Stereoselective Synthesis of 23-Deoxyantheridiol[1]

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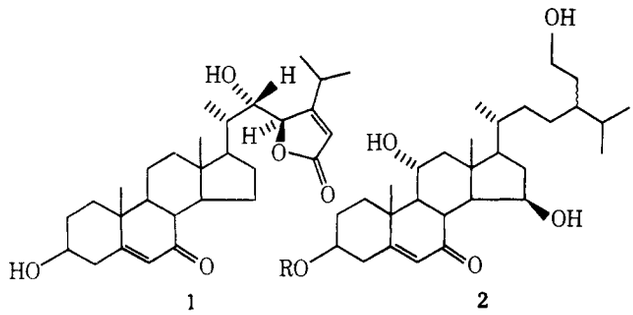
A highly stereoselective synthesis of (22*R*)-3β,22-dihydroxy-7-oxostigmasta-5,24(28)-dien-29-oic acid δ-lactone (23-deoxyantheridiol), a steroid produced by the aquatic fungus *Achlya*, has been achieved. Reaction of readily prepared 3β-acetoxycholesta-5,22(*E*)-dien-24-one with alkaline hydrogen peroxide gave almost exclusively the (22*S*,23*R*)-epoxide, which was converted to the (22*R*)-hydroxy-24-ketone with aluminum amalgam. The lactone ring was constructed by a novel intramolecular Wittig–Horner reaction. Esterification of the hydroxyl with bromoacetyl bromide followed by reaction with triethyl phosphite gave the phosphonate. Treatment of the phosphonate with sodium hydride yielded the unsaturated δ-lactone. The 7-ketone was introduced by photooxygenation followed by treatment with cupric acetate. 23-Deoxyantheridiol showed weak biological activity in *Achlya*.

The sexual reproductive process in *Achlya*, a widely distributed genus of saprophytic aquatic fungi, is initiated and coordinated by the steroid hormones antheridiol (**1**) and the oogoniols (**2**; R = H, $(CH_3)_2CHCO$, CH_3CH_2CO, or CH_3CO).[2,3]



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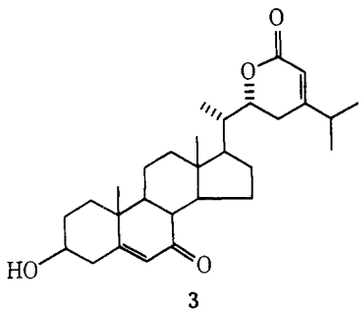

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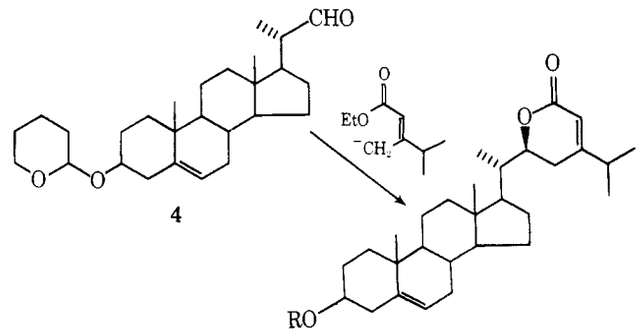
Antheridiol, which is secreted into the surrounding water by vegetative hyphae of female strains of the fungus, induces the formation of many antheridial branches or male sex organs on hyphae of male strains. The male is also stimulated to secrete the oogoniols which act on the female, causing the formation of oogonia or female sex organs. Developing oogonia are believed to secrete greater amounts of antheridiol than vegetative hyphae, and this results in chemotropic growth of the antheridial branches to the oogonia in order for conjugation of the sex organs to occur.[4]

Culture liquids of several female strains of *Achlya* have been found to contain antheridiol as well as the closely related steroid 23-deoxyantheridiol (**3**).[5] The structure of the latter steroid was confirmed by synthesis of its C-22 epimer by Green and Edwards some time ago.[5]

23-Deoxyantheridiol was slightly active in the induction of antheridial branches, but the activity was thought to be caused by traces of antheridiol. The two steroids possess similar mobilities on thin-layer chromatography, which was used for their isolation, so it was not possible to achieve complete separation of **1** from **3**. The synthetic C-22 epimer was found to be biologically inactive, but since correct stereochemistry at C-22 in antheridiol is important for full biological activity,[6] it seemed desirable to develop a synthetic route to **3** itself. We would then have sufficient amounts of pure natural product to make an accurate evaluation of its biological properties.

The synthetic method used by Green and Edwards involved aldol condensation of the tetrahydropyranyloxy derivative of 22,23-dinorchol-5-en-24-al (**4**) with the anion of ethyl *trans*-3,4-dimethyl-2-pentenoate, which gave the unsaturated lactone **5a**. Photooxygenation of **5a** followed by treatment with cupric acetate gave the corresponding 7-ketone **6**. Com-



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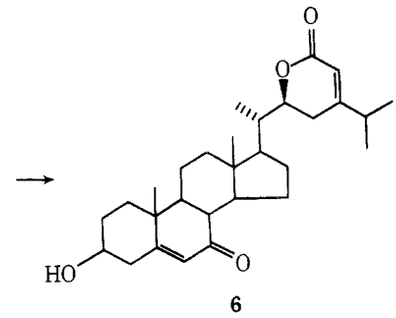
5a, R = H
b, R = Ac



