Synthesis of Antheridiol and Some Observations on the Chemistry of Butenolides

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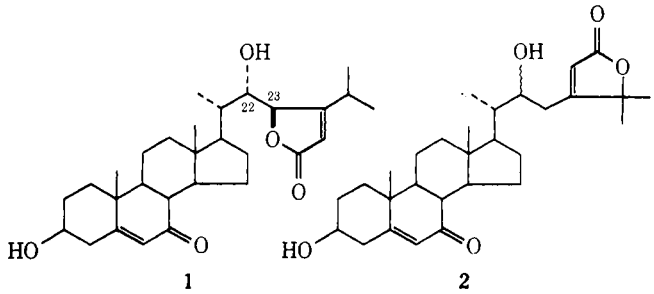
The fungal sex hormone, antheridiol, has been synthesized in an overall yield of ~20% by aldol condensation of 3β-acetoxy-22,23-bisnor-Δ⁵-cholenaldehyde and the carbanion of β-isopropylbut-2-enolide. This yielded the acetate of 7-deoxy-7-dihydroantheridiol (22*S*,23*R*) which was hydrolyzed and then subjected to photooxygenation and rearrangement to give antheridiol. The major product of the aldol condensation, the acetate of 7-deoxy-7-dihydroantheridiol (22*R*,23*S*), was converted to the desired isomer (22*S*,23*R*) by Jones oxidation followed by autoxidation and then borohydride reduction. The scope of the aldol condensation involving butenolides has been investigated.

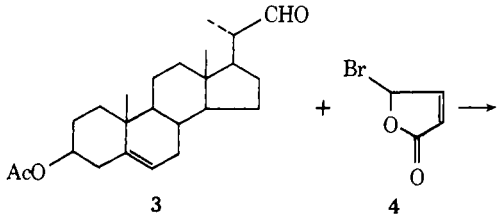
Antheridiol (**1**) is a hormonal substance which is secreted by female strains of the aquatic fungus, *Achlya*, and which acts on male strains causing the formation of antheridial hyphae, or male sex organs. Its isolation was reported in 1967[2] and the elucidation of its structure in 1968.[3] Shortly thereafter a synthesis was reported by workers at the Syntex Corp., Calif.[4] This paper gives details of work carried out at the New York Botanical Garden which has led to a practical synthesis of the hormone.[5]

Initially our objective was to synthesize the structure **1** without regard to the stereochemistry at C_{22} and C_{23}. The stereochemistry, which was unknown in 1968, has been determined mainly from synthetic experiments by the Syntex group. We planned to use a Reformatsky reaction to link a C_{22} aldehyde directly to a C_7 butenolide. A similar method had been used successfully to prepare the structure **2**, which at one time was suspected of being the structure of antheridiol itself.[6]



1 **2**

Model experiments were first carried out with 3β-acetoxy-22,23-bisnor-Δ⁵-cholenaldehyde (**3**) and the readily



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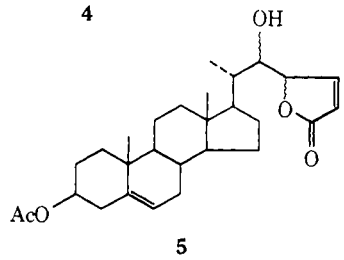
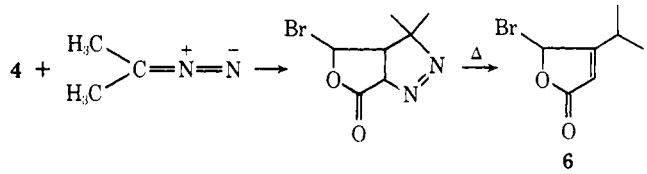


5

prepared bromobutenolide (**4**). When a solution of these compounds in benzene was heated with activated zinc dust, a very low yield (~5%) of a condensation product could be obtained. The crystalline product (mp 185–200°) had spectral properties consistent with structure **5**.

Thus the infrared spectrum had ν_{max} 3420 (hydroxyl), 1799, 1767, 1733 cm^{-1} (lactone and acetate), and the nmr spectrum showed, in particular, a doublet at δ 3.63 (J = 4.5 Hz) assigned to 22-H, a broad singlet at δ 5.9 assigned to 23-H and doublets at δ 6.2 (J = 6 Hz) and 7.2 (J = 6 Hz) assigned to 25-H and 24-H, respectively. The mass spectrum of **5** had the base peak at m/e 312 (M − CH_3COOH − 84) indicating ready cleavage at the C_{22}–C_{23} bond with transfer of one hydrogen to the lactone, similar to the case of antheridiol.

Encouraged by the success of the Reformatsky reaction, we proceeded to prepare γ-bromo-β-isopropylbut-2-enolide (**6**). This was accomplished by treating γ-bromobut-2-enolide (**4**) with an ethereal solution of 2-diazopropane.[7] An unstable pyrazoline was formed which on heating in xylene decomposed to give the desired compound in 35% yield. Two other methods of making **6** were tried but were unsuccessful. One method was allylic bromination of β-isopropylbut-2-enolide (**7**), which gave a monobromo derivative containing bromine exclusively in the side chain. The other involved acid-catalyzed pyrolysis of 2,5-diacetoxy-3-isopropyl-2,5-dihydrofuran. No 2-acetoxy-4-isopropylfuran could be isolated. Only isomer **8** was obtained and this, on bromination, gave γ-bromo-α-isopropylbut-2-enolide (**9**).[8]



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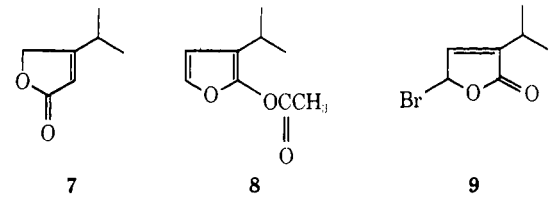
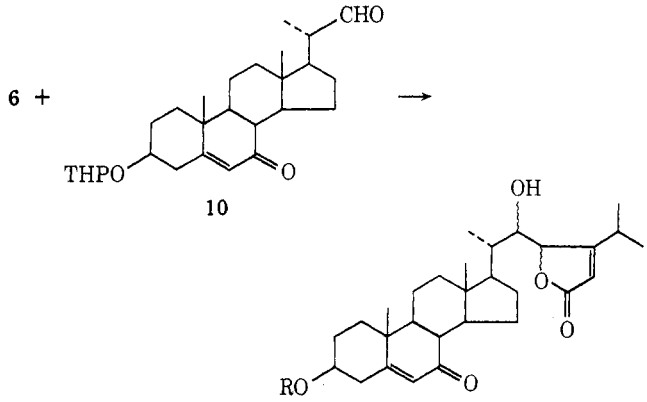


7 **8** **9**

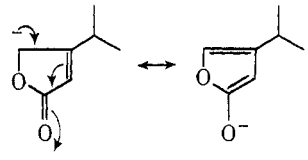
Reformatsky reaction of **6** and the aldehyde (**10**), which had been synthesized previously,[6] yielded a product which possessed approximately 1% of the biological activity of antheridiol. However, neither antheridiol nor any of its isomers could be isolated from acid hydrolysis of the product.

