5%), 341 (M^+–CO_2–CH_2OH; 18),







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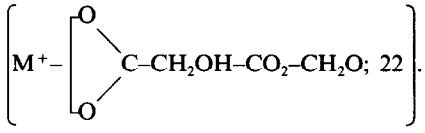


Continued elution furnished 220 mg of *6β,19-epoxy-21-hydroxy-4-pregnene-3,20-dione-18,11β-lactone* (**15b**) which, after crystallization from acetone, had m.p. 229–231°C (dec.); λ_{max}^{KBr} 2.90, 5.66, 5.85 and 5.97 μ; δ 3.16 (dd, J = 11;6, 12-H_2), 3.52, 4.47 (ABq, J = 8.2, 19-H_2), 4.235 (d, J = 18.4, 21-H), 4.48 (d, J = 18.2, 21-H′), 4.730 (d, J = 4, 6α-H), 4.907 (d, J = 6.2, 11α-H) and 5.883 (s, 4-H) ppm.

The 20-one **15b** (42 mg) could also be obtained by hydrolysis of **15a** (80 mg) in dioxan (3 ml) containing 5% HCl (0.5 ml) at room temperature for 48 h, followed by the usual workup.

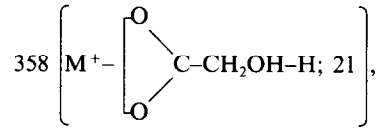
19,21-Dihydroxy-4-pregnen-3,20-dion-20-ethylene ketal-18,11β-lactone (**16a**)

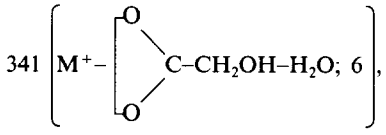
A solution of 300 mg of the oxidolactone **15a** in 20 ml of acetic acid was diluted with 2 ml of water and, with mechanical stirring, treated on the steam bath over a 6 min period with 7 g of zinc powder. The mixture was cooled in ice to room temperature, filtered with suction, the zinc washed with 3 × 5 ml portions of acetic acid, the combined filtrates were distilled *in vacuo* at 38°C and the residual semisolid was worked up with water and MDC. The combined MDC extracts were washed with aq. $NaHCO_3$, dried, evaporated *in vacuo* and the residue was crystallized from acetone to furnish 72 mg of **16a**, m.p. 225–227°C; λ_{max}^{KBr} 2.90 5.69 and 6.06 μ; δ 3.46 (dd, J = 12.0; 7.7, 21-H), 3.52 (dd, J = 12.0; 5.3, 21-H′), 3.99 (m, 20-dioxolane), 3.63 (dd, J = 12.7; 9.5, 19-H), 4.08 (dd, J = 12.7; 6.3, 19-H′), 4.93 (d, J = 6.1, 11α-H) and 5.87 (s, 4-H) ppm; EI: *m/z* 388 (M^+–CHOH; 80%), 370 (M^+–CHOH–H_2O; 7), 358 (M^+–2CHOH; 100) and 314 (M^+–2CHOH–CO_2; 6); CI: 419 ($M^+ + 1$; 31%), 401 ($M^+ + 1$–H_2O; 11) and 389 ($M^+ + 1$–CHOH; 100).

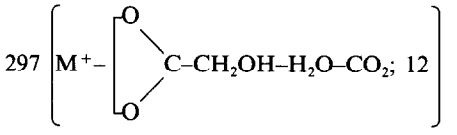
19,21-Dihydroxy-5-pregnene-3,20-dione-3,20-di-(ethylene ketal)-18,11β-lactone (**17**)

A. Reduction of **15a** *with zinc-acetic acid-isopropanol.* A solution of 2.35 g of **15a** in 350 ml of isopropanol and 30 ml of acetic acid was refluxed with stirring for 40 min with 60 g of zinc powder (activated by washing with 5% HCl, isopropanol and air-dried). The mixture was cooled, filtered with suction and the solid washed well with isopropanol. The combined filtrates were distilled to dryness *in vacuo*, the residue treated with 200 ml of water and extracted with 4 × 100 ml portions of MDC. The extracts were washed with aq. $NaHCO_3$, dried and evaporated to a gum (2.36 g) containing a mixture of *19,21-dihydroxy-4-pregnene-3,20-dion-20-ethylene ketal-18,11β-lactone* (**16a**) and its 5-ene isomer **16b** in variable proportions.

B. Ketalization with ethylene glycol. A mixture of **16a** and **16b** (2.86 g) obtained as above was treated with 200 ml of ethylene glycol and 280 mg of *p*-toluenesulfonic acid, and distilled *in vacuo* with mechanical stirring at 60–65°C over a 3.5 h period until a volume of about 50 ml remained. The mixture was cooled, treated with 100 ml of saturated aq. $NaHCO_3$ and 20 ml of saturated NaCl solution, and extracted with 100 ml and 3 × 70 ml portions of MDC. The dried extracts were evaporated *in vacuo* and the residual semisolid was chromatographed on 250 g of silica gel using $CHCl_3$–ethanol 98:2 with a trace of Et_3N as the eluting agent to afford 950 mg of the diketal **17**, m.p. 211–213°C, resolidifying and melting at 228°C (EtOAc); λ_{max}^{KBr} 2.82 and 5.68 μ; δ 2.977 (dd, J = 11.5;6.6, 12-H), 3.47 (dd, J = 12.0;8, 21-H), 3.50 (dd, J = 11.5;6, 21-H′), 3.630 (dd, H = 13;9, 19-H), 3.758 (dd, J = 13;5.8, 19-H′), 3.976 (m, 3 and 20-dioxolanes), 4.971 (d, J = 6.2, 11α-H) and 5.51 (brdd, J ~ 5; 1.5, 6-H) ppm; EI: *m/z* 462 (M^+, 1%), 432 (M^+–CHOH; 73), 402 (M^+–2CHOH; 100),







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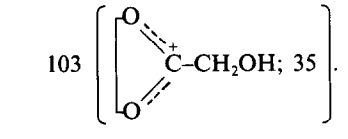

Purification of small amounts by TLC could be simplified by adding Reichstein's Compound S and cortisol as fluorescing markers running, respectively, in front and behind **17** in $CHCl_3$–ethanol 30:2.

11β,18-Epoxy-18,19,21-trihydroxy-5-pregnene-3,20-dione-3,20-di-(ethylene ketal) (*3,20-diketal of 19-hydroxyaldosterone*) (**18**)

The procedure used for the preparation of **6** was slightly modified: a mechanically stirred solution of 742 mg of **17** in 20 ml of dry MDC was treated in a N_2 atmosphere at −30°C with 15 ml of 1 M DIBAH solution in toluene from a syringe through a rubber septum. The mixture was slowly stirred at −20°C for 1 h, cooled to −30°C, treated again with 15 ml of DIBAH solution, stirred at −20°C for another h, treated at −40°C with 50 ml of MDC, then with 20 ml of 2 M isopropanol solution in toluene, and at 0°C with 1.5 ml of water. Next 10 g of celite and 15 g of Na_2SO_4 were added and after stirring for 15 min the mixture was filtered with suction through a large sintered glass funnel. The gelatinous precipitate was washed with 100 ml of MDC, transferred into a column (30 mm dia) and eluted with a total of 4 l of THF, applying suction to maintain a desirable dripping rate. The filtrate and eluate were evaporated *in vacuo* and the oily residue chromatographed on 65 g of silica gel with $CHCl_3$–ethanol, 98:2 containing a trace of Et_3N. The suitable fractions were pooled, the solvent was evaporated *in vacuo* and crystallization of **18** initiated by addition of 7 ml of EtOAc. The product was washed with more EtOAc, weighed 295 mg and had the m.p. 196–199.5°C; λ_{max}^{KBr} 2.94 μ; δ 3.5–3.7 (m, 21-H_2), 3.7–3.9 (m, 19–H_2), 4.0 (m, 3 and 20-dioxolanes), 4.461 (d, J = 6.2, 11α-H), 4.753 (d, J = 6.6,11α-H, minor isomer), 4.597 (s, 18–H, minor isomer), 5.168 (s, 18-H), 5.49 (brd, J = 3, 6-H) and 5.53 (brd, 6-H, minor isomer) ppm; EI: *m/z* 446 (M^+–H_2O; 3%), 417 (M^+–H_2O–CHO; 97), 399 (M^+–2H_2O–CHO; 100) and 389 (M^+–CH_2OH–CO_2; 18).

Further elution of the column furnished 128 mg of a compound of unknown structure, m.p. 165–180°C (acetone), λ_{max}^{KBr} 2.85–3.12 μ, the NMR spectrum of which is devoid of the 11α-H peaks.

19-Hydroxyaldosterone (**20**) *and 11β,18;18,21-diepoxy-19-hydroxy-4-pregnene-3,20-dion-20-ethylene ketal* (**19**)

A solution of 51 mg of diketal **18** in 4 ml of dioxan was treated with 0.2 ml of 5% HCl and stored at 19°C for 10 h, at which time TLC indicated the presence of mere traces of the starting material. Saturated aq. $NaHCO_3$ solution (10 ml) and MDC (50 ml) were added and the aqueous phase was reextracted with 4 × 15 ml portions of MDC. The dried (Na_2SO_4) extracts were evaporated *in vacuo* at 35°C, the residue was applied to 8 TLC plates 0.2 mm thick and developed with $CHCl_3$–ethanol, 30:2.5. The appropriate zone was eluted with 10 ml of methanol each and the combined solutions were evaporated *in vacuo*. The gum was dissolved in a little MDC, the solution was filtered through a cotton plug to remove residual silica, evaporated in a stream of N_2 and scratched with ether to afford 19 mg of crystalline **20**, pure in TLC. The compound is moderately soluble in warm ether and very soluble in water: by washing the glassware and the cotton plug with water and evaporating in a desiccator over H_2SO_4 additional 2 mg of pure **20** was obtained. The compounds melts at 110–120°C; λ_{max}^{KBr} 2.93, 5.82 (w) and 6.02 μ (Fig. 3); δ 4.065 (brd), 3.99 (brd, J = 13, 21-H_2), 4.40, 4.245 (d,d, J = 18;18, 21-H_2: this system only partially seen), 4.757 (d, J = 6.3, 19-H_2, one isomer), 4.969 (d, J = 5.6, 19–H_2, another isomer), 5.115 (s, 18-H, one isomer), 5.489 (s, 18-H, another isomer) and 5.827 (s, 4-H) ppm; MS—see Fig. 4.

The second product isolated from the plates was the less polar compound **19**, which was eluted with methanol. Evaporation gave a solid which, on recrystallization from EtOAc, melted at 262–5°C, 18 mg; λ_{max}^{KBr} 2.66 and 5.99 μ; δ 3.48 (m, 21-H_2), 3.77 (dd, J = 11.0;4.2, 19-H), 4.00 (dd, 19-H′), 4.0 (m, 20-dioxolane), 4.0 (m, 19-**OH** probably), 4.793 (d, J = 6.3, 11α-H), 4.817 (s, 18-H) and 5.810 (s, 4-H) ppm. Acetylation gave a product with δ 1.97 (s, 19-OAc), 3.434 (dd, J = 12;1.6, 21-H), 3.460 (d, J = 12.3, 21-H′), 3.88 and 4.02 (m, 4H, 20-dioxolane), 4.25 (d, J = 12, 19-H), 4.760 (d, J = 12, 19-H′), 4.662 (d, J = 6.4, 11α-H), 4.725 (s, 18-H) and 5.900 (s, 4-H) ppm.

11β,18-Epoxy-19-hydroxy-4-pregnene-17β,18-carbolactone (*19-hydroxyaldosterone-γ-etiolactone*) (**21**)

A solution of 5 mg of 19-hydroxyaldosterone (**20**) in 0.5 ml of water was treated with a solution of 50 mg of $NaIO_4$ in 0.5 ml of water. After 15 min the precipitated solid was collected, water-washed, air-dried and the lactone **21** was extracted from it with $CHCl_3$; evaporation and trituration with ether furnished 2.5 mg, m.p. 250–253°C; λ_{max}^{KBr} 2.79, 2.88, 3.02, 5.65 and 6.04 μ (Fig. 3); δ 3.5 (ABq, 19-H_2), 4.02 (d, J = 10, 19-**OH**), 5.052 (d, J = 5.8, 11α-H), 5.575 (s, 18-H) and 5.843 (s, 4-H) ppm; EI: *m/z* 316 (M^+–CO; 12%) 299 (M^+–CO_2–H; 16) and 239 (M^+–OCHOCO–CH_2OH; 22). The acetate had δ 2.000 (s, 19-OAc), 2.950 (dd, J = 11;6, 12-H_2), 4.319 (d, J = 12.1, 19-H), 4.692 (dd, J = 12;1.5, 19-H′), 4.948 (d, J = 5.2, 11α-H), 5.515 (s, 18-H) and 5.925 (d, J = 1.5, 4-H) ppm.

RESULTS AND DISCUSSION

The key intermediate employed in the synthesis was the ketal **2** which we had synthesized earlier [21] (Fig. 1). It was obvious that the 18,11β lactone should be preserved as a relatively insensitive moiety up to the penultimate step of the synthesis, that is the reduction to the hemiacetals **6** and **18**.

For the synthesis of HAA (**8**) first the 5,6-double bond had to be introduced, preferably by enol-acetylation of **2**, followed by sodium borohydride reduction [23, 24]. However, attempts at conversion

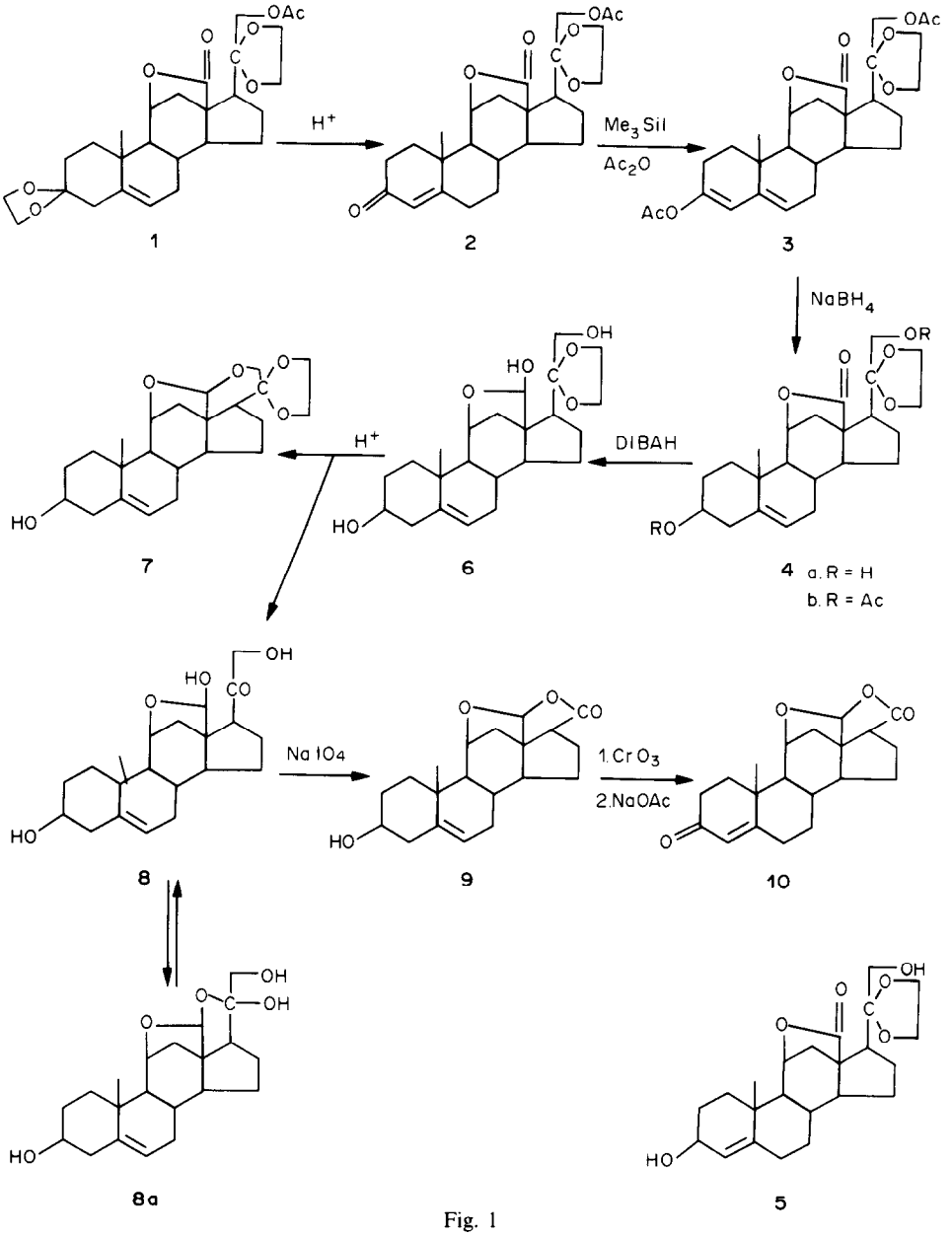

Fig. 1

of **2** into **3** were met with failure due to the sensitivity of the side-chain: acetic anhydride or isopropenyl acetate with a variety of acidic catalysts led mostly to splitting off of the 20-ketal group; also acetyl chloride or acetic anhydride in warm pyridine caused extensive decomposition. On the other hand, the mild enol acetylation method employing acetic anhydride, trimethylchlorosilane and NaI at room temperature [25] proved to be eminently suitable for the preparation of **3**, which was best not purified but directly treated with sodium borohydride to afford the ketal **4a** in 86% overall yield from **2**. For comparison, the 3β-hydroxy-4-ene isomer **5** was prepared by direct reduction of **2** with sodium borohydride; its oxidation with Sarett's reagent gave back the starting ketone **2**.

The lactone **4a** was next reduced to the hemiacetal **6** with DIBAH in cyclohexane–MDC at low temperature, in analogy with a corresponding reaction in the 3α,5α-tetrahydroaldosterone series [26]. Hydrolysis of **6** with dilute HCl in dioxan afforded HAA (**8**), crystallizing on contact with water to a solid of m.p. 114–125°C to 138–145°C. Its i.r. spectrum in KBr (Fig. 3) exhibits no carbonyl absorption showing that in the solid state the compound, like 3β,5α-tetrahydroaldosterone [21], exists solely in the cyclic form **8a**. The [^{1}H]NMR spectrum in $CDCl_3$ shows the presence of two isomeric forms in a ratio of 4:5, according to the 19-methyl singlets. Finally, an unequivocal correlation was established by periodate oxidation of HAA to the corresponding etiolactone **9** (i.r. spectrum in Fig. 3), followed by the Jones oxidation and isomerization of the 5,6-double

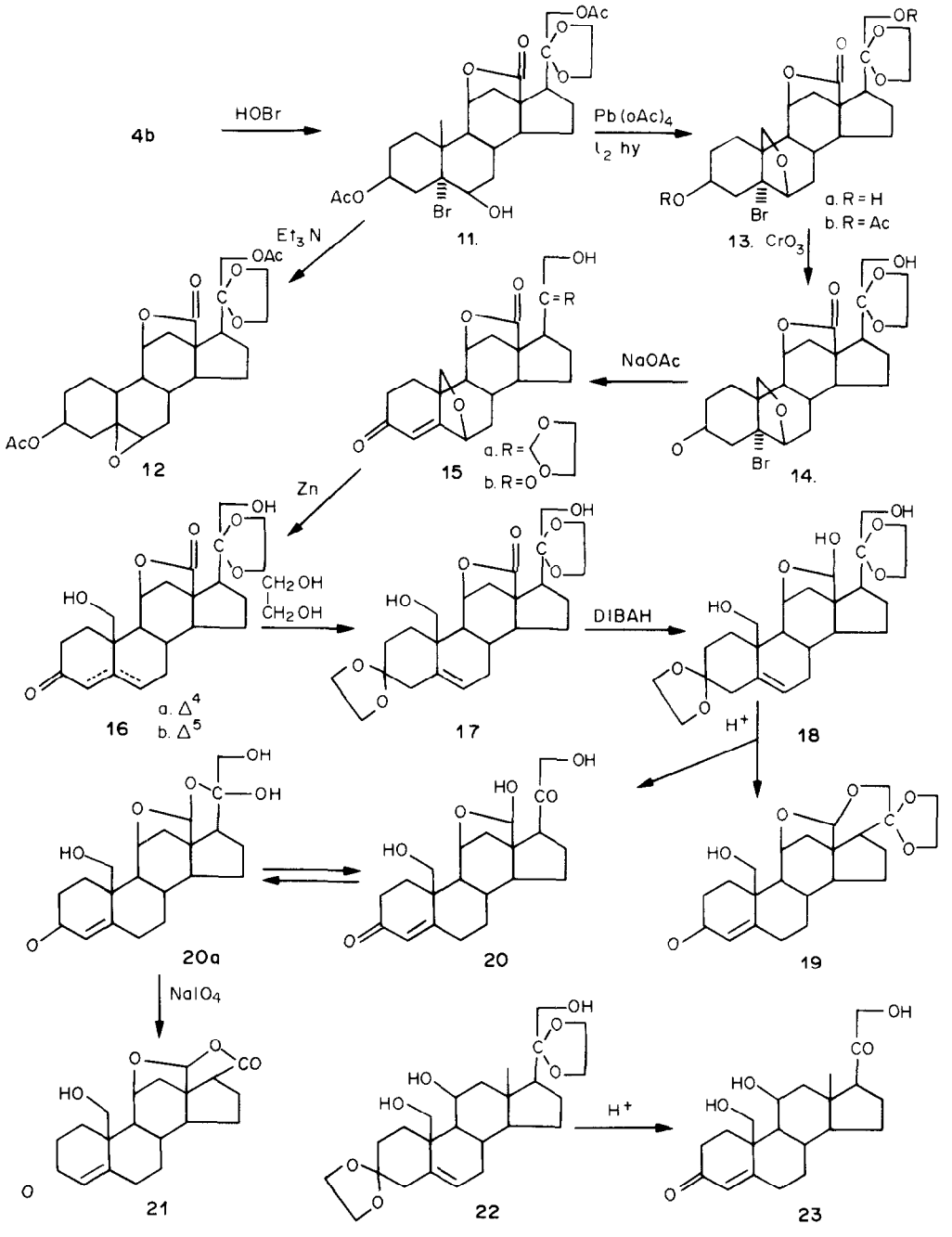

Fig. 2

bond with sodium acetate to furnish aldosterone-γ-etiolactone (**10**) [22], confirming the 17β-configuration in **8**.

The second compound obtained from the hydrolysis of **6** was the expected [26] ether **7**.

For the synthesis of 19-hydroxyaldosterone (**20**), introduction of the hydroxyl into the 19-position was the next major objective. One line of approach was concerned with microbiological hydroxylation by means of *Pellicularia filamentosa* [27–29] using steroid substrates already possessing oxygen functions at positions **11** and **18**. However, the results, which will be the subject of another publication, were not encouraging and therefore a purely chemical route employing the hypoiodite reaction [30] was undertaken.

While the ketal **1** was a desirable starting material for such a synthesis, survival of the ketal group at C_3, under the acidic conditions of addition of hypobromous acid across the double bond, was in doubt. As the second best alternative, the 3β-ol **4a** was acetylated and the diacetate **4b** reacted with *N*-bromoacetamide and perchloric acid to furnish the bromohydrin **11**, the ketal group at C_{20} being sta-

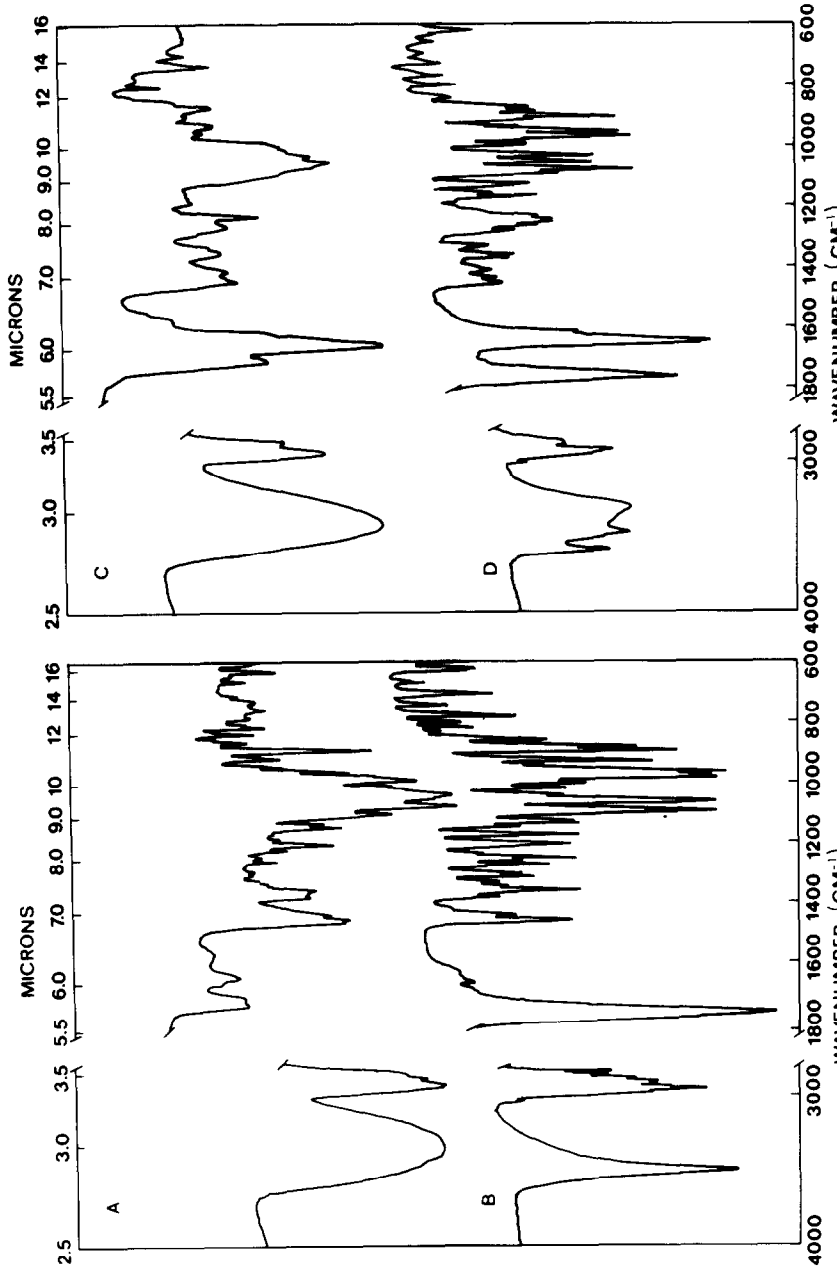

Fig. 3. I.r. spectra in KBr. *A*, HAA (**8a**); *B*, HAA-γ-etiolactone (**9**); *C*, 19-hydroxyaldosterone (**20,20a**); *D*, 19-hydroxyaldosterone-γ-etiolactone (**21**).

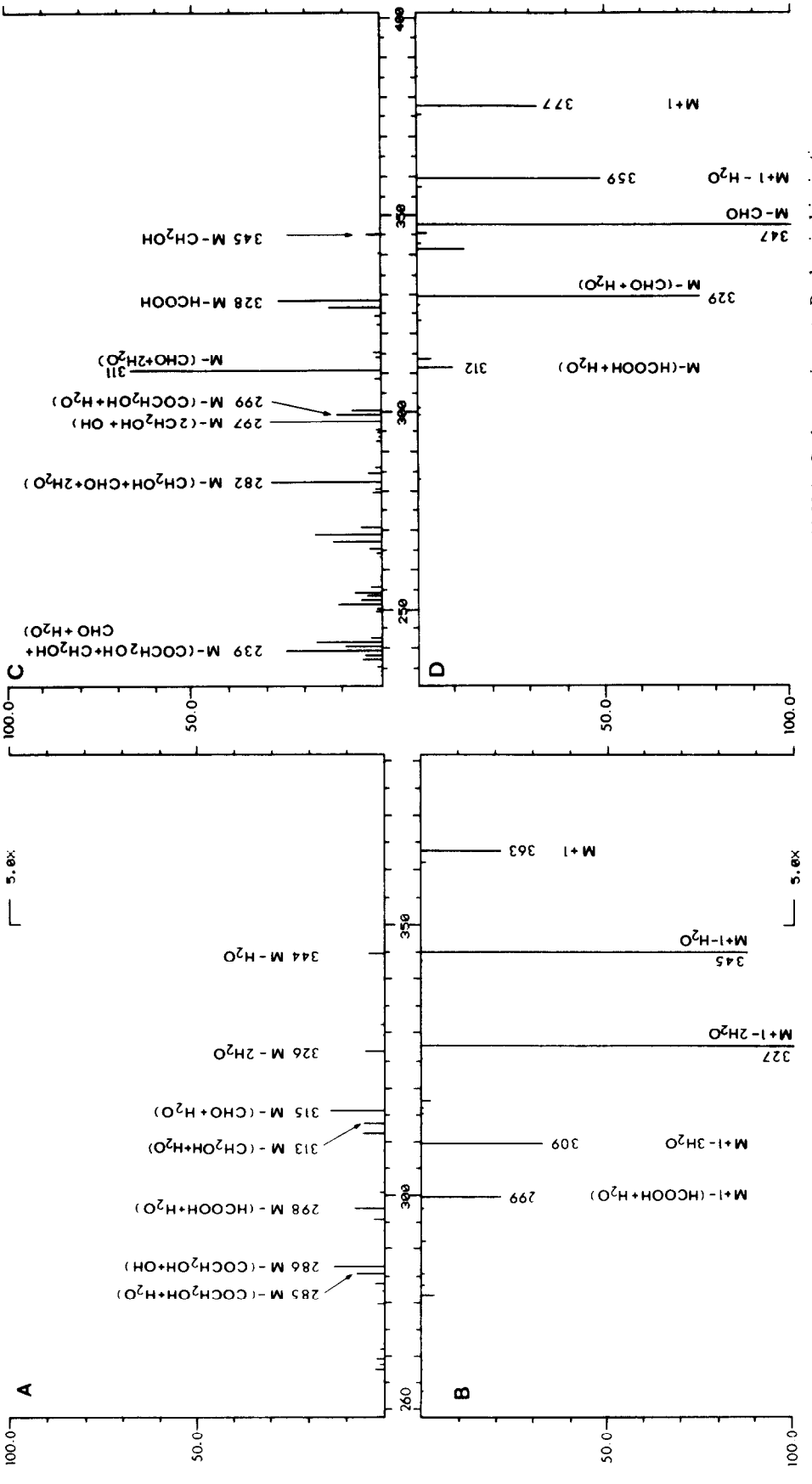

Fig. 4. Mass spectra. HAA (**8,8a**): *A*, electron impact; *B*, chemical ionization. 19-Hydroxyaldosterone (**20,20a**): *C*, electron impact; *D*, chemical ionization.

bilized by the presence of the acetate at C_{21} (Fig. 2). The bromohydrin easily lost HBr during the isolation with formation of the epoxide **12**, and even more readily with Et_3N or sodium acetate. The crude **11** was, therefore, directly reacted with lead tetraacetate, iodine an and $CaCO_3$ under illumination: chromatographic purification gave the cyclic derivative **13b** in 60% overall yield from **4b**.

The diacetate **13b** was next saponified with boiling bicarbonate and the diol **13a** selectively oxidized with the Jones reagent at C_3, the secondary hydroxyl having preference over the primary C_{21}-hydroxyl, without extensive hydrolysis of the ketal at C_{20}. The resulting bromoketone **14** was unstable and therefore directly dehydrobrominated with sodium acetate in ethanol: chromatography afforded the unsaturated ketone **15a** in 43% overall yield from **13b**, and a small amount of the 20-one **15b**.

Reductive ring opening of 6,19-oxides is commonly performed with zinc and acetic acid, when partial acetylation of the 19-hydroxyl may take place. Since acetylation can be prevented by the use of aqueous acetic acid [31], the ketal **15a** was subjected to a brief treatment with zinc and 90% acetic acid, when a 23% of the desired 4-en-19-ol **16a** was obtained. Zinc [32] or amalgamated zinc [33] in refluxing isopropanol were ineffective; however zinc in a mixture of isopropanol and acetic acid, used with much success in a synthesis of 19-hydroxydeoxycorticosterone [34], proved to be a useful solvent also in the present synthesis, allowing conversion of **15a** into a mixture of **16a** and its 5-ene isomer **16b**, which was directly ketalized with ethylene glycol and *p*-toluenesulfonic acid to yield the diketal **17** in 30% overall yield from **15a**.

Reduction of the lactone function in **17** to the hemiacetal **18** with DIBAH proceeded less cleanly than the case of **4** (*vide supra*) or in the 3α,5α-tetrahydroaldosterone series [26]. A large excess of the reagent was required and the product was accompanied by more polar by-products. Furthermore, the products of decomposition of DIBAH with isopropanol and water were tightly bound to the triol **18** which, in distinction from the other two series, could be desorbed with difficulty, preferably with a large volume of THF. The product was purified by chromatography and proved to be a mixture of the two C_{18} epimers, as indicated in the NMR spectrum by two sets of protons at positions 6, 11α and 18.

At this stage no serious difficulties were expected in completing the synthesis of 19-hydroxyaldosterone (**20**) by a mild hydrolysis of its diketal **18**, since a comparable conversion of 19-hydroxycorticosterone diketal (**22**) to 19-hydroxycorticosterone (**23**) was smoothly carried out almost 25 years ago [35]. In the event, two compounds were now obtained from **18**: the desired 19-hydroxyaldosterone (**20**) and the 18,21-anhydro compound **19** in roughly equal amounts. In this connection two points deserve a comment. (a) The hydroxyl at position **19** promotes the hydrolysis of the ketal at C_3, the rate being faster than in the diketal of aldosterone [26], and (b) acid-catalyzed cyclization to the 18,21-anhydro structure in the three aldosterone series (3α,5α-tetrahydro [26], 3β-hydroxy-5-ene **6** and 19-hydroxy **18**) occurs much more readily when the 20-keto group is protected as a ketal than when it is free. Consequently the three anhydro compounds isolated from the hydrolyses were the 20-ketals.

19-Hydroxyaldosterone is a crystalline solid, m.p. 110–120°C, soluble in most organic solvents and *freely in water*, from which it can be recovered unchanged by evaporation in a desiccator over sulfuric acid. It adheres tenaciously to residual silica from PTLC, from which it can be readily desorbed with water. Its i.r. spectrum in KBr (Fig. 3) exhibits a weak saturated carbonyl, showing that in the solid state the molecule exists mostly in the cyclic 18,20-epoxy form **20a**. [^{1}H]NMR spectra indicate that in $CDCl_3$ 19-hydroxyaldosterone exists mainly in two isomeric forms in the ratio 7:5, as judged by the 18-H singlets; in D_2O the ratio is 5:1.

Finally, $NaIO_4$ oxidation of 19-hydroxyaldosterone gave the expected 19-hydroxyaldosterone-γ-etiolactone (**21**), proving the 17β configuration. As anticipated, its [^{1}H]NMR spectrum shows the presence of a single isomer only.

The *biological activity* of HAA and 19-hydroxyaldosterone will be reported in detail at a later date. Preliminary results show that HAA in the Kagawa bioassay system [36] is a very active mineralocorticoid at 2.5 μg per rat, possessing both antinatriuretic and kaliuretic activity and is approx 1/10 to 1/25 as active as aldosterone. 19-Hydroxyaldosterone demonstrated full mineralocorticoid activity, causing both antinatriuresis and kaliuresis at 25 μg per rat. In short-circuit current measurements carried out by μs and Professor Bernard Rossier at the University of Lausanne, the latter compound showed mineralocorticoid activity approaching that of aldosterone.

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