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parison of the spectral properties of the synthetic product with those of **3** showed that the two compounds were isomeric at C-22. Thus, aldol condensation with the aldehyde **4** gave almost exclusively a product having the 22*S* configuration. The stereochemical course of the reaction is similar to that reported for condensations involving related aldehydes, for example, in the synthesis of α-ecdysone[7] and antheridiol.[8]

It was therefore necessary to use a different approach to introduce an oxygen function at C-22 with the desired configuration. Sucrow and co-workers have demonstrated that epoxidation of 3β-acetoxy-27-nor-5α-cholesta-7,23(*E*)-dien-24-one with alkaline hydrogen peroxide is highly stereoselective. They were able to isolate in 85% yield the (22*S*,23*R*)-epoxide.[9] A similar result was reported by Popplestone and Unrau, who converted 3β-acetoxycholesta-5,22(*E*)-dien-24-one (**7**) to the (22*S*,23*R*)-epoxide **8** by reaction with alkaline hydrogen peroxide.[10] In the former case the configuration at C-22 was established by reduction with hy-

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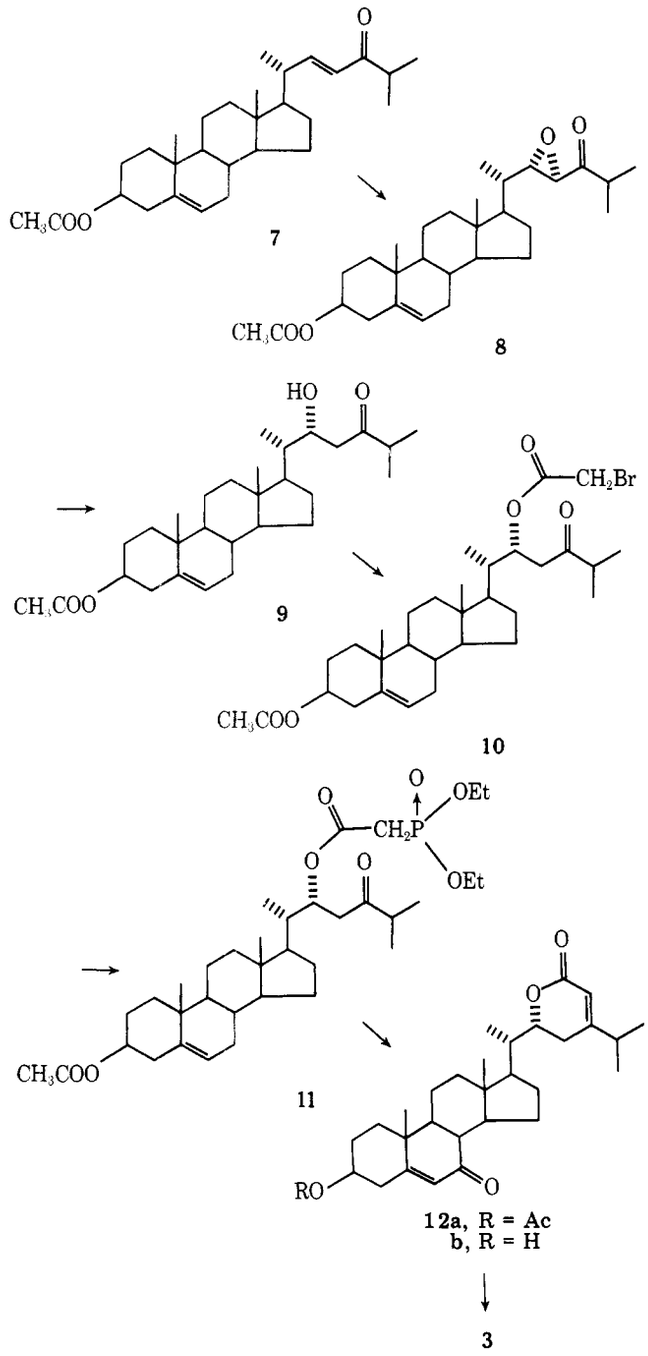

drazine to give (22*R*)-3β-acetoxy-27-nor-5α-cholesta-7,23(*E*)-dien-22-ol, which was then subjected to a Horeau analysis. The remarkable stereoselectivity of the epoxidation reaction may be due to a favored conformation of the side chain which results in one side of the double bond being less hindered than the other and therefore more susceptible to attack by hydroperoxide anion. However, examination of Dreiding models does not reveal any preferred conformation which would explain the observed stereoselectivity. It is interesting to note that the same stereoselectivity has been reported for the reaction of (22*E*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-one with dimethyloxosulfonium methylide, which gave exclusively, and in high yield, the (22*S*,23*S*)-cyclopropyl ketone.[11] Reductive cleavage of the epoxide **8** would be expected to yield the hydroxy ketone **9** possessing the appropriate functionality for constructing a lactone ring with the same stereochemistry as that in **3.**

Wittig reaction of 3β-acetoxy-22,23-dinorchol-5-en-24-al[12] with the phosphorane prepared from 1-bromo-3-methylbutan-2-one, itself conveniently obtained by direct bromination of the corresponding ketone,[13] furnished the α,β-unsaturated ketone **7** in 87% yield. The latter was smoothly epoxidized with 30% hydrogen peroxide and dilute sodium hydroxide to give, after reacetylation of the product, a high yield (94%) of α,β-epoxy ketone. The NMR spectrum indicated that it was actually a mixture of **8** and the isomeric epoxide in a ratio of approximately 95:5. Attempts at reductive opening of the epoxide ring with chromium(II) acetate[14] gave some hydroxy ketone but also products from elimination (i.e., **7**) and possibly retro-aldol cleavage. However, reduction of **8** dissolved in ethanol–ether with aluminum amalgam gave an 86% yield of the desired hydroxy ketone **9.**[15]