Other ways were therefore sought for condensing the butenolide with the aldehyde. An attempt was made to prepare the carbanion of **7**, which is the intermediate in the Reformatsky reaction above, by treatment of **7** with trityllithium. We believed the carbanion should form



11, R = THP
12, R = H

readily because the electron-withdrawing effect of the lactone oxygen would enhance the acidity of the allylic hydrogen on the adjacent carbon atom. Resonance stabilization of the anion would also favor its formation.

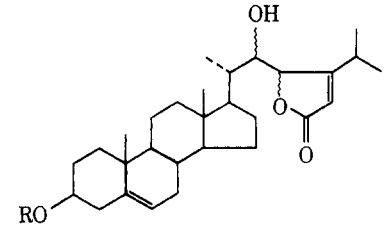


In the event, addition of a solution of **7** in tetrahydrofuran to a blood red solution of tritylllithium at −30° in an argon atmosphere resulted in rapid discharge of the color. The resulting pale yellow solution was immediately cooled to −70° and a solution of the aldehyde **10** in tetrahydrofuran added. The mixture was kept at −70° for 30 min; then the temperature was allowed to rise gradually to 0° during 30 min. The solution was then added to an excess of cold (0°) solution of dilute hydrochloric acid.

The product after chromatography (50% yield) exhibited biological activity about 10% that of antheridiol. The aldol reaction of **10** and **7** results in the creation of two new asymmetric centers (C_{22} and C_{23}), so that four stereoisomers were to be expected, provided no epimerization occurred at C_{20} in the reaction. In a similar condensation involving a C_{22} aldehyde, the Syntex workers found no evidence of epimerization at C_{20}.[4] Repeated chromatography resolved the product into two components, and these on gentle acid hydrolysis afforded mainly two isomers of antheridiol. Thus one of the hydrolyzed components had the same properties as those reported for the erythro isomer of antheridiol.[4] Recrystallization gave crystals with lower biological activity while the residue from the mother liquor had higher activity. However, no pure antheridiol could be obtained from this residue despite repeated recrystallization and chromatography. The erythro isomer and antheridiol (which also has the erythro configuration at C_{22},C_{23}) have the same R_f values in different solvent systems and could not be separated by chromatography. The other hydrolyzed component was later shown to be a mixture of threo isomers containing mainly the 22*R*,23*R* isomer.

Repetition of the aldol condensation of β-isopropylbut-2-enolide with 3β-acetoxy-22,23-bisnor-Δ⁵-cholenaldehyde yielded the product **13** (70%) and the corresponding diol **14** (5%).

The product was resolved by chromatography and fractional crystallization into the following components. The acetate of 7-deoxy-7-dihydroantheridiol (**13**, 22*S*,23*R*), mp 164–166°; the erythro isomer (**13**, 22*K*,23*S*), mp 219–223°; the threo isomer (**13**, 22*R*,23*R*), mp 202–208°; and the threo isomer (**13**, 22*S*,23*S*), mp 175–180°, 195–198°. These



13, R = Ac
14, R = H

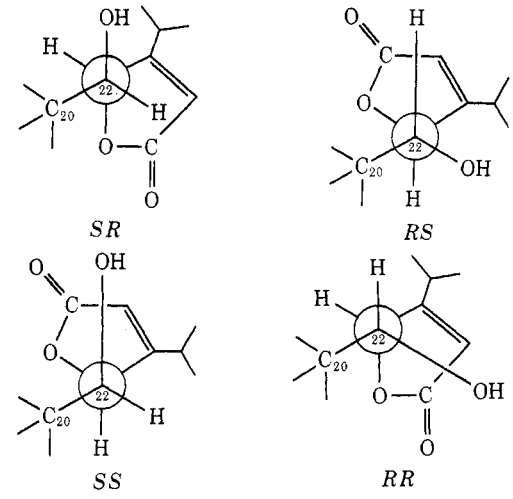
isomers were formed in a ratio of approximately 1:10:2:1 (see Experimental Section). The more polar fractions from the chromatography were a mixture of isomers of 7-deoxy-7-dihydroantheridiol, containing mainly the erythro isomer (**14**, 22*R*,23*S*). The acetates were cleanly resolved on thin layer chromatography (tlc) using ethyl acetate–petroleum ether or benzene–ether as solvents. The evidence for the assignment of stereochemistry to the four $C_{22}C_{23}$ stereoisomers is presented later. The mass spectra of the acetates were very similar. All showed the highest peak at *m/e* 438, which corresponds to loss of acetic acid from the molecular ion (M^+ 498). The most intense peaks occurred at *m/e* 312 and 126 and result from a McLafferty rearrangement involving cleavage of the C_{22}–C_{23} bond and transfer of a hydrogen to the lactone fragment. This cleavage is characteristic of the antheridiol side chain.

The infrared (ir) spectra of the acetates taken in KBr showed several differences from each other. The butenolide and acetate carbonyl absorptions were partly merged, the former appearing as a shoulder in the acetate peak in the *SR* and *SS* isomers, but as a distinct peak in the *RR* isomer. The carbonyl region in the *RS* isomer was more complex, four distinct peaks being observed: 1818 (w), 1767 (s), 1739 (m), and 1715 cm^{-1} (s).

