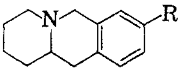

I

and hypotensive activity, we have prepared a few benzo[*b*]quinolizidine derivatives. It has been found that sodium borohydride may be used for the reduction of acridizinium bromide to I (R = H).

EXPERIMENTAL[3]

2-Aldoximino-1-(3-methoxy)benzylpyridinium bromide. To a flask containing 5 ml. of dimethyl formamide, 4.88 g. of pyridine 2-aldoxime and 8.04 g. of *m*-methoxybenzyl bromide were added. The flask was warmed gently on the steam bath until solution was complete, then stoppered and allowed to stand for 24 hr. at room temperature. The colorless crystals were triturated with ethyl acetate and collected. The yield was 11.41 g. (88%), m.p. 178–182°. The analytical sample melted at 180–182°.

Anal. Calcd. for $C_{14}H_{15}BrN_2O_2$: C, 52.03; H, 4.68; N, 8.67. Found: C, 52.44; H, 4.84; N, 8.55.

8-Methoxyacridizinium perchlorate. To a solution containing 1 g. of the oximino quaternary salt in 8 ml. of absolute alcohol, 6 ml. of concentrated hydrochloric acid was added, and the mixture refluxed for 5 hr. After vacuum evaporation of the solvents the residual yellow solid was washed with ethyl acetate and then dissolved in a small quantity of water. The perchlorate was precipitated by addition of perchloric acid. Recrystallization of the product from methanol yielded 0.41 g. (44%) of yellow platelets, m.p. 222–224° (lit.[4] 218–219°).

8-Hydroxyacridizinium bromide. The oximino quaternary salt (5.9 g.) was placed in 30 ml. of 48% hydrobromic acid and the mixture refluxed for 45 min. The mixture was vacuum evaporated and the residue crystallized from a concentrated ethanol solution. The yield was 3.9 g. (97%),[5] m.p. 246–248° (lit.[4] 250–252°).

8-Methoxybenzo[b]quinolizidine (I, R = OCH_3) *hydroperchlorate.* To a suspension of 5.2 g. of 8-methoxyacridizinium perchlorate in 300 ml. of methanol, 100 mg. of platinum oxide was added and hydrogenation was carried out at room temperature and atmospheric pressure until the theoretical amount of hydrogen had been absorbed. The solution was filtered, concentrated, and cooled; 4.4 g. (83%) of colorless crystals, m.p. 175–177° was obtained.

Anal. Calcd. for $C_{14}H_{20}ClNO_4$: C, 52.92; H, 6.35; N, 4.41. Found: C, 53.10; H, 6.07; N, 4.50.

8-Methoxybenzo[b]quinolizidine (I. R = OCH_3) was recrystallized from ethanol, m.p. 50–51°.

Anal. Calcd. for $C_{14}H_{19}NO\cdot{}^1/_3H_2O$: C, 75.30; H, 8.88; N, 6.27. Found: C, 75.58; H, 8.69; N, 6.15.

8-Hydroxybenzo[b]quinolizidine (I. R = OH) *hydrochloride.* The reduction of 1.7 g. of the 8-hydroxyacridizinium salt was carried out as in the case of the methyl ether. Concentration of the methanol solution yielded 1.51 g. (88%), decomposes 268–290°. The analytical sample consisted of colorless prisms, decomposes 276–318°.

Anal. Calcd. for $C_{13}H_{18}ClNO$: C, 65.13; H, 7.57; N, 5.81. Found: C, 65.37; H, 7.69; N, 5.70.

8-Hydroxybenzo[b]quinolizidine (I. R = OH) was obtained as a colorless powder, m.p. 230–231°.

Anal. Calcd. for $C_{13}H_{17}NO$: C, 76.82; H, 8.43; N, 6.90. Found: C, 76.51; H, 8.35; N, 7.04.