We proposed to construct an unsaturated lactone ring on the side chain of **9** by reaction with the anion of triethyl phosphonoacetate. In another investigation it had been found that 1-hydroxy-2-methylpentan-3-one reacted readily with the anion of triethyl phosphonoacetate to give 4-ethyl-5-methyloxacyclohex-3-en-2-one in moderate yield.[16] However, when the condensation was attempted with **9,** an extensive retro-aldol reaction occurred giving back the starting aldehyde which then reacted with phosphonoacetate anion.

The C-22 hydroxyl in **9** was therefore protected by esterification with bromoacetyl bromide and pyridine, and the bromo ester **10** was heated with triethyl phosphite to give the phosphonate **11** in an overall yield of 75%. Intramolecular condensation occurred readily when sodium hydride was added to a solution of **11** in tetrahydrofuran and the mixture heated. A good yield (86%) of the unsaturated δ-lactone **12a** was thus obtained. Attempts were also made to obtain **12a** from the triphenylphosphonium salt instead of the phosphonate, but the Wittig reaction failed, possibly for steric reasons. Reformatsky reaction of the bromo ester **10** also failed to yield any condensation product. Although the intramolecular Wittig–Horner reaction has been used for the construction of butenolides,[17] to our knowledge this is the first successful application to the preparation of unsaturated δ-lactones such as **12a.**

Completion of the synthesis involved hydrolysis of the acetate group of **12a** with dilute potassium carbonate solution in methanol to give the corresponding alcohol **12b.** Photooxygenation of the latter in pyridine solution with hematoporphyrin as a sensitizer afforded the 5α-hydroperoxide, which was converted to **3** by treatment with cupric acetate in pyridine. The overall yield for the synthesis starting from a readily available aldehyde, 3β-acetoxy-22,23-dinorchol-5-en-24-al, was about 30%.

The properties of synthetic 23-deoxyantheridiol were the same as those which have been reported for the natural product. Further confirmation of the structure was obtained by comparing the synthetic compound with its C-22 epimer prepared according to the method of Green and Edwards.[5] The NMR spectra of the two compounds show distinct differences as reported earlier. The H-22 signal in the spectrum of **3** appears as a doublet of triplets centered at δ 4.38 (J = 12 and 4.5 Hz), while that in the epimer occurs as a doublet of doublets at δ 4.40 (J = 13 and 4 Hz). The doublet for H-21 is fully visible (at δ 1.05) in the spectrum of the epimer **6,** but only the high-field arm of the doublet for H-21 (centered at δ 1.03) is observed in the spectrum of **3.** The low-field arm is hidden by one of the isopropyl hydrogen signals at δ 1.06.

Measurement of the CD curves indicated the difference in stereochemistry of the lactone rings in **5b** and **12a,** the former giving a negative Cotton effect at 254 nm and the latter a positive Cotton effect at 251 nm.

Fourier transform ^{13}C NMR spectra of steroids belonging to the natural and unnatural stereochemical groups were also recorded. It was possible to make definite spectral assignments for virtually all of the carbons in each of the compounds by the use of off-resonance decoupled spectra and by comparison

Table I. ^{13}C NMR Chemical Shifts (ppm Relative to Me_4Si) in 23-Deoxyantheridiol (3) and a Related Series of Steroids[a]

carbon	12a	12b	3	5b	5a	6
1	37.0	37.3	36.4	37.0	37.3	36.4
2	27.8	31.6	31.1	27.8	31.6	31.1
3	73.9	71.7	70.3	73.9	71.6	70.4
4	38.1	42.3	41.9	38.1	42.2	41.8
5	139.8	140.9	165.8	139.7	140.9	165.5
6	122.4	121.4	125.7	122.5	121.4	126.1
7	31.9	31.9	201.6	32.0[b]	32.0[b]	201.7
8	31.9	31.9	45.4	31.9[b]	31.8[b]	45.4
9	50.1	50.2	49.6[b]	49.9	50.0	49.8[b]
10	36.6	36.5	38.4	36.6	36.5	38.2
11	21.0	21.1	21.2	21.0	21.1	21.3
12	39.7	39.8	38.7	39.7	39.6	38.6
13	42.9	42.9	43.5	42.3	42.2	43.1
14	56.3	56.4	49.9[b]	56.4	56.4	50.0[b]
15	24.3	24.3	26.3	24.2	24.2	26.2
16	27.4	27.4	27.7	27.8	27.7	28.2
17	52.3	52.3	51.1	51.4	51.4	50.2[b]
18	11.8	11.8	11.9	11.8	11.7	11.8
19	19.3	19.4	17.3	19.3	19.4	17.4
20	38.9	38.9	38.7	39.7	39.6	39.8
21	13.4	13.5	13.6	13.3	13.2	13.4
22	79.9	79.9	79.9	79.6	79.6	79.6
23	25.2	25.2	25.1	29.6	29.6	29.7
24	166.6	166.3	166.8	166.8	167.0	166.9
25	34.8	34.8	34.8	34.7	34.7	34.7
26	19.9	19.9	19.9	19.9	19.8	19.9
27	20.4	20.4	20.4	20.4	20.4	20.4
28	113.6	113.5	113.4	113.5	113.4	113.4
29	166.2	166.2	166.3	166.2	166.4	166.3
CH_3 (acetate)	21.4			21.4		
C=O (acetate)	170.4			170.4		