The nmr spectra of the acetates afforded the best means of distinguishing the isomers. In particular, the coupling constant between 22-H and 23-H in the erythro isomers was 8.5–9.0 Hz, while for the threo isomers 23-H appeared as a broad singlet indicating only weak coupling with 22-H. These coupling constants are expected for erythro and threo isomers from a consideration of nonbonded interactions.[9]

It is clear from the Newman projections (Scheme I) shown that the conformer which contributes most (to the mixture of the three staggered forms) will in both cases, *SR* and *RS*, have a dihedral angle of 180° between the protons, leading to a large coupling constant. In the threo isomers the conformer which contributes most will have a dihedral angle of 60°, leading to a small coupling con-

Scheme I



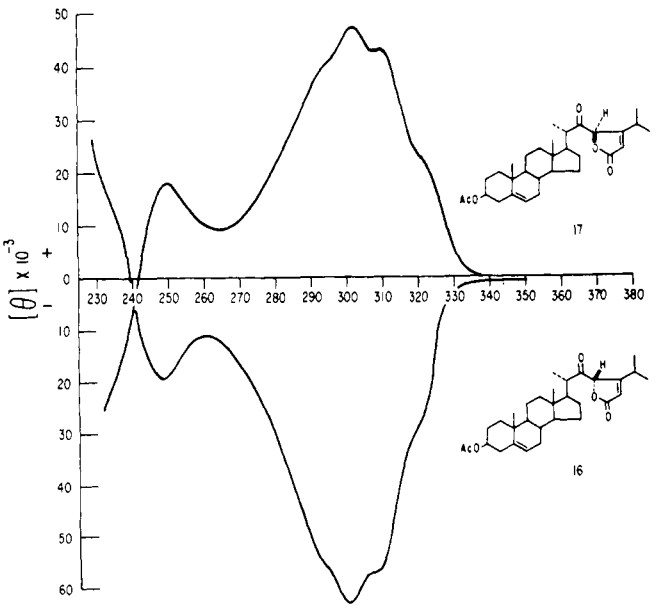


Figure 1.

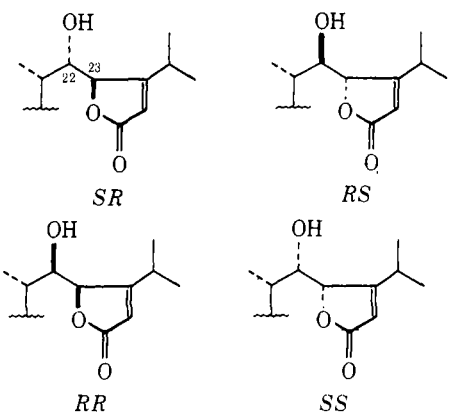
stant, as is actually observed. In fact, the large difference in the observed values for the erythro and threo isomers suggests that each isomer exists mainly in a single conformation.

The coupling constant between 22-H and 20-H appeared to be small for all except the 22*S*,23*S* isomer in which a value of 5 Hz was observed. The C_{26} and C_{27} methyl groups appeared as a pair of doublets (J = 7 Hz) in all four compounds.

The condensation of the aldehyde **3** and the butenolide **7** is quite stereoselective, one major product, the 22*R*,23*S* isomer, being formed. The stereoselectivity at C_{22} is similar to that obtained in other reported condensations involving steroidal C_{22} aldehydes. For example, an attempted synthesis of 23-deoxyantheridiol yielded only the C_{22} epimer (22*S*).[10] The configuration at C_{22} is correctly predicted by Cram's rule as noted by Barton and coworkers.[11]

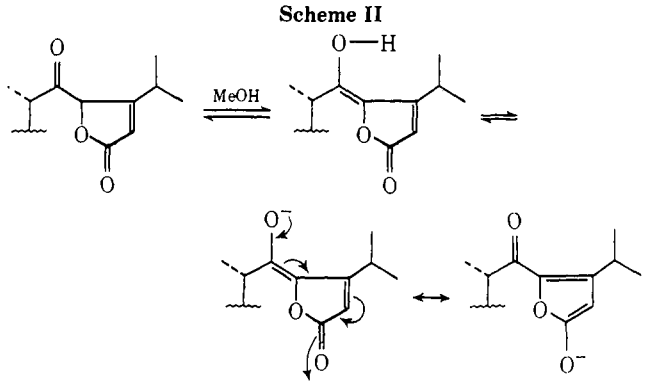
Since the yield of the isomer possessing the same side-chain stereochemistry as antheridiol (*i.e.*, 22*S*,23*R*) was low, attempts were made to change the stereochemistry at C_{22} and C_{23} in the other isomers by an oxidation–reduction sequence. Thus Jones oxidation of **13** (22*R*,23*S*) gave an almost quantitative yield of the 23*S* ketone **16** (mp 154–158°, crude product). Similarly Jones oxidation of **13** (22*R*,23*R*) gave the 23*R* ketone **17**, mp 156–160°. The nmr spectra of the two ketones differ particularly in the chemical shifts of the C_{18} and C_{21} methyl groups and of the 23-H proton. The circular dichroism curves are most distinctive, the curve for the 23*S* ketone being practically the mirror image of that of the 23*R* isomer (Figure 1). The very high values observed for the molar ellipticity indicate that rotation about the C_{22}–C_{23} bond is quite restricted and one rotamer predominates in the case of the 23*R* isomer and one in the case of the 23*S* isomer.[12] The situation is similar to that in the 22-hydroxy compounds (**13**) discussed above.

If the octant rule is applied to models of the two ketones arranged so that steric interactions are at a minimum, the butenolide ring will fall in a positive octant for the 23*R* isomer and in a negative octant for the 23*S* isomer. Therefore, the CD curves predict the same configurations for the isomers as is found from the following independent evidence. The acetate **13**, mp 164–166°, which can be converted directly to antheridiol, and the acetate **13**, mp 219–223°, are erythro isomers from nmr evidence described above. In addition, the latter compound can be hydrolyzed to give a diol which has the same properties as those reported for the erythro isomer of 7-deoxy-7-dihydroantheridiol.[4] The configuration at C_{22} and C_{23} in antheridiol has been definitely assigned as 22*S*,23*R*.[13] The erythro isomer **13**, mp 219–223°, which must therefore be 22*R*,23*S*, will give on oxidation a ketone possessing the *S* configuration at C_{23}. Since oxidation of **13**, mp 202–208°, yields a different ketone from that obtained from **13**, mp 219–223°, the former ketone has the 23*R* configuration and the parent compound is a threo isomer (22*R*,23*R*). The fourth isomer **13**, mp 175–180°, 195–198°, has the threo configuration (22*S*,23*S*) also.