The *methiodide* was prepared in 92% yield by refluxing a methanol solution of the base for 1 hr. with excess methyliodide. It formed colorless needles from ethanol, m.p. 274–275°.

Anal. Calcd. for $C_{14}H_{20}INO$: C, 48.72; H, 5.84; N, 4.06. Found: C, 48.56; H, 5.91; N, 4.25.

Benzo[b]quinolizidine (I. R = H) *methiodide.* (a) *From the hydrobromide.* Benzo[b]quinolizidine hydrobromide[2] was converted to the free base by action of ammonia, and the crude base obtained by ethereal extraction was methylated with methyl iodide. The product was obtained from ethanol as colorless irregular crystals, m.p. 290–291°.

(b) *From the sodium borohydride reduction product.* To a solution of 2 g. of acridizinium bromide in 45 ml. of water an aqueous suspension 0.68 g. of sodium borohydride was added. The mixture was heated on the steam bath until the evolution of hydrogen ceased and a red oil separated. The oil was taken up in ether, the solution dried and concentrated, and the residue heated with methyl iodide on the steam bath for 2 hr. The product melted at 267–268° and the melting point did not change on recrystallization. When a sample of the product was dissolved in ethanol and seeded with a single crystal of product obtained by Procedure a, the entire material crystallized in irregular clusters, m.p. 290–291°. The infrared spectrum of this material was identical with that of the product obtained by Procedure a.

Anal. Calcd. for $C_{14}H_{20}IN$: C, 51.07; H, 6.12; N, 4.25. Found[6]: C, 51.27, 50.97; H, 5.85, 5.96; N, 4.13, 4.55.

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[3] All melting points were taken on a Fisher-Johns hot stage and are uncorrected. All analyses were performed by Drs. Weiler and Strauss, Oxford, England.

[4] C. K. Bradsher and J. H. Jones, *J. Am. Chem. Soc.*, **79**, 6033 (1957).

[5] Only a 37% yield of 8-hydroxyacridizinium was reported earlier [ref. (4)] for the cyclization of crude 1-(3-methoxybenzyl)-2-formylpyridinium bromide.

(6) Values are for the product of Procedure *a* and for the low-melting form (m.p. 267–268°) obtained by Procedure *b*.

9(11)-Dehydrocortical Steroids. Synthesis of 9(11)-Anhydro-17α-hydroxycorticosterone Acetate and 9(11)-Anhydrocorticosterone Acetate

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The elimination of the 11β-hydroxyl group from the steroidal nucleus to give 9(11)-dehydro compounds is easily accomplished because of the favorable conformation of the 11β-hydroxyl group and the 9α-hydrogen atom (di-axial-trans).[1] Reichstein and his co-workers[2] have reported the conversion of 11β-hydroxylated cortical steroids without a 17α-hydroxyl function to 9(11)-anhydro derivatives using phosphorus oxychloride and pyridine or refluxing acetic-hydrochloric acid mixtures. In the

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(1) W. A. Cranshaw, H. B. Henbest, and E. R. Jones, *J. Chem. Soc.*, **73** (1954); H. L. Herzog, C. C. Payne, and E. B. Hershberg, *J. Am. Chem. Soc.*, **76**, 930 (1954).

(2) (a) C. W. Shoppee, *Helv. Chim. Acta*, **23**, 740 (1940); (b) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **24**, 351 (1941); (c) P. Hegner and T. Reichstein, *Helv. Chim. Acta*, **26**, 715 (1943); (d) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **26**, 1316 (1943).

case of the steroidal hormones where an α,β-unsaturated ketone is present, the usual reagents for dehydration lead to complications. Graber, Haven, and Wendler[3] have reported the preparation of 17α-hydroxy-21-acetoxy-4,9(11-)pregnadiene-3-20-dione, [9(11)-anhydro-17α-hydroxycorticosterone acetate], by an indirect method from 11β-hydroxy-20-cyano-21-acetoxy-17(20)-pregnen-3-one. The dehydration of this latter compound was effected with phosphorus oxychloride and pyridine. In compounds where the Δ^4-3-ketone structure is absent, these latter authors[3] have shown that dehydration with phosphorus oxychloride-pyridine proceeds without complications to give the anhydro compound in good yields—e.g., 11β,17α-dihydroxy-21-acetoxypregnane-3,20-dione gives 17-α-hydroxy-21-acetoxy-9(11)-pregnene-3,20-dione in 73% yield.