[a] ^{13}C NMR spectra were recorded at room temperature in $CDCl_3$. The concentrations of the solutions ranged from 0.05 to 0.2 M. Spectral parameters were the following: acquisition time, 1 s; pulse delay, 0.3 s; number of transients, 8–60 K; data points, ~8000. [b] Assignments may be reversed in vertical column.

with literature data.[18] The assignments are given in Table I. They indicate that the carbons most affected by a change in stereochemistry at C-22 are C-23 and to a lesser extent C-20 and C-17. These results are in accord with expectation.[19]

Synthetic 23-deoxyantheridiol has been tested for biological activity and found to be weakly active. The lowest concentration of **3** which induced significant antheridial branch formation was approximately 50 ng/mL. This is more than one thousand times greater than the minimum concentration of antheridiol required for biological activity. Thus, it appears that 23-deoxyantheridiol is not needed by the organism, at least not for the induction of antheridial hyphae. At present one can only speculate about the function of **3.** It could possibly be a metabolite or, perhaps more likely, a biosynthetic precursor of antheridiol. Its structure indicates that it might be formed from the trienoic acid **13,** which has been shown to be a precursor of antheridiol.[20] Ring closure in the trienoic acid would give the δ-lactone in **3,** and oxidation at C-7 would complete its biosynthesis. If reopening of the lactone ring can occur in the cell, then **3** could be a potential precursor of antheridiol. There is also a possibility that **3** may be involved in some other stage of the sexual process in *Achlya.* We plan to investigate these possibilities.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Spectra were obtained on the following instruments: Varian EM 390 (^1H NMR), Varian CFT 20 (^{13}C NMR), Perkin-Elmer 550 spectrophotometer (UV), Beckman IR 18A-X (IR), LKB 9000 (mass spectra), and a Cary 61 circular dichrometer coupled with a Texas Instruments 980A computer. NMR results are reported in parts per million (or δ) using Me_4Si as an internal standard (δ = 0). The ionizing voltage for mass spectra was 70 eV. Infrared spectra were taken of KBr pellets unless otherwise specified. Elemental analyses were performed by Pascher Laboratories, Bonn, W. Germany.

Isobutyrylmethylenetriphenylphosphorane.[21] Method A. To a stirred suspension of methyltriphenylphosphonium iodide (16.15 g, 40 mmol) in dry ether (250 mL) under argon was added a solution of phenyllithium in benzene–ether (17 mL, 2.4 M). The resulting yellow-orange mixture was stirred for 2 h and cooled in an ice bath. To it was added a solution of isobutyryl bromide (2.1 mL) in dry ether (50 mL) during 15 min. After stirring overnight, the mixture was filtered through a sintered glass funnel. The residue was mixed thoroughly with chloroform and the insoluble material discarded. The chloroform solution was washed with 5% HCl and 5% NaOH (3 × 40 mL), dried ($MgSO_4$), and evaporated to yield a yellow oil. NMR analysis showed this oil to be a mixture of the desired phosphorane and a methyltriphenylphosphonium halide. The oil was vigorously stirred with three portions of hot benzene to separate the soluble phosphorane and the insoluble phosphonium salt. The benzene solutions were decanted, combined, and evaporated to yield the crystalline phosphorane (4.90 g, 72%). The original ether layer yielded additional product if dilute HCl (1–2 mL) was added and the mixture was shaken for 5 min and then filtered to isolate the salt of the phosphorane. This could be converted to the phosphorane by dissolving in chloroform, shaking with dilute NaOH (excess), and removing the chloroform. Crystallization from ethyl acetate gave pure phosphorane: mp 172–175 °C; NMR ($CDCl_3$) δ 1.15 (d, J = 7 Hz, $(CH_3)_2C$), 2.50 (m, J = 7 Hz, CH), 3.66 (broad d, J = 27 Hz, P=CH), 7.5 (m, 15 aromatic H).

Method B. Bromine (12.8 mL) was added in one portion to a stirred solution of methyl isopropyl ketone (21.5 g) in dry methanol (250 mL). After the solution became colorless (ca. 20 min), $NaHCO_3$ (21 g) in H_2O (250 mL) was added carefully and the mixture was stirred until most of the bubbling had stopped. The solution was extracted with hexane (3 × 170 mL), and the hexane extracts were combined, washed with dilute $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated in a stream of nitrogen. The resulting liquid was distilled to yield a fraction (17.1 g) boiling at 72–82 °C (25 mm) that was shown by NMR analysis to be a mixture of 1-bromo-3-methylbutan-2-one (93%) and 3-bromo-3-methylbutan-2-one (7%).[13] The mixture of bromo ketones (17.1 g) in dry benzene (75 mL) was added to a solution of triphenylphosphine (29.0 g) in a minimum of dry benzene. After standing for 17 h, the mixture was filtered and the white crystals were washed several times with hot benzene. This phosphonium salt [31.2 g; NMR ($CDCl_3$) δ 1.11 (d, J = 7.5 Hz, $(CH_3)_2C$), 3.26 (m, J = 7.5 Hz, CH), 5.90 (d, J = 12 Hz, P–CH_2), 7.8 (m, 15 aromatic H)] was dissolved in chloroform, and the solution was shaken well with 10% NaOH (75 mL). The chloroform layer was separated, washed with H_2O, dried ($MgSO_4$), and evaporated to yield 24.26 g (30% overall yield from methyl isopropyl ketone) of crystalline phosphorane.