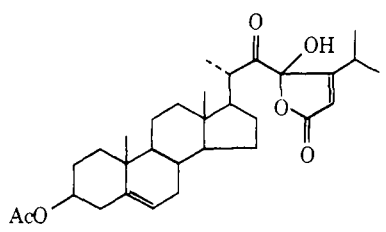


The CD curves of the ketones **16** and **17** were measured in chloroform or dioxane in which the compounds are stable. In solution in methanol the ketones both showed uv maxima at 321 and 363 nm. Addition of a drop of alkali caused a sharp increase (over tenfold) in the intensity of the 363-nm maximum and disappearance of the 321-nm maximum, indicating that the former was produced by the enolate anion shown (Scheme II). In agreement, acidification resulted in immediate disappearance of the 363-nm peak and reappearance of the 321-nm peak.

Scheme II



When the ketone **16** or **17** was run on tlc with ethyl acetate–petroleum ether, a single spot with an R_f greater than that of the parent alcohol **13** was observed. However, if the developing solvent was chloroform–methanol, at least three spots resulted. Examination of these spots



18

showed that the ketone was undergoing autoxidation and the main product was identified as the 23-hydroxy ketone **18**.

The yield of **18** could be increased to about 80% by stirring a solution of **16** or **17** in tetrahydrofuran–methanol (3:1) with silica gel overnight. A by-product of the reaction was 3β-acetoxy-22,23-bisnor-Δ^5-cholenic acid, formed by oxidative cleavage of the C_{22}–C_{23} bond in **16** or **17**.

The ketones undergo autoxidation very readily because the enolate anion can form in the presence of methanol which acts as a base. The reaction of an enolate anion with molecular oxygen is well known, and a mechanism can be written which explains the formation of the two products.[14,15]

The formation of the hydroxy ketone **18** was important because this compound could be cleanly reduced with sodium borohydride to one having the hydroxy butenolide side chain of antheridiol. Treatment of **16** itself with sodium borohydride in ethanol gave a yellow solution of the enolate anion and no hydroxy butenolide could be isolated. It is interesting to note that butenolides containing hydrogen on the γ carbon such as antheridiol and β-isopropylbut-2-enolide are attacked by borohydride with reduction of the double bond. When the γ position is fully substituted as in β,γ,γ-trimethylbut-2-enolide, no reduction of the double bond occurs. Presumably the presence of a γ hydrogen will result in the formation of a carbanion in the presence of base. Rearrangement gives a carbonyl (*via* an enol) which is reduced by the borohydride.

Different conditions for the reduction of **18** were tried. The most satisfactory was reaction of excess sodium borohydride with a solution of **18** in tetrahydrofuran–ethanol (3:1) at 10° for 20 hr. A nearly quantitative yield of **13** was obtained which contained approximately 20% of the 22*S*,23*R* isomer. This isomer could be separated and the remaining material put through the oxidation–reduction sequence again in order to produce more of the desired isomer. In this way the yield of **13** with the side-chain stereochemistry of antheridiol could be increased considerably.

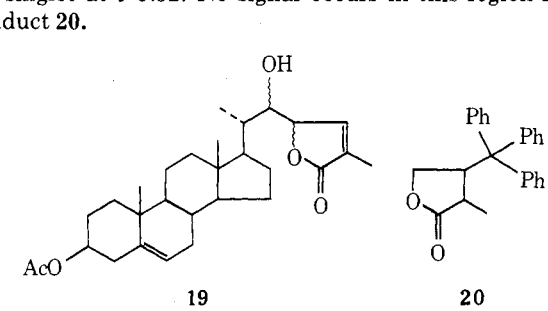
An attempt was also made to invert the configuration at C_{22} in the isomer **13** (22*R*,23*R*). Treatment of the latter with methanesulfonyl chloride gave the 22-mesylate as well as much elimination product. However when the mesylate was heated with tetrabutylammonium formate, only elimination occurred.[16] This result is not surprising since the C_{22} position is hindered and elimination will be favored over substitution. Elimination also produces an extended conjugated system, another reason in its favor.

In order to complete the synthesis of antheridiol, the acetate **13** (22*S*,23*R*) was hydrolyzed to the diol. Conditions had to be carefully chosen since the hydroxy butenolide structure was sensitive to base and to hydrochloric acid in methanol. However dilute sulfuric acid in dioxane gave a very high yield of the diol.

The final step in the synthesis was the introduction of the 7-ketone. The most convenient method was that first employed by the Syntex group.[4] Photooxygenation of the diol **14** in the presence of a sensitizer, hematoporphyrin, gave a high yield of the 5α-hydroperoxide. The hydroperoxide from the 22*R*,23*S* isomer was quite unstable and was easily converted to the 7-ketone in about 60% yield by treatment with cupric chloride in pyridine for 24 hr. The hydroperoxide from the 22*S*,23*R* isomer was obtained crystalline. It was more stable and longer treatment and more cupric chloride were required in order to obtain a 50% conversion to the corresponding 7-ketone (antheridiol).

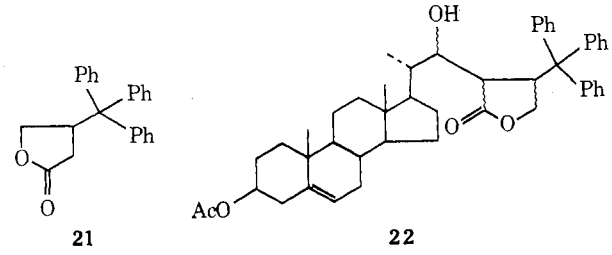
Another method of introducing the 7-ketone involved conversion of the diol **14** to the bistetrahydropyranyl ether. The latter was then oxidized with Collins reagent to give the 7-ketone.[17] Careful acid hydrolysis removed the tetrahydropyranyl protecting groups though some elimination of the 3β-hydroxyl could not be prevented. The yield in this method was about 35%. The synthetic antheridiol had the same properties, including biological activity, as the natural hormone. The method of synthesis described here affords pure antheridiol in an overall yield of approximately 20% from the readily accessible 3β-acetoxy-22,23-bisnor-Δ^5-cholenaldehyde (**3**). The results of biological tests of antheridiol and certain synthetic intermediates in *Achlya* and other systems will be reported elsewhere.

The scope of the aldol condensation of butenolides such as **7** with aldehydes has been investigated further. When excess of α-methylbut-2-enolide[18] was added to tritylllithium and the resulting pale yellow solution treated with the aldehyde **3**, a low yield (~30%) of condensation product was obtained. Spectral and analytical properties support the structure **19**. As in the case of **13** a mixture of four stereoisomers is formed, the main component being probably the 22*R*,23*S* isomer. The major product (50%) from the condensation was a crystalline compound whose spectral properties indicated the structure **20**. Thus the mass spectrum showed the molecular ion at *m/e* 342 and base peak at *m/e* 243 ($C_{19}H_{15}^+$), the latter due to the triphenylmethyl ion. The lactone **20** results from nucleophilic attack of trityl carbanion on the β carbon of the butenolide.[19] The hindered tertiary carbanion appears to be involved rather than one of the ring carbons as observed in the reaction of tritylllithium and benzophenone.[20] In the latter case the nmr spectrum of the adduct showed a signal at δ 5.53. Triphenylmethane itself shows a methine-H singlet at δ 5.52. No signal occurs in this region for the adduct **20**.



