Synthesis of an Isomer of Antheridiol

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The syntheses are described of 3β-acetoxy-22,25-dihydroxy-$\Delta^{5,24(28)}$-stigmastadien-29-oic acid γ-lactone (III) and of its 7-keto analog (II) which is isomeric with the fungal sex hormone antheridiol. The key reaction was a Reformatsky condensation of a C_{22} aldehyde with a C_7 bromo butenolide.

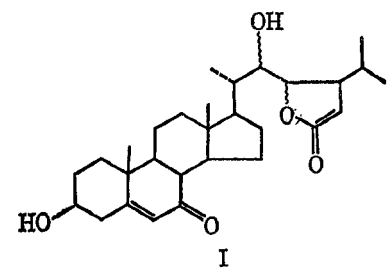
The role hormones play in sexual reproduction in fungi is well established.[1] In the case of the aquatic fungus *Achlya bisexualis*, the mechanism of hormonal control of the sexual process was postulated by Raper several years ago.[2] The substance which initiates the process and which is secreted by the female mycelium was called hormone A. It was isolated in crystalline form in 1965 and renamed antheridiol.[3] The hormone has been shown to be a steroid with the structure I[4] and this structure has now been confirmed by a synthesis carried out by Fried and coworkers of the Syntex Corp.[5]

During the structural investigation, before a satisfactory nuclear magnetic resonance (nmr) spectrum had been obtained,[6] the isomeric structure II for antheridiol was favored and a synthesis of this structure was first undertaken. This paper describes the synthesis of the model steriod III, and the isomer of antheridiol II. The synthetic work provided important evidence for the structure of antheridiol itself.

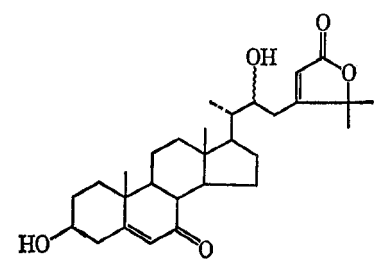
The starting material for the synthesis of III was 3β-acetoxy-22,23-bisnorcholenic acid, which was reduced to the aldehyde IV by a modification of the method of Staab.[7] Treatment with an excess of N-N'-carbonyldiimidazole in boiling tetrahydrofuran gave the imidazolid, which was reduced with lithium tri-*t*-butoxyaluminium hydride in tetrahydrofuran at room temperature to give 3β-acetoxy-22,23-bisnor-Δ^5-cholenaldehyde (IV), mp 113–116°,[8] in high yield.

cis-4-Hydroxy-3,4-dimethyl-2-pentenoic acid lactone was prepared essentially by the method of Stewart and Woolley.[9] It gave, on treatment with N-bromosuccinimide in refluxing carbon tetrachloride under illumination, a mixture of products separated by chromatography into unchanged lactone, the bromo lactone V (R_1 = Br; R_2 = H), mp 41°, and the dibromo lactone V (R_1 = R_2 = Br), mp 120°.

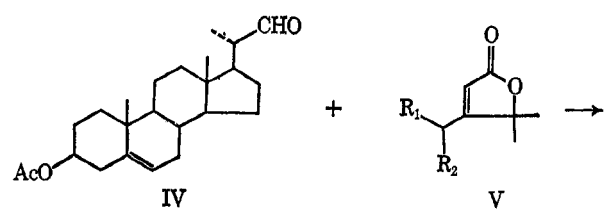
Reformatsky reaction of the aldehyde IV and the bromo lactone V (R_1 = Br; R_2 = H) with activated zinc dust in benzene followed by chromatography of the product afforded 3β-acetoxy-22,25-dihydroxy-