3β-Acetoxycholesta-5,22(*E*)-dien-24-one (7). This compound was prepared from the condensation of 3β-acetoxy-22,23-dinorcholenaldehyde[12] and isobutyrylmethylenetriphenylphosphorane in 87% yield according to the procedure of Fryberg et al.:[21] mp 138–140 °C; NMR ($CDCl_3$) δ 0.72 (s, 3, H-18), 1.02 (s, 3, H-19), 1.10 (d, 9, J = 7 Hz, H-21, H-26, and H-27), 2.02 (s, 3, OAc), 2.80 (m, 1, J = 7 Hz, H-25), 4.6 (broad m, 1, H-3), 5.36 (m, 1, H-6), 6.03 (d, 1, J = 16 Hz, H-23), 6.70 (d of d, 1, J = 8.5 and 16 Hz, H-22); IR 2940, 1740, 1700, 1630, 1250, 1040 cm^{-1}.

(22*S*,23*R*)-3β-Acetoxy-24-oxocholest-5-ene 22,23-Epoxide (8). To a stirred warm solution (35 °C water bath) of unsaturated ketone **7** (253 mg) in absolute ethanol (16 mL) was added quickly 30% hydrogen peroxide (1.05 mL) and 4 N NaOH (0.55 mL). The warm water bath was removed after 1 h and the mixture stirred for an additional hour. The solvent was partially removed (~40%) in a nitrogen stream, and the mixture was extracted twice with ether. The ether extracts were combined, washed with water and brine, dried ($MgSO_4$), and evaporated to yield a clear oil. NMR analysis of the crude product showed it to be a mixture of **8** and the corresponding 3β-alcohol. Acetylation of the mixture with acetic anhydride and pyridine yielded the epoxide **8** (0.24 g, 94%): mp 125–126 °C; NMR ($CDCl_3$) δ 0.68 (s, 3, H-18), 1.01 (s, 3, H-19), 1.03, 1.06, 1.11, 1.14 (H-21, H-26, and H-27), 2.01 (s, 3, OAc), 2.80 (m, 1, H-22), 3.25 (d, 0.95, J = 2 Hz, H-23, (22*S*,23*R*)-epoxide), 3.36 (d, 0.05, J = 2 Hz, H-23, (22*R*,23*S*)-epoxide), 4.60 (broad m, 1, H-3), 5.36 (m, 1, H-6); IR (CH_2Cl_2) 1730 cm^{-1}.

(22*R*)-3β-Acetoxy-24-oxocholest-5-en-22-ol (9). To the epoxy ketone **8** (2.95 g) dissolved in dry ether (125 mL) and absolute ethanol (60 mL) was added Al(Hg) (made from 4.6 g of Al foil)[22] and 5 drops

of H_2O. The mixture was stirred vigorously for 4 h and suction filtered, and the residue was washed with ether. The combined ether filtrates were washed with water and brine and dried ($MgSO_4$), and the solvent was evaporated to yield a crystalline solid (2.84 g). Recrystallization from methanol afforded **9** as white plates (2.55 g, 86%): mp 142–144 °C; NMR ($CDCl_3$) δ 0.73 (s, 3, H-18), 0.93 (d, 3, J = 7 Hz, H-21), 1.03 (s, 3, 19-H), 1.12 (d, 6, J = 7 Hz, H-26 and H-27), 2.00 (s, 3, OAc), 4.06 (m, 1, 22-H), 4.56 (broad m, 1, H-3), 5.30 (m, 1, H-6); IR 3530, 2980, 1742, 1700, 1255, 1045 cm^{-1}; MS m/e 398 (27, M^+ − HOAc), 380 (17), 312 (100).

(22*R*)-3β-Acetoxy-22-(bromoacetoxy)cholest-5-en-24-one (10). The ketol **9** (740 mg) was dissolved in dry ether (30 mL) and dry pyridine (162 mg). This stirred solution was cooled in an ice bath, and bromoacetyl bromide (400 mg) in ether (3 mL) was added dropwise during 5 min. A white precipitate formed during the addition. The ice bath was removed after 10 min and the mixture stirred for 12 h. Thin-layer chromatography showed some starting material still present, so more pyridine (0.25 mL) was added. After stirring 3 h more, the mixture was suction filtered and the residue washed with ether. The combined ether filtrates were washed twice with 10% HCl, H_2O, and brine, dried ($MgSO_4$), and evaporated to yield a crystalline solid (875 mg). Recrystallization from methanol afforded **10** (800 mg, 86%) as white plates. A second recrystallization from methanol gave pure **10:** mp 154–156 °C; NMR ($CDCl_3$) δ 0.70 (s, 3, H-18), 0.97 (d, 3, J = 7 Hz, H-21; only high-field arm visible), 1.00 (s, 3, H-19), 1.08 (d, 6, J = 7 Hz, H-26 and H-27), 2.00 (s, 3, OAc), 3.75 (s, 2, C(=O)CH_2Br), 4.6 (broad m, 1, H-3), 5.36 (m, 1, H-6), 5.43 (m, 1, H-22); IR 2950, 1760, 1740, 1720, 1295, 1257 cm^{-1}; MS m/e 520 (0.8, M^+ − HOAc), 518, 379 (1.3, M^+ − HOAc − $HOOCCH_2Br$), 43 (100).