19 **20**

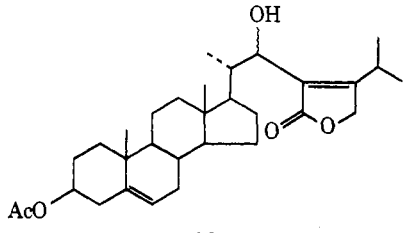
The reaction which gives **20** competes well with abstraction of a γ hydrogen from the butenolide. When aldol condensation was attempted with but-2-enolide,[18] no product analogous to **13** could be isolated. As expected, lactone **21**, the Michael adduct of triphenylmethane and but-2-enolide, was obtained, though in low yield (10%). The major product (70%) was a steroid which is assigned the structure **22**.



21 **22**

Because but-2-enolide has no methyl group which can hinder the approach of the trityl anion and also contribute an inductive effect, adduct formation proceeds almost exclusively in this case. The intermediate α anion then reacts with the steroid aldehyde to give **22**. The condensation product **22** has been partially resolved.

Another interesting possibility in condensations involving butenolides was observed in snythesis of **13**. A byproduct (5%) which was present in chromatographic fractions containing **13** (22*R*,23*S*) had properties consistent with the structure **23**. In particular, the nmr spectrum



23

showed a singlet at δ 4.74 for two protons α to oxygen in the butenolide ring. β-Isopropylbut-2-enolide (**7**) shows a corresponding signal at δ 4.80 (d, J = 2 Hz). Oxidation of **23** with Jones reagent gave the 22-ketone, λ_{max} 232 nm (ϵ 11,900). The uv spectrum was not affected by alkali (*cf.* **16**). The formation of **23** involves addition of the α carbanion of **7** to the aldehyde **3** followed by rearrangement of the intermediate anion to give the α,β-unsaturated butenolide.[21]

Experimental Section[22]

Reformatsky Reaction of 3β-Acetoxy-22,23-bisnor-Δ^5-cholenaldehyde (3) and γ-Bromobut-2-enolide (4). The method was the same as that used for the preparation of **2** described in an earlier paper.[6] Chromatography of the crude product with ethyl acetate–petroleum ether gave a fraction which crystallized on adding methanol. The crystals of **5** obtained in a yield of 5% from the aldehyde appeared homogeneous by tlc: mp 185–200°; ir 3420, 1799, 1767, 1733 cm^{-1}; nmr δ 0.71 (18 H), 1.03 (19 H), 1.04 (d, J = 6 Hz, 21 H), 2.03 (acetate), 3.63 (d, J = 4.5 Hz, 22 H), 5.37 (m, 6 H), 5.9 (m, 23 H), 6.22 (d, J = 6 Hz, 25 H), 7.21 (d, J = 6 Hz, 24 H); mass spectrum m/e 396 (M − 60), 312 (M − 60 − 84), 84 ($C_4H_4O_2^+$).

β-Isopropylbut-2-enolide (7). To a three-necked flask in an ice–salt bath were added acetic anhydride (2 l.), potassium acetate (260 g), and 3-methylbut-1-ene (50 g). The mixture was stirred and powdered $KMnO_4$ (340 g) added gradually during 3 hr.[23] Care was taken to maintain the temperature of the mixture below 5°. The stirring was continued for a further 5 hr at ~5° and then cold ethyl acetate (2 l.) was added, followed by an ice-cold solution of sodium bisulfite (450 g) in water (3.5 l.). The organic layer was separated and the aqueous solution neutralized with sodium bicarbonate and extracted with ethyl acetate. The combined ethyl acetate extract was washed with saturated $NaHCO_3$ solution, water, then dried (Na_2SO_4), and distilled to give 1-acetoxy-3-methylbutan-2-one (37 g): bp 90° (2 mm); ir (neat) 1730 cm^{-1}; nmr δ 1.12 (d, J = 7 Hz, isopropyl CH_3), 2.13 (acetate), 2.72 (m, methine H), 4.74 (s, methylene H). An alternative method for making this compound involved reaction of isopropylmagnesium bromide with glycolonitrile and acetylation with acetic anhydride–pyridine of the product, 1-hydroxy-3-methylbutan-2-one.[24] The ketoacetate (37 g) and ethyl bromoacetate (45 g) in dry benzene (180 ml) were added gradually to activated zinc dust,[6] and the mixture was gently warmed. When the reaction became vigorous, the heating was stopped and the remaining solution added at such a rate as to maintain the reflux. The mixture was refluxed for a further 2 hr. It was cooled, diluted with benzene, and acidified with dilute H_2SO_4. The benzene layer was separated, washed with water, and dried (Na_2SO_4); the solvent was removed, leaving an oil which was chromatographed, then distilled under reduced pressure to give **7**, (10 g): bp 90° (1.75 mm); ir 1775, 1740, 1626 cm^{-1}; nmr δ 1.22 (d, J = 7 Hz, isopropyl CH_3), 2.75 (m, methine H), 4.84 (d, J = 2 Hz, methylene H), 5.83 (q, vinyl H).

The butenolide **7** (126 mg) in carbon tetrachloride (3 ml) was heated with *N*-bromosuccinimide (178 mg) under illumination from a 100-W lamp. The reaction was complete within 30 min. The mixture was cooled and filtered, and the solvent was removed from the filtrate leaving a yellow oil. This gave a single spot on tlc and the nmr indicated that bromine was present exclusively in the allylic position on the side chain: nmr (CCl_4) δ 2.05 (s, CH_3), 5.02 (d, J = 2 Hz, methylene H), 4.95 (t, J = 2 Hz, vinyl H).