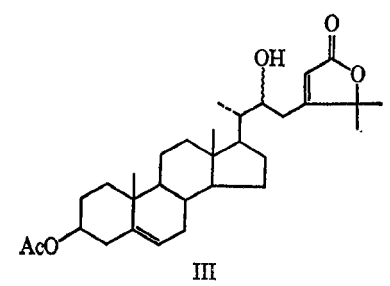
I



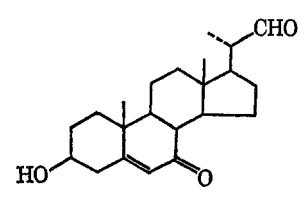
II



IV V



III



VI

$\Delta^{5,24(28)}$-stigmastadien-29-oic acid γ-lactone (III), mp 224–227°, in a yield of 15% (from IV). The substance gave one spot on thin layer chromatography (tlc) as did its product of acetylation, mp 205–207°, and product of deacetylation, mp 240–244°. This indicated that the product III was a single epimer.

It is worth noting that the lactone ring of III was unchanged after treatment with alkali and subsequent acidification. In contrast, when an alcoholic solution

(1) See, *inter alia*, (a) L. Machlis in "The Fungi," Vol. II, G. C. Ainsworth and A. S. Sussman, Ed., Academic Press Inc., New York, N. Y., 1966, p 415; (b) W. H. Nutting, H. Rapoport, and L. Machlis, *J. Amer. Chem. Soc.*, **90**, 6434 (1968); (c) H. van den Ende, *J. Bacteriol.*, **96**, 1298 (1968).

(2) J. R. Raper, *Amer. J. Bot.*, **26**, 639 (1939).

(3) T. C. McMorris and A. W. Barksdale, *Nature*, **215**, 320 (1967).

(4) G. P. Arsenault, K. Biemann, A. W. Barksdale, and T. C. McMorris, *J. Amer. Chem. Soc.*, **90**, 5635 (1968).

(5) J. A. Edwards, J. S. Mills, J. Sundeen, and J. H. Fried, *ibid.*, **91**, 1248 (1969).

(6) This was in part due to the poor solubility of antheridiol in suitable organic solvents. Eventually, a mixed solvent, 4:1 $CDCl_3$–CD_3OD, proved to be best.

(7) H. A. Staab and H. Braünling, *Justus Liebigs Ann. Chem.*, **654**, 119 (1962).

(8) A. P. Centolella, F. W. Heyl, and M. E. Herr, *J. Amer. Chem. Soc.*, **70**, 2953 (1948).

(9) J. M. Stewart and D. W. Woolley, *ibid.*, **81**, 4951 (1959).

of antheridiol was treated with a trace of sodium hydroxide solution, the ultraviolet spectrum changed in 3 hr from a single maximum at 220 mμ to maxima at 237 and 278 mμ. This change was not fully investigated because of lack of material, but it probably involved rearrangement of the α,β-butenolide to the β,γ isomer[10] as well as partial elimination of the 3β-hydroxyl group. The loss of the α,β-unsaturated lactone chromophore exposed the peak at 237 mμ owing to the Δ^5-7 ketone present in antheridiol.

For the synthesis of II, stigmasteryl acetate was oxidized with anhydrous sodium chromate in acetic acid–acetic anhydride at 35–40° to give 7-ketostigmasteryl acetate, mp 181–183°.[11] Treatment with dilute potassium carbonate solution then gave the keto alcohol. Controlled ozonolysis of this compound in a solution of methylene chloride containing 1% pyridine at −78°, followed by reductive work-up (Zn–CH_3COOH),[12] gave a 40% yield of 3β-hydroxy-Δ^5-7-ketocholen-22,23-bisnoraldehyde (VI), mp 226–229°.

The mass spectrum of the aldehyde VI (mol wt 344) was very similar to that of antheridiol, thus providing further evidence for the steroid nucleus of the latter compound. Antheridiol (mol wt 470) readily loses a large fragment, *m/e* 126, giving the base peak *m/e* 344 which corresponds to the molecular ion of VI. The only significant difference in the spectra was in the few additional peaks, *viz.*, at *m/e* 470, 452, 434 (all very low intensity), 126, and 111, in the spectrum of antheridiol.