(22*R*)-3β-Acetoxy-22-(diethylphosphonoacetoxy)cholest-5-en-24-one (11). The bromoacetate **10** (1.09 g) was heated with excess triethyl phosphite (4 mL) in an oil bath (130 °C) for 3 h. A stream of nitrogen was used to blow off the excess triethyl phosphite, leaving a clear oil which crystallized on standing. Recrystallization from hexane yielded **11** (1.05 g, 88%) as white needles: mp 117.5–119 °C; NMR ($CDCl_3$) δ 0.70 (s, 3, H-18), 0.96 (d, 3, J = 7 Hz, H-21; only high-field arm visible), 1.02 (s, 3, H-19), 1.08 (d, J = 7 Hz, H-26 and H-27), 1.33 (t, 6, J = 7.5 Hz, P(OC–CH_3)$_2$), 2.02 (s, 3, OAc), 2.89 (d, 2, J = 21 Hz, CH_2–P), 4.16 (m, 4, J = 7.5 Hz, P(O–CH_2C)$_2$), 4.6 (broad m, 1, H-3), 5.36 (m, 1, H-6), 5.43 (m, 1, H-22); IR 2940, 1735, 1720, 1245, 1025 cm^{-1}; MS m/e 576 (1.2, M^+ − HOAc), 440 (1.4, M^+ − $C_6H_{13}O_5P$), 380 (100, M^+ − HOAc − $C_6H_{13}O_5P$).

(22*R*)-3β-Acetoxy-22-hydroxystigmasta-5,24(28)-dien-29-oic Acid δ-Lactone (12a). To a stirred solution of phosphonate **11** (507 mg) in dry tetrahydrofuran (25 mL) was added 57% NaH (34 mg). After stirring for 5 min at room temperature and 1 h at reflux, the solution was cooled and diluted with H_2O, and the solvent volume was reduced in a stream of nitrogen. The resulting suspension was extracted with ether. The ether extract was washed twice with water and brine, dried ($MgSO_4$), and evaporated to leave a clear oil (405 mg). Chromatography on silica gel with dichloromethane–petroleum ether yielded **12a** (330 mg, 86%) and **7** (28 mg, 8%). Crystallization from hexane–methanol afforded **12a** as white needles: mp 163.5–165 °C; NMR ($CDCl_3$) δ 0.73 (s, 3, H-18), 1.02 (s, 3, H-19), 1.03 (d, 3, J = 6 Hz, H-21), 1.10 (d, 6, J = 7 Hz, H-26 and H-27), 2.02 (s, 3, OAc), 4.40 (d of t, 1, J = 12 and 4.5 Hz, H-22), 4.60 (broad m, 1, H-3), 5.39 (m, 1, H-6), 5.78 (broad s, 1, H-28); IR 2940, 1739, 1718, 1640, 1245 cm^{-1}; MS m/e 482 (0.3, M^+), 422 (27, M^+ − HOAc), 43 (100); CD (*c* ca. 0.0005 g/mL, CH_3OH) $[\theta]_{300}$ +160, $[\theta]_{290}$ +740, $[\theta]_{280}$ +3160, $[\theta]_{270}$ +9260, $[\theta]_{260}$ +18 390, $[\theta]_{251}$ max +23 090, $[\theta]_{250}$ +22 720, $[\theta]_{240}$ +12 960, $[\theta]_{230}$ +410, $[\theta]_{220}$ +6810.

(22*R*)-3β,22-Dihydroxystigmasta-5,24(28)-dien-29-oic Acid δ-Lactone (12b). To a stirred solution of **12a** (290 mg) in tetrahydrofuran (3 mL) and methanol (40 mL) was added a 10% K_2CO_3 solution (60% CH_3OH, 40% H_2O; 1 mL). After stirring for 23 h, 10% NH_4Cl (1.5 mL) and H_2O (30 mL) were added and the volume was reduced by ca. 50% in a stream of nitrogen. Filtration afforded **12b** (243 mg, 92%). Crystallization from ethyl acetate–chloroform yielded white needles: mp 260–263 °C; NMR ($CDCl_3$) δ 0.72 (s, 3, H-18), 1.00 (s, 3, H-19), 1.02 (d, 3, J = 6 Hz, H-21; only high-field arm visible), 1.10 (d, 6, J = 7 Hz, H-26 and H-27), 3.52 (broad m, 1, H-3), 4.37 (d of t, 1, J = 12 and 4.5 Hz, H-22), 5.35 (m, 1, H-6), 5.77 (broad s, 1, H-28); IR 3500, 2930, 1711, 1640, 1065 cm^{-1}; MS m/e 440 (21, M^+), 422 (32, M^+ − H_2O), 407 (19, M^+ − H_2O − CH_3), 139 (100).