Pyrolysis of 2,5-Diacetoxy-3-isopropyl-2,5-dihydrofuran. 4-Isopropyl-2-furoic acid was prepared according to the method of Elming.[25] Decarboxylation by the method of Piers and Brown[26] gave better yields of β-isopropylfuran than those reported by Elming.[25] Treatment of the furan with lead tetraacetate afforded the diacetoxy dihydro derivative.[27] The latter compound (250 mg) and a crystal of toluenesulfonic acid[28] were heated at 100° for 10 min in a sublimation tube under reduced pressure (2 mm). The liquid darkened rapidly and a distillate was formed which collected in a trap cooled with Dry Ice–acetone. The distillate (~100 mg) was a mixture of acetic acid and 2-acetoxy-3-isopropylfuran (**8**). The nmr spectrum (CCl_4) of **8** had δ 1.14 (d, J = 7 Hz, isopropyl CH_3), 2.25 (acetate), 2.65 (quintet, J = 7 Hz, methine H), 6.23 (d, J = 2 Hz, 4 H), 6.98 (d, J = 2 Hz, 5 H). The structure was confirmed by adding a solution of bromine in carbon tetrachloride to one of the furan in carbon tetrachloride (cooled to −10°) until the bromine color just persisted. Excess of bromine was avoided by adding a little more of the furan.[27] The nmr spectrum (CCl_4) of the product, γ-bromo-α-isopropylbut-2-enolide (**9**) had δ 1.24 (d, J = 7 Hz, isopropyl CH_3), 2.75 (quintet, J = 7 Hz, methine H), 6.83 (t, J = 1 Hz, γ H), 7.1 (t, J = 1 Hz, β H).

γ-Bromo-β-isopropylbut-2-enolide (6). 2-Acetoxyfuran was converted to γ-bromobut-2-enolide[27] which was purified by distillation followed by chromatography with ethyl acetate–petroleum ether: nmr (CCl_4) δ 6.2 (pair of d, J = 5 and 1 Hz, α H), 7.0 (t, J = 1 Hz, γ H), 7.70 (pair of d, J = 5 and 1 Hz, β H). The bromobutenolide (2.2 g) was treated with an excess of an ethereal solution (0°) of 2-diazopropane[7] (100 ml) prepared from acetonehydrazone (5 g). Reaction was rapid as indicated by immediate discharge of the red color. Toward the end of the addition the color persisted and some oily material separated. The reaction mixture was kept at 0° overnight; then benzene (100 ml) was added and the mixture concentrated to one-half the volume. (If all the solvent was removed the pyrazoline rapidly polymerized to a black tar and gas was evolved.) Xylene (100 ml) was next added and the mixture again concentrated to one-half the volume. Finally more xylene (50 ml) was added and the mixture refluxed for 3 hr. Most of the xylene was distilled off [40–50° (1.0 mm)] and the residue chromatographed with ethyl acetate–petroleum ether (1:9) to give **6** in 35% yield: ir 1799, 1767 sh, 1637 cm^{-1}; nmr (CCl_4) δ 1.18, 1.33 (pair of d, J = 7 Hz, isopropyl CH_3), 2.89 (m, methine H), 5.83 (d, J = 1 Hz, α-H), 6.75 (s, γ-H); mass spectrum m/e 204 (M^+), 206.

Anal. Calcd for $C_7H_9O_2Br$: C, 40.98; H, 4.39; O, 15.61; Br, 39.02. Found: C, 40.74; H, 4.53; O, 15.79: Br, 39.26.

Reformatsky Reaction of 3β-Tetrahydropyranyloxy-22,23-bisnor-Δ^5-7-ketocholenaldehyde (10) and γ-Bromo-β-isopropylbut-2-enolide (6). The aldehyde (800 mg) and the bromobutenolide (400 mg) were dissolved in dry benzene (20 ml), and the solution was refluxed with activated zinc dust (200 mg) as described earlier.[6] The crude product had about 1% of the biological activity of antheridiol. A portion of the product was treated with dilute HCl in methanol (0.08 ml of 6 *N* HCl in 100 ml of methanol) to remove the tetrahydropyranyl group and then chromatographed with ethyl acetate–petroleum ether. However, no crystalline antheridiol or any of its isomers could be isolated.

Condensation of Aldehyde 10 with β-Isopropylbut-2-enolide (7). Butyllithium (0.6 g, 90% in hydrocarbon, obtained from Ventron Corp., Beverly, Mass.) was weighed into a three-necked flask previously flushed out with argon. Extreme care was taken to avoid contact with air during the weighing. (The butyllithium was stored in a desiccator with an argon atmosphere.) Triphenylmethane (2.86 g) in tetrahydrofuran (10 ml) freshly distilled from lithium aluminum hydride, was added dropwise during 10 min to the butyllithium in the flask (argon atmosphere), cooled to *ca.* −30° in an acetone bath containing enough Dry Ice to maintain the temperature. A deep red solution formed immediately. The solution was stirred (magnetic stirrer) at −30° for 30 min, then allowed to come gradually to room temperature, and stirred for a further 30 min. It was then cooled to −30° and a solution of **7** (1.0 g) in tetrahydrofuran (15 ml) was added dropwise with stirring. Towards the end of the addition, which required only a few minutes, the red color was discharged. The pale yellow solution was quickly cooled to *ca.* −72° and the aldehyde **10** (1.25 g) in tetrahydrofuran (25 ml) added gradually. The reaction mixture was stirred at −72° for a further 30 min and then allowed to come to room temperature during 30 min. It was added to an excess of dilute HCl (0°) with vigorous stirring. A semisolid precipitate was

obtained, so the mixture was extracted with ether and the extract washed (H_2O) and dried (Na_2SO_4). The ether was distilled off and the residue chromatographed with ethyl acetate–petroleum ether. Early fractions gave triphenylmethane. The crystalline condensation product **11** (0.7 g) possessed about 10% of the biological activity of antheridiol. It was resolved by further chromatography into two main components. One component (0.4 g) was recrystallized from methanol: mp 221–223°; ir 1767, 1660, 1626 cm^{-1}; nmr δ 0.70 (18 H), 1.04 (d, J = 7 Hz, 21 H), 1.20 (19 H), 1.19, 1.23 (pair of d, J = 7 Hz, 26 and 27 H), 3.64 (d, J = 9 Hz, 22 H), 4.90 (d, J = 9 Hz, 23 H), 5.68 (s, 6 H), 5.75 (t, J = 1 Hz, 28 H).

Anal. Calcd for $C_{34}H_{50}O_6 \cdot 0.25CH_3OH$: C, 73.07; H, 9.13; O, 17.77. Found: C, 73.06; H, 9.55; O, 17.25.