The aldehyde was converted to its tetrahydropyranyl ether (dihydropyran–*p*-toluenesulfonic acid) and then condensed with the bromo lactone V (R_1 = Br; R_2 = H) to give the tetrahydropyranyl ether of II in 15% yield from VI. This product, mp 220–225°, α_D −74°, appeared to be homogeneous by tlc. Removal of the tetrahydropyranyl group by gentle acid treatment afforded 3β,22,25-trihydroxy-$\Delta^{5,24(28)}$-stigmastadien-7-on-29-oic acid γ-lactone (II), mp 241–244°.

The mass spectra of II and antheridiol proved to be almost identical. However, the fragment ion from the side chain of the latter compound gave a much stronger peak at *m/e* 126 than the corresponding fragment from II. Both spectra showed intense peaks at *m/e* 111 [$(C_7H_{10}O_2 - CH_3)^+$]. Like antheridiol, compound II was easily converted into a $\Delta^{3,5}$-7 ketone (λ_{max} 278 mμ) on treatment with acid.

The isomer of antheridiol (II) was inactive in the biological assay for hormone A.

Experimental Section[13]

3β-Acetoxy-22,23-bisnorcholenaldehyde (IV).—3β-Acetoxybisnorcholenic acid (20 g)[13b] was added to dry tetrahydrofuran (200 ml) followed by N,N′-carbonyl diimidazole (25 g). The mixture was heated to boiling, when complete solution occurred, and refluxed for 45 min. It was then cooled and poured into water, giving a white precipitate which was separated by filtration, washed thoroughly with water, and dried over P_2O_5, yield 22 g, mp 220–223°. This imidazolid (22 g) was suspended in dry tetrahydrofuran (300 ml) and a solution of lithium tri-*t*-butoxyaluminum hydride (20 g) in tetrahydrofuran (200 ml) was added dropwise during a 1-hr period at room temperature. The resulting solution was concentrated *in vacuo* at room temperature to about 100 ml and poured into dilute 1 *N* hydrochloric acid (400 ml) with vigorous stirring. The precipitate was separated by filtration, washed with water, and air dried, yield 23 g, mp 114–118°. Tlc showed it to be mainly one compound, the aldehyde, with small amounts of alcohol formed by reduction of the aldehyde and unchanged imidazolid. The product was, therefore, purified by chromatography on silica gel (0.05–0.20 mm) with chloroform, giving 14 g of aldehyde, mp 113–116°. This aldehyde has been prepared by ozonolysis of stigmasteryl acetate dibromide by Centolella, *et al.*,[8] who give a melting point of 113–116°. The latter method was tried but was found not to be so convenient as the one described above. Spectral data for compound IV follow: ir 2725 and 1733 cm^{-1}; nmr δ 0.70 (H-18), 1.03 (H-19), 1.13 (d, *J* = 7 Hz, H-21), 5.40 (broad peak, H-6), and 9.61 (d, *J* = 3.5 Hz, H-24).

***cis*-4-Hydroxy-3,4-dimethyl-2-pentenoic Acid Lactone (V, R_1 = R_2 = H).**—The preparation was similar to the one described in the literature,[9] except for the following differences. 2-Hydroxy-2-methylbutan-3-one was acetylated by refluxing with excess acetic anhydride and a little zinc dust for 3 hr. The liquid was cooled and poured into ice–water, causing separation of the fragrant, oily acetate. Sodium bicarbonate was added to neutralize the acetic acid and the acetate was then extracted into ether. The extract was dried (Na_2SO_4) and distilled, and the fraction with a boiling point of 77° (21 mm) was collected and used in the Reformatsky reaction with ethyl bromoacetate. The product of this reaction after treatment with sodium hydroxide solution was acidified and then extracted with ether. The extract was dried (Na_2SO_4), the ether was removed, and the residual brown, viscous liquid was chromatographed on alumina with 5:1 benzene–ether. The lactone was nicely crystalline: mp 42–44°; uv max 208 mμ (ε 13,000); ir 1760 cm^{-1}; nmr δ 1.45 (2 CH_3), 2.03 (d, *J* = 2 Hz, 1 CH_3), and 5.70 (q, *J* = 2 Hz, 1 H).