The preparation of 9-halo derivatives of 11β,17α-dihydroxy-21-acetoxy-4-pregnen-3,20-dione (hydrocortisone acetate) demanded a ready supply of the 9(11)-anhydro compound, 17α-hydroxy-21-acetoxy-4,9(11)-pregnadien-3,20-dione, and a direct method of dehydration of the readily available hydrocortisone acetate was sought. Two reagents, methanesulfonyl chloride and methyl chlorosulfite, were found which bring about this dehydration smoothly and in good yield. One of these reagents was mentioned briefly without details in the preparation of 17α-hydroxy-21-acetoxy-1,4,9(11)-pregnatrien-3,20-dione from 11β,17α-dihydroxy-21-acetoxy-1,4-pregnadien-3,20-dione (prednisolone acetate)[4] and we are prompted to communicate our experience with this dehydration.

Initially, hydrocortisone acetate in pyridine reacted with methanesulfonyl chloride at room temperature in the course of 24 hr. to give a 30% yield of the 9(11)-anhydro compound. Further study of this reaction lead to optimum conditions whereby the dehydration was carried out with methanesulfonyl chloride in pyridine-dimethylformamide solution at 80–85° to give 75–80% yield of product.

The method appears to be generally applicable and has been employed in the dehydration of corticosterone acetate.

A more vigorous dehydrating agent was methyl chlorosulfite.[5] This reagent effected dehydration of hydrocortisone acetate in tetrahydrofuran-pyridine solution at −10 to −5° over a period of 4 hr. or in dimethylacetamide solution at 20–25° over a period of 30 min. The yield with this reagent was 65–89% of theory. It is of interest that in the dehydration of cholesterol to cholestadiene with methyl chlorosulfite reported by Berti[5] the methyl sulfonyl ester was first obtained and isolated and the dehydration was achieved only by heating at 185–270° under 20 mm. vacuum. In the present work despite our efforts to isolate the intermediate esters no trace of these intermediates could be isolated.

EXPERIMENTAL

17α-Hydroxy-21-acetoxy-4,9(11)-pregnadien-3,20-dione. (a) Methanesulfonyl chloride method. To a slurry of 10 g. (0.0247 mol.) of 11β,17α-dihydroxy-21-acetoxy-4-pregnen-3,20-dione (hydrocortisone acetate) in 50 ml. of dry dimethylformamide[6] and 8.8 ml. of dry pyridine was added dropwise with stirring 6.43 g. (4.4 ml., 0.0564 mol.) of methanesulfonyl chloride. The reaction mixture was stirred and the temperature maintained at 80–85° for 1 hr. after all the methanesulfonyl chloride had been added. At the end of the reaction period the temperature was brought to 25–30° and the mixture was diluted with 200 ml. of methanol. After cooling (ice bath) for 1/2 hr. the product was filtered and washed with methanol. Recrystallization from methylene chloride–methanol afforded 7.4 g. (77.5%) of product, m.p. 228–238°; infrared identical with an authentic sample. An analytical sample melted 232.5–236.5° (lit.[3] m.p. 231.5–234.5°).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.47; H, 7.38. Found: C, 71.08; H, 7.93.