This component was hydrolyzed by treatment with dilute HCl in methanol (0.08 ml of 6*N* HCl in 100 ml of methanol at 0° for 30 min). The crystalline product obtained in high yield was also ~10% as active as antheridiol. It gave a single spot on tlc which had the same R_f as antheridiol in all solvent systems studied, *e.g.* in $CHCl_3$–MeOH (15:1), R_f 0.43. Recrystallization from methanol gave long needles, mp 260–265° dec. The spectral properties were identical with those of the erythro isomer of antheridiol (see later). The biological activity of the crystals was less than that of the starting material, while that of the residue from the mother liquor was higher. However, despite further crystallization and chromatography of the residue, no pure antheridiol could be isolated. The major component of the condensation product is thus the erythro isomer (22*R*,23*S*). The other main component (0.17 g) on hydrolysis gave crystals of the threo isomer (22*R*,23*R*) of antheridiol (see later).

Condensation of 3β-Acetoxy-22,23-bisnor-Δ⁵-cholenaldehyde (3) with β-Isopropylbut-2-enolide (7) *n*-Butyllithium (1.68 g) was treated with excess of triphenylmethane (8.6 g) in tetrahydrofuran (35 ml) to form trityllithium as described above. The red solution was treated with an excess of **7** (2.71 g in 20 ml of tetrahydrofuran) and the resulting carbanion allowed to react with the aldehyde **3** (6.32 g in 40 ml of tetrahydrofuran). The gummy product (17.6 g) was chromatographed with ethyl acetate–petroleum ether, 100-ml fractions being collected. Early fractions contained triphenylmethane. Unreacted aldehyde (~1.2 g) was eluted in fractions 13–17. It was followed by a product (~0.5 g) formed from elimination of the 22-OH in **13**. Unreacted butenolide, **7**, was eluted in the first fractions, 21–23, containing **13** (22*S*,23*R*). Fractions 23–25 contained almost pure **13** (22*S*,23*R*) (0.47 g) which was recrystallized from ethyl acetate–petroleum ether: mp 164–166°; ir 1760 sh, 1733, 1637 cm^{-1}; nmr δ 0.70 (18 H), 1.02 (19 H), 1.17, 1.22 (pair of d, J = 6.5 Hz, 26 and 27 H), 2.03 (acetate), 3.61 (d, J = 9 Hz, 22 H), 4.94 (d, J = 9 Hz, 23 H), 5.4 (m, 6 H), 5.78 (t, J = 1 Hz, 28 H); mass spectrum m/e 438, 420, 312, 126; CD max $[\theta]_{218nm}$ +37,350° (dioxane).

Anal. Calcd for $C_{31}H_{46}O_5$: C, 74.66; H, 9.30. Found C, 74.24; H, 9.45.

Fractions 26 and 27 contained the isomer **23** which was obtained pure after recrystallization from methanol (50 mg): mp 169–171°, on further heating it solidified then melted again at 180–181°; ir 1740, 1667 cm^{-1}; nmr δ 0.70 (18 H), 1.02 (19 H), 1.12 (d, J = 6.5 Hz, 26 and 27 H), 2.02 (acetate), 3.49 (d, J = 3 Hz, 22 H), 4.74 (s, 28 H), 5.37 (m, 6 H); mass spectrum m/e 438, 420.

Anal. Found: C, 74.75; H, 9.52.

Fractions 26–39 were mainly **13** (22*R*,23*S*) (4.29 g) which on recrystallization from methanol or ethyl acetate–petroleum ether gave pure compound: mp 219–223°; ir 1818 (w), 1767 (s), 1739 (w), 1715 cm^{-1} (s); nmr δ 0.72 (18 H), 1.03 (19 H), 1.05 (d, J = 6.5 Hz, 21 H), 1.18, 1.23 (pair of d, J = 7 Hz, 26 and 27 H), 2.03 (acetate), 3.6 (d, J = 8.5 Hz, 22 H), 4.91 (d, J = 8.5 Hz, 23 H), 5.4 (m, 6 H), 5.77 (t, J = 1 Hz, 28 H); mass spectrum m/e 438, 420, 312, 126; CD max $[\theta]_{218nm}$ −49,800° (dioxane).

Anal. Found: C, 74.54; H, 9.33.

Fractions 45–55 were mainly **13** (22*R*,23*R*) (0.9 g) which on recrystallization from ethyl acetate–petroleum ether gave pure compound: mp 204–208°; ir 1760, 1733, 1637 cm^{-1}; nmr δ 0.73 (18 H), 1.03 (19 H), 1.13 (d, J = 6 Hz, 21 H), 1.17, 1.25 (pair of d, J = 7 Hz, 26 and 27 H), 2.03 (acetate), 3.9 (broad s, 22 H), 4.9 (broad s, 23 H), 5.4 (m, 6 H), 5.82 (t, J = 1 Hz, 28 H); mass spectrum m/e 438, 420, 312, 126.

Anal. Found: C, 74.63; H, 9.03.

Fractions 56–58 contained mainly **13** (22*S*,23*S*) (~0.5 g) but with a small amount of the isomer **13** (22*R*,23*R*). Preparative tlc followed by recrystallization gave pure **13** (22*S*,23*S*): mp 175–180°, 195–198°; ir 1736, 1637 cm^{-1}; nmr δ 0.75 (18 H), 1.03 (19 H), 1.13 (d, J = 7 Hz, 21 H), 1.18, 1.26 (pair of d, J = 7 Hz, 26 and 27 H), 2.02 (acetate), 3.89 (d, J = 4.5 Hz, 22 H), 5.07 (broad s, 23 H), 5.4 (m, 6 H), 5.82 (t, J = 1 Hz, 28 H); mass spectrum m/e 438, 420, 312, 126.

Anal. Found: C, 74.35; H, 9.31.

Later fractions from the chromatography contained a mixture of isomers of 7-deoxy-7-dihydroantheridiol (**14**) (~0.2 g), but these could be separated only by preparative tlc with multiple development.

Hydrolysis of the 3-Acetate of 7-Deoxy-7-dihydroantheridiol (13). A solution of the acetate **13** (22*S*,23*R*) (45 mg) in dioxane (20 ml) was refluxed with 5% H_2SO_4 (5 ml) for 1 hr. Most of the solvent was removed under reduced pressure and cold water added, giving a crystalline precipitate of the diol **14** (38 mg). On crystallization from methanol it had mp 234–238°: ir 1740 cm^{-1}; nmr δ 0.70 (18 H), 1.02 (19 H), 1.17, 1.22 (pair of d, J = 6.5 Hz, 26 and 27 H), 3.61 (d, J = 9 Hz, 22 H), 4.94 (d, J = 9 Hz, 23 H), 5.4 (m, 6 H), 5.78 (t, J = 1 Hz, 28 H).