Reaction of V (R_1 = R_2 = H) with N-Bromosuccinimide.—The lactone (1 g) was dissolved in dry carbon tetrachloride (60 ml), and N-bromosuccinimide (1.4 g) was added. The mixture was refluxed under illumination from a 250-W lamp for 30 min and cooled, and the liquid was filtered away from the succinimide. Removal of the solvent gave an oil (2 g) which was chromatographed on silica gel with 1:3 ethyl acetate–petroleum ether (bp 60–90°) to give, first, the dibromo lactone V (R_1 = R_2 = Br): mp 120°; ir 1748 cm^{-1}; nmr δ 1.63 (s, 2 CH_3), 6.23 (br s, 1 H), and 6.48 (br s, 1 H); mass spectrum *m/e* 282 (M^+), 284, and 286. The bromo lactone V (R_1 = H; R_2 = Br) was eluted next: mp 41°; ir 1760 cm^{-1}; nmr δ 1.58 (s, 2 CH_3), 4.21 (d, *J* = 1.5 Hz, 2 H), and 6.20 (t, *J* = 1.5 Hz, 1 H); mass spectrum *m/e* 204 (M^+), 206, 235, and 250. The last two peaks were presumably formed by loss of bromine from the molecular ion followed by dimerization of the resulting radical ion ($C_{14}H_{18}O_4$ = 250) and loss of a methyl ($C_{13}H_{15}O_4$ = 235).

Anal. Calcd for $C_7H_9O_2Br$ (mol wt, 205.05): C, 41.00; H, 4.39; O, 15.61; Br, 39.00. Found: C, 40.90; H, 4.25; O, 15.73; Br, 39.74.

Late fractions from the chromatography gave unchanged lactone V (R_1 = R_2 = H).

3β-Acetoxy-22,25-dihydroxy-$\Delta^{5,24(28)}$-stigmastadien-29-oic Acid γ-Lactone (III).—A solution of 3β-acetoxy-22,23-bisnorcholenaldehyde (500 mg) and the bromo lactone V (R_1 = H; R_2 = Br) (275 mg) in dry benzene (8 ml) was refluxed together with activated zinc dust for 2 hr. (The zinc dust was activated by treating it with dilute 6 *N* hydrochloric acid for 5 min, washing it several times with water and then with acetone, and drying it at 100° *in vacuo*.) The mixture was diluted with benzene shaken with dilute 2 *N* hydrochloric acid for several minutes and then with water and dried (Na_2SO_4), and the solvent was removed. The residue (700 mg) was chromatographed on silica gel with 1:1 ethyl acetate–petroleum ether to give III: yield 93 mg; mp 224–227°; ir 1757, 1742 (sh), and 1718 cm^{-1} (acetate and lactone); nmr δ 0.70 (H-18), 1.00 (H-19) (the H-21 signal appeared as two inflections on the side of the H-19 singlet), 1.43

(10) The isomerization of α,β-butenolides with alkali is well known in the cardenolide field. See L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 739.

(11) L. F. Feiser, M. Fieser, and R. N. Chakravarti, *J. Amer. Chem. Soc.*, **71**, 2226 (1949).

(12) This method was first used for ozonolysis of stigmastadienone by G. Slomp, Jr., and J. L. Johnson, *ibid.*, **80**, 915 (1958).