(22*R*)-3β,22-Dihydroxy-7-oxostigmasta-5,24(28)-dien-29-oic Acid δ-Lactone (3). A stirred solution of **12b** (137 mg) in dry pyridine (14 mL) containing hematoporphyrin (15 mg) was irradiated with four 15-W fluorescent lamps for 39 h, during which time oxygen was bubbled through the solution. The reaction mixture was diluted with ether, treated with charcoal, and filtered through Celite, and the ether was blown off. $Cu(OAc)_2$·H_2O (115 mg) was added to the pyridine solution, and the mixture was stirred for 8 h. After dilution with ethyl acetate, the solution was washed with dilute phosphoric acid, dilute $NaHCO_3$, and H_2O, dried ($MgSO_4$), and evaporated. Chromatography of the crude product on silica gel with dichloromethane–methanol yielded **3** (100 mg, 70%), mp 260–264 °C. An analytical sample was prepared by preparative LC (Waters Model M-6000A; μ Porasil; hexane–dichloromethane–methanol, 30:10:1; 4.2 mL/min; 5000 psi; followed by crystallization from ethyl acetate): mp 268–270 °C; NMR ($CDCl_3$) δ 0.72 (s, 3, H-18), 1.03 (d, 3, J = 6 Hz, H-21), 1.10 (d, 6, J = 7 Hz, H-26 and H-27), 1.20 (s, 3, H-19), 3.6 (broad m, 1, H-3), 4.38 (d of t, 1, J = 12 and 4.5 Hz, H-22), 5.67 (s, 1, H-6), 5.73 (broad s, 1, H-28); IR 3470, 2940, 1705, 1675, 1635 cm^{-1}; MS m/e 454 (90, M^+), 436 (34, M^+ − H_2O), 316 (66, M^+ + H-lactone), 245 (100, $C_{16}H_{21}O_2$); UV λ_{max} (CH_3OH) 226 nm (ε 18 800); CD (*c* ca. 0.0006 g/mL, CH_3OH) $[\theta]_{300}$ +0, $[\theta]_{290}$ +970, $[\theta]_{280}$ +5700, $[\theta]_{270}$ +12 000, $[\theta]_{260}$ +19 000, $[\theta]_{256}$ max +21 900, $[\theta]_{250}$ +17 500, $[\theta]_{240}$ −670, $[\theta]_{230}$ −34 000, $[\theta]_{220}$ −65 300.

Anal. Calcd for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 76.30; H, 9.20.

(22*S*)-3β,22-Dihydroxystigmasta-5,24(28)-dien-29-oic Acid δ-Lactone (5a). This compound was prepared essentially by the procedure of Green et al.[5] except for a few modifications. 3β-Acetoxy-22,23-dinorcholenaldehyde was used rather than **4.** Workup and column chromatography gave **5a** directly (35%) and **5b** (48%). **5b** could be converted to **5a** following the procedure previously described for the 22*R* isomer. **5a** had the following properties: mp 220–222 °C; NMR ($CDCl_3$) δ 0.70 (s, 3, H-18), 1.00 (s, 3, H-19), 1.10 (d, 6, J = 7 Hz, H-26 and H-27), 3.53 (broad m, 1, H-3), 4.40 (d of d, 1, J = 13.5 and 3 Hz, H-22), 5.33 (m, 1, H-6), 5.73 (broad s, 1, H-28); IR 3450, 2945, 1721, 1645, 1265 cm^{-1}; MS m/e 440 (14, M^+), 422 (27, M^+ − H_2O), 407 (11, M^+ − H_2O − CH_3), 139 (100).

5b had the following properties: mp 210–212 °C; NMR ($CDCl_3$) δ 0.70 (s, 3, H-18), 1.00 (s, 3, H-19), 1.07 (d, 6, J = 7 Hz, H-26 and H-27), 2.01 (s, 3, OAc), 4.40 (d of d, 1, J = 13.5 and 3 Hz, H-22), 4.60 (broad m, 1, H-3), 5.35 (m, 1, H-6), 5.75 (broad s, 1, H-28); IR 2940, 1734, 1721, 1645, 1260 cm^{-1}; MS m/e 482 (0.4, M^+), 422 (100, M^+ − HOAc); CD (*c* ca. 0.0006 g/mL, CH_3OH) $[\theta]_{300}$ −290, $[\theta]_{290}$ −8700, $[\theta]_{280}$ −12 100, $[\theta]_{270}$ −16 200, $[\theta]_{260}$ −25 400, $[\theta]_{254}$ max −28 500, $[\theta]_{250}$ −25 400, $[\theta]_{240}$ −6700, $[\theta]_{230}$ +11 200, $[\theta]_{220}$ +2500.

5b and its C-22 epimer, **12a,** were found to have quite different mobilities when subjected to LC (Waters Model M-6000A, μ Porasil; hexane–dichloromethane–methanol, 30:10:1; 4.2 mL/min; 5000 psi). **12a** was eluted with a retention time of 4.9 min, while **5b** was eluted with a retention time of 5.6 min. On coinjection baseline separation was evident.

A sample of **12a** prepared from recrystallized bromoacetate **10** and phosphonate **11** showed no **5b** (<1%) on LC. But in a sample of **12a** prepared without recrystallizing **10** and **11,** a small amount of **5b** (~5%) could be detected.

A sample of **5b** prepared by the method of Green et al.[5] showed a peak with the same retention time as **12a** (~2%) on LC.