Anal. Calcd for $C_{29}H_{44}O_4 \cdot 0.25CH_3OH$: C, 75.65, H, 9.70. Found: C, 75.98; H, 9.95.

The erythro isomer **14** (22*R*,23*S*) was obtained in the same way: mp 209–211°; ir 7167 sh, 1745 cm^{-1}; nmr δ 0.72 (18 H), 1.02 (19 H), 1.04 (d, J = 7 Hz, 21 H), 1.18, 1.23 (pair of d, J = 7 Hz, 26 and 27 H), 3.61 (d, J = 8.5 Hz, 22 H), 4.93 (d, J = 8.5 Hz, 23 H), 5.38 (m, 6 H), 5.79 (t, J = 1 Hz, 28 H); mass spectrum m/e 456 (M^+), 438, 420, 405, 330, 312, 297, 284, 271, 255, 213.

Anal. Calcd for $C_{29}H_{44}O_4 \cdot 0.25CH_3OH$: C, 75.65; H, 9.70. Found: C, 75.56, H, 9.98.

Similar hydrolysis of the threo isomer **13** (22*R*,23*R*) gave the corresponding diol: mp 192–196° (methanol); ir 1748 cm^{-1}; nmr δ 0.73 (18 H), 1.03 (19 H), 1.13 (d, J = 6 Hz, 21 H), 1.17, 1.25 (pair of d, J = 7 Hz, 26 and 27 H), 3.94 (broad s, 22 H), 4.91 (broad s, 23 H), 5.38 (m, 6 H), 5.83 (t, J = 1 Hz, 28 H).

Anal. Calcd for $C_{29}H_{44}O_4 \cdot CH_3OH$: C, 73.73; H, 9.90. Found: C, 73.84; H, 9.69.

Hydrolysis of the threo isomer **13** (22*S*,23*S*) gave the corresponding diol: mp 248–252° (ethyl acetate); ir 1745 cm^{-1}; nmr ($CDCl_3$–CD_3OD, 3:1) δ 0.78 (18 H), 1.03 (19 H), 1.13 (d, J = 7 Hz, 21 H), 1.21, 1.28 (pair of d, J = 7 Hz, 26 and 27 H), 4.05 (broad peak, 22 H), 5.11 (broad s, 23 H), 5.33 (m, 6 H), 5.82 (t, J = 1 Hz, 28 H).

Anal. Calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 75.76; H, 9.61.

Antheridiol (1). A solution of 130 mg of the diol **14** (22*S*,23*R*) and 13 mg of hematoporphyrin in 18 ml of pyridine contained in a Pyrex tube 2 cm × 15 cm was irradiated for 24 hr with two 15-W fluorescent lamps placed close to the tube while oxygen was passed through the solution.[4] The dark brown solution was diluted with ether (100 ml), stirred with activated charcoal, and then filtered through Celite. Removal of solvent from the filtrate gave the crystalline hydroperoxide which from tlc appeared nearly pure. It was dissolved in 10 ml of pyridine and, after adding 6 mg of $CuCl_2 \cdot 2H_2O$, was allowed to stand at room temperature for 3 days. (Shorter times resulted in lower yields of antheridiol.) The pyridine was removed *in vacuo* and the residue chromatographed (preparative tlc, two silica gel plates, $CHCl_3$–MeOH, 15:1) to give 66 mg of pure antheridiol: mp 244–248° dec (methanol): ir 1740, 1672, 1626 cm^{-1}; nmr ($CDCl_3$–CD_3OD, 3:1) δ 0.72 (18 H), 1.23 (19 H), 1.17, 1.23 (pair of d, J = 7 Hz, 26 and 27 H), 3.6 (broad d, J = 8 Hz, 22 H), 4.98 (broad d, J = 8 Hz, 23 H), 5.70 (s, 6 H), 5.79 (broad s, 28 H).

The erythro isomer of antheridiol was prepared similarly from the diol **14** (22*R*,23*S*) (100 mg). The intermediate hydroperoxide was less stable and could not be isolated crystalline. Treatment with $CuCl_2 \cdot 2H_2O$ (3 mg) for 24 hr gave the 7-ketone (62 mg): mp 260–263° (methanol); ir 1759, 1650, 1631 cm^{-1}; nmr ($CDCl_3$–CD_3OD, 3:1) δ 0.74 (18 H), 1.06 (d, J = 6.5 Hz, 21 H), 1.23 (19 H), 3.6 (d, J = 8.5 Hz, 22 H), 4.96 (d, J = 8.5 Hz, 23 H), 5.7 (s, 6 H), 5.78 (t, J = 1 Hz, 28 H).

Anal. Calcd for $C_{29}H_{42}O_5 \cdot 0.25CH_3OH$: C, 73.37; H, 9.06. Found: C, 73.41; H, 9.13.

The threo isomer (22*R*,23*R*) of antheridiol (29 mg) was prepared from the diol **14** (22*R*,23*R*) (42 mg): mp 209–213° (ethyl acetate–petroleum ether); ir 1761, 1742, 1678, 1637 cm^{-1}.

Anal. Calcd for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 74.32; H, 9.11.

The threo isomer (22*S*,23*S*) (18 mg) was prepared from the diol **14** (22*S*,23*S*) (25 mg): mp 260–263°; ir 1742, 1672, 1637 cm^{-1}; nmr ($CDCl_3$–CD_3OD, 4:1) δ 0.78 (18 H), 1.23 (19 H), 1.21, 1.26 (pair of d, J = 7 Hz, 26 and 27 H), 5.13 (broad s, 23 H), 5.71 (s, 6 H), 5.85 (t, J = 1 Hz, 28 H).

An alternative route to antheridiol and its isomers was as fol-

lows: 50 mg of the diol **14** (22*R*,23*S*) was converted to the bis-tetrahydropyranyl ether with dihydropyran and a trace of *p*-toluenesulfonic acid. The ether was dissolved in methylene chloride (3 ml), and a slurry of 400 mg of Collins reagent[17] in methylene chloride (2 ml) was then added. Tarry material formed quickly. The mixture was stirred for 18 hr, more Collins reagent (150 mg) added, and the stirring continued for 7 hr. The mixture was filtered through a column of silica gel and the column eluted with ethyl acetate. Removal of solvent from the combined filtrate gave 35 mg of the 7-ketone. It was treated with dilute HCl in methanol (0.08 ml of 6*N* HCl in 100