(13) (a) Melting points were taken on a Kofler hot stage and are uncorrected. Infrared spectra were determined in KBr disks with a Perkin-Elmer Model 21 spectrophotometer and ultraviolet spectra were determined in ethanol with a Perkin-Elmer Model 450 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian A-60 A spectrometer, using tetramethylsilane as internal reference. Microanalyses were carried out by Dr. F. Pascher, Bonn, Germany. (b) Purchased from Mann Research Laboratories, Inc., New York, N. Y. 10006.

(H-26 and -27), 1.98 (acetate), 3.99 (broad peak, H-22), 4.52 (very broad peak, H-3), 5.32 (br s, H-6), and 5.80 (s, H-28); mass spectrum *m/e* 438 (M − 60), 312 (M − 60 − 126), and 111.

Anal. Calcd for $C_{31}H_{46}O_5$: C, 74.66; H, 9.30; O, 16.04; mol wt, 498.68. Found: C, 74.50; H, 9.51; O, 16.01; mol wt, 500 (cryoscopic).

This substance gave a single spot on tlc with different solvent systems. Acetylation with acetic anhydride and pyridine gave a product, mp 205–207°, which was also homogeneous by tlc. Likewise, hydrolysis of the acetate group by treatment of the alcoholic solution with 10% K_2CO_3 solution gave a crystalline product, mp 240–244°, ir 1745 cm^{-1}, which was homogeneous.

7-Ketostigmasterol.—The following method gave better yields than that described in the literature.[11] Stigmasteryl acetate (5 g) was dissolved in acetic acid (500 ml) and acetic anhydride (50 ml). The solution was stirred at 35–40° with sodium chromate (5 g) for 48 hr, concentrated under reduced pressure to a small volume, and poured into water with vigorous stirring. The precipitate was removed by filtration, washed with water, air dried, and crystallized from ethyl acetate–petroleum ether, yielding *ca.* 2 g, mp 176–179°. Recrystallization from methanol raised the melting point to 181–183°.

The keto acetate in methanol (200 ml) was stirred overnight with 10% K_2CO_3 solution (20 ml). Most of the solvent was removed *in vacuo* and water was added to the residue. The insoluble product was collected and dried: it melted partially at 122–124° and completely at 143–145°; uv max 237 mμ (ε 12,200); ir 1675 and 1634 cm^{-1}.

3β-Hydroxy-Δ⁵-7-keto-22,23-bisnorcholenaldehyde (VI).—7-Ketostigmasterol (1 g), dissolved in methylene chloride (100 ml) and pyridine (1 ml), was ozonized at −78° for 45 min. (This reaction time was found to be most suitable for the conditions used. Shorter reaction times gave mixtures of unchanged 7-ketostigmasterol and aldehyde which were not readily separated, while longer reaction times led to attack of the nuclear double bond.) A white suspension formed. This was stirred with zinc dust (2 g) and acetic acid (2 ml) for 2 hr, during which time it warmed to room temperature. It was then washed several times with water and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a crystalline residue which was shaken with a little ethyl acetate and separated by filtration: yield 330 mg; mp 226–229° (recrystallization from chloroform–ethyl acetate did not change the melting point); α_D −124° (*c* 0.5, MeOH); uv max 238 mμ (ε 12,800); ir 3484, 2710, 1727, 1664, and 1626 cm^{-1}; nmr δ 0.73 (H-18), 1.13 (d, *J* = 7 Hz, H-21), 1.20 (H-19), 5.73 (broad peak, H-6), and 9.63 (d, *J* = 3 Hz, H-24); mass spectrum *m/e* 344 (base peak, M^+).

Anal. Calcd for $C_{22}H_{32}O_3$ (mol wt, 344.48): C, 76.70; H, 9.36; O, 13.93. Found: C, 75.74; H, 9.41; O, 14.73. A satisfactory analysis has not been obtained, possibly because the crystals still contained solvent after being heated to constant weight *in vacuo* at 100°.