Biological assays were carried out according to the method of Barksdale et al.[6]

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Registry No.—3, 32212-69-4; **5a,** 32212-70-7; **5b,** 67237-33-6; **6,** 32212-71-8; **7,** 32230-64-1; **8,** 42261-08-5; **9,** 67237-34-7; **10,** 67237-35-8; **11,** 67237-36-9; **12a,** 67237-30-3; **12b,** 67237-31-4; isobutyrylmethylenetriphenylphosphorane, 19753-67-4; methyltriphenylphosphonium iodide, 2065-66-9; isobutyryl bromide, 2736-37-0; methyl isopropyl ketone, 563-80-4; 1-bromo-3-methylbutan-2-one, 19967-55-6; 3-bromo-3-methylbutan-2-one, 2648-71-7; triphenylphosphine, 603-35-0; 3β-acetoxy-22,23-dinorcholenaldehyde, 10211-88-8; bromoacetyl bromide, 598-21-0; triethyl phosphite, 122-52-1.

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Studies on the Synthesis of Cardiotonic Steroids. 4.[1] Synthesis of Strophanthidin

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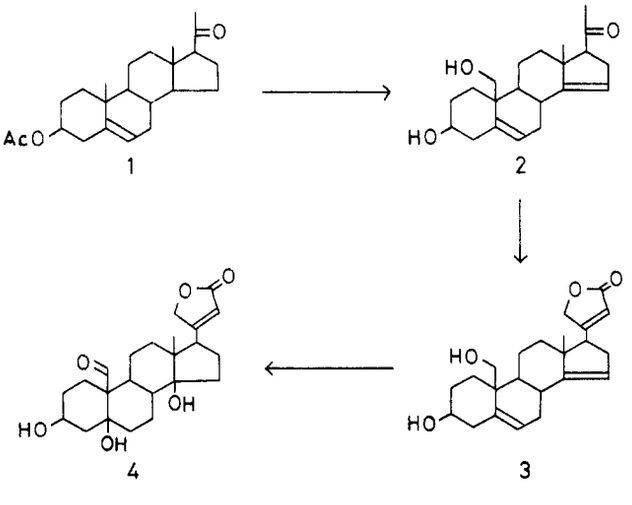
The synthesis of strophanthidin (4) starting with pregnenolone acetate (1) is described. 19-Hydroxylation and introduction of a 14 double bond afforded 9, which was then transformed into the cardatrienolide 13. By stepwise introduction of 5β- and 14β-hydroxy groups, strophanthidol (22) was obtained. Conversion of strophanthidol to strophanthidin was successfully carried out by the oxidation with chromic trioxide in hexamethylphosphoric triamide.

Since Sondheimer's first synthesis of digitoxigenin in 1962,[2] there have been recorded the syntheses of several other natural cardenolides—periplogenin,[3a,4] xysmalogenin,[3b] uzarigenin,[3c,4] and canarigenin.[3d,4] However, the synthesis of more complex 19-oxygenated cardenolides represented by strophanthidin (4)[5] has not been accomplished, seemingly due to the difficulty in assembling unstable functionalities on the steroid nucleus.[6] We now describe the synthesis of strophanthidin, starting with readily available pregnenolone acetate (1).

Our synthetic approach to strophanthidin involved the following principal phases of conversion (Scheme I): (1) derivation of a 19-hydroxy group and a 14 double bond from 1 leading to the dihydroxydienone 2, (2) transformation of 2 to the cardatrienolide 3 without affecting functional groups in the steroid skeleton, (3) formation of two tertiary β-hydroxy groups at the 5 and 14 positions, followed by selective oxidation of the 19-hydroxymethyl moiety to an aldehyde group.

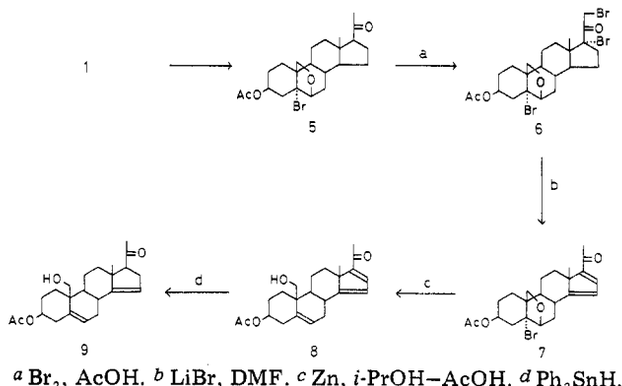
Scheme I



Preparation of the 3-acetate of the dihydroxy ketone 2 is outlined in Chart I. 5-Bromo-6,19-oxidopregnenolone acetate (5) prepared from pregnenolone acetate (1) by an established method[7] was brominated with 2 equiv of bromine to give the 17,21-dibromo compound 6 in 63% yield. It was then subjected to lithium bromide catalyzed dehydrobromination in *N*,*N*-dimethylformamide[8] to furnish the conjugated dienone 7 in 70% yield. Treatment of 7 with zinc dust in weakly acidic 2-propanol at reflux temperature generated the 5 double bond,[7a] yielding the trienone 8 in 89% yield. Selective hydrogenation of the 16 double bond of 8 leading to the dienone 9 was accomplished in 82% yield by heating with triphenylstannane in toluene, a convenient method for the partial reduction of conjugated dienones developed in our laboratory.[9]

The next task—construction of the cardatrienolide structure 13—was then performed by the reaction sequence (Chart II) which had been developed during our digitoxigenin synthesis.[9] First, the 21-methylthio derivative 10 was obtained in 44% yield by the base-catalyzed reaction of 9 with diethyl oxalate followed by the reaction of the resulting 21-oxalyl derivative with methyl thiotosylate in the presence of excess

Chart I



[a] Br_2, AcOH. [b] LiBr, DMF. [c] Zn, *i*-PrOH–AcOH. [d] Ph_3SnH, PhMe.

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