(b) Methyl chlorosulfite method. Four grams (0.01 mol.) of 11β,17α-dihydroxy-21-acetoxy-4-pregnene-3,20-dione (hydrocortisone acetate) was dissolved in 20 cc. of dry dimethylacetamide. To this solution was added with stirring 11 g. (0.084 mol.) of methyl chlorosulfite.[5] During the addition the temperature was kept between 20–25° by external cooling. During the course of the addition the mixture set to a semi-solid mass. When the addition of the methyl chlorosulfite was complete (30 min.), 70 ml. of methanol was added and the mixture was aged in an ice bath for 1/2 hr. The product was filtered and washed with methanol, 2.5 g. (65%), m.p. 205–225°. Recrystallized from chloroform-methanol, the product melted 237–240°. Identity was established by infrared comparison and mixed melting point.

A 4-g. sample (0.01 mol.) of 11β,17α-dihydroxy-21-acetoxy-4-pregnene-3,20-dione was dissolved by heating in 150 ml. of dry tetrahydrofuran and the solution was cooled to −10°. Dry pyridine (8 ml.) was added. To the cooled solution was added with stirring 11.5 g. (0.088 mol.) of methyl chlorosulfite.[5] The temperature was kept between −10 and −5° during the addition of the reagent. The reaction mixture was then allowed to come to room temperature over a period of 4 hr and the product was precipitated by the addition of 150 ml. of ice water. The product was filtered after aging 1/2 hr. and washed with cold water, yield 3.45 g. (89%), m.p. 226–230°. The identity of the substance was confirmed by mixed melting point and comparison of the infrared spectrum.

21-Acetoxy-4,9(11)-pregnadien-3,20-dione. A 4-g. sample (0.013 mole) of 11β-hydroxy-21-acetoxy-4-pregnene-3,20-dione (corticosterone acetate) was dissolved in 20 ml. of dry dimethylformamide. To this solution was added 1.6 ml. of dry pyridine and 2 ml. of methanesulfonyl chloride. The reaction mixture stood at room temperature 3 days. At the end of this time 60 ml. ice water was added. The precipitated product was filtered, washed with water, and twice recrystallized from methanol, m.p. 153–157° (lit.[2d] m.p. 159–160°); infrared 5.75 μ (OAc), 5.82 μ (>C=O), 6.01 μ, 6.19 μ (—C=C—C=O), no OH band.

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(3) R. P. Graber, A. C. Haven, Jr., and N. L. Wendler, *J. Am. Chem. Soc.*, **75**, 4722 (1953).

(4) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restino, A. Borman, and F. M. Singer, *J. Am. Chem. Soc.*, **77**, 4181 (1955).

(5) Berti, *J. Am. Chem. Soc.*, **76**, 1214 (1954).

(6) The steroid initially all goes into solution and then reprecipitates as a dimethylformamide complex.

Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.50; H, 8.16. Found: C, 74.71; H, 8.22.

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16-Hydroxylated Steroids. XIII.[1] 9α-Fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione

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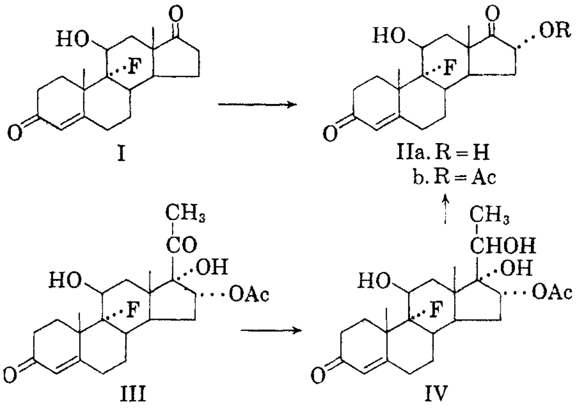
The research program of this laboratory on the preparation of 16-hydroxylated steroids has been extended to include C19-steroids. We wish to report on the synthesis of 9α-fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa).[2]

9α-Fluoro-11β-hydroxy-4-androstene-3,17-dione (I)[3] on microbiological hydroxylation with *Streptomyces roseochromogenus* (Lederle AE 409)[4] afforded 9α-fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa). The structure of the fermentation product was established as follows:

Compound IIa exhibited a positive Blue Tetrazolium test indicative of the 16,17-ketol moiety.[5] Acetylation gave the monoacetate IIb, in turn, synthesized from 16α-acetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (III).[6] Reduction of III in methanol at 0° with sodium borohydride gave 16α-acetoxy-9α-fluoro-11β,17α,20-trihydroxy-4-pregnen-3-one (IV).[7] The latter on the basis of elemental analyses was apparently obtained in a pure state. However, its ultraviolet absorption spectrum, $\lambda_{max}^{methanol}$ 240 mμ (ε 11,000), revealing a low molecular extinction coefficient,[8] indicated that reduction in part of the C3-carbonyl and/or the C4-5-double bond had occurred.[9] The material as such in methanol was treated with an aqueous solution of sodium periodate at room temperature to give after partition chromatography 16α-acetoxy-9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (IIb), identical in all respects with the acetylated fermentation product.

Bioassay. Rosemberg and Dorfman[10] have recently cited 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (I) as the first instance of a highly active sodium retaining substance in the C19-series. These same investigators have now found in a preliminary assay that 9α-fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa) was inactive in the electrolyte assay (saline load, six hours) on adrenalectomized rats at 6, 25, and 100 μg. dose levels.[11]

Flow Sheet



EXPERIMENTAL

All melting points are uncorrected.

9α-Fluoro-11β,16α-dihydroxy-4-androstene-3,17-dione (IIa). Forty 500 ml. flasks were charged with 100 ml. each of the following medium: corn steep liquor (30 g.), glucose (30 g.), soybean oil (5 g.), and calcium carbonate (5 g.) in 1 l. of distilled water. Each flask, after the addition of 25 mg. of 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (I) dissolved in 1 ml. of methanol, was inoculated with 4 ml. of a 48 hr. (28°) mycelial growth of *Streptomyces roseochromogenus* (Lederle AE 409). The fermentation was carried out for 78 hr. at 28° with shaking (rotary shaker, 240 RPM).

The pooled fermentation mixture was filtered, and the filtrate was extracted twice with 4 l.-portions of chloroform. The combined extracts were washed with water, treated with animal charcoal, dried and evaporated. The crude residue was subjected to partition chromatography on 300

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(1) Paper XII, S. Bernstein, R. Littell, J. J. Brown, and I. Ringler, *J. Am. Chem. Soc.*, **81**, 4573 (1959).

(2) G. H. Thomas and R. W. Thoma, U. S. Patent 2,853,502 (Sept. 23, 1958), have also described the preparation of IIa by microbiological 16α-hydroxylation.

(3) S. Bernstein and R. H. Lenhard, *J. Am. Chem. Soc.*, **77**, 6665 (1955); J. Fried and E. F. Sabo, *J. Am. Chem. Soc.*, **79**, 1130 (1957).

(4) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz, and A. Borman, *Recent Progr. Hormone Research*, **9**, 149 (1955); R. W. Thoma, J. Fried, S. Bonanno and P. Grabowich, *J. Am. Chem. Soc.*, **79**, 4818 (1957).

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(6) S. Bernstein, J. J. Brown, L. I. Feldman, and N. E. Rigler, *J. Am. Chem. Soc.*, **81**, 4956 (1959).

(7) The reduction of the C20-carbonyl group presumably provided the 20β-hydroxyl group; see, D. K. Fukushima and E. D. Meyer, *J. Org. Chem.*, **23**, 174 (1958).

(8) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

(9) F. Sondheimer, M. Velasco, E. Batres and G. Rosenkranz, *Chem. & Ind.* (London), 1482 (1954); J. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955).

(10) E. Rosemberg and R. I. Dorfman, *Proc. Exptl. Biol. and Med.*, **99**, 336 (1958).

(11) We wish to thank the Worcester Foundation group for carrying out this assay, the details of which will be reported by them elsewhere.