3β,22,25-Trihydroxy-Δ⁵,²⁴⁽²⁸⁾-stigmastadien-7-on-29-oic Acid γ-Lactone (II).—The keto aldehyde VI (300 mg) was stirred for 30 min with dihydropyran (6 ml) and a small crystal of *p*-toluenesulfonic acid. The resulting solution was concentrated *in vacuo* and chromatographed on silica gel with 1:3 ethyl acetate–petroleum ether. The crystalline fractions of the tetrahydropyranyl ether were combined. The nmr spectrum of this material was similar to that of the starting aldehyde VI, except for increased resonances in the region of δ 1.6 and 3.6 and a broad peak at δ 4.73, all due to the protons of the tetrahydropyran ring. The aldehyde VI could be recovered unchanged by treating the tetrahydropyranyl ether with a solution of dilute HCl in methanol (0.1 ml of concentrated HCl in 100 ml of methanol) for 3 hr at room temperature. Thus no epimerization occurred at C-20 in the formation of the tetrahydropyranyl ether.

The tetrahydropyranyl ether and the bromo lactone V (R_1 = Br, R_2 = H) (180 mg) were dissolved in dry benzene (3 ml), activated zinc dust (60 mg) was added, and the mixture was refluxed for 1.5 hr. It was then cooled, diluted with benzene, washed with dilute hydrochloric acid and water, and dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel with 1:1 ethyl acetate–petroleum ether, giving the tetrahydropyranyl ether of II: yield 66 mg; mp 220–225°; α_D −74° (*c* 0.2, MeOH); uv max 215 mμ (ε 19,000) and 233 (inflection, 16,000); ir 3472, 1739, 1675, and 1634 cm^{-1}; nmr δ 0.70 (H-18), 0.97 (broad peak, H-21[14]), 1.20 (H-19), 1.45 (H-26 and -27), 5.70 (broad s, H-6), and 5.87 (s, H-28).

Anal. Calcd for $C_{34}H_{50}O_6$: C, 73.61; H, 9.09; O, 17.31; mol wt, 554.75. Found: C, 73.38; H, 9.02; O, 17.74; mol wt, 510 (tensimetric).

This substance appeared to be homogeneous by tlc. The tetrahydropyranyl group was removed by adding 4 ml of a solution of dilute HCl in methanol (0.08 ml of 6 *N* HCl in 100 ml of methanol) to 18 mg of III. It dissolved on shaking, the solution was kept for 2 hr at room temperature, a few drops of sodium bicarbonate solution were added, and the methanol was evaporated in a stream of N_2. Water was added to the residue and the insoluble material was separated, washed with water, dried, and recrystallized from ethyl acetate to give II: mp 241–244°; uv max 214 mμ (ε 18,600) and 232 (inflection, 15,000); ir 3425, 1727, and 1656 cm^{-1}; nmr δ 0.71 (H-18), 1.00 (broad peak, H-21[14]), 1.21 (H-19), 1.47 (H-26 and -27), 5.71 (br s, H-6), and 5.90 (s, H-28).

Anal. Calcd for $C_{29}H_{42}O_5$ (mol wt, 470.63): C, 74.01; H, 9.00; O, 17.00. Found: C, 74.28; H, 9.01; O, 17.14.

When the tetrahydropyranyl ether of II was allowed to stand with the dilute hydrochloric acid and methanol overnight, extensive elimination of the 3β substituent occurred and the Δ³,⁵-7 ketone was isolated: mp 237–240°; uv max 210 mμ (ε 14,900) and 278 (22,200); ir 3410, 1754, 1645, and 1623 cm^{-1}.

Registry No.—II, 22336-99-8; tetrahydropyranyl ether of II, 22287-16-7; III, 22287-17-8; IV, 10211-88-8; V (R_1 = R_2 = H), 4182-41-6; V (R_1 = R_2 = Br), 22319-54-6; V (R_1 = H; R_2 = Br), 22287-47-4; VI, 22287-19-0.

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(14) This broad peak is probably a result of virtual coupling. For an explanation of this effect, see N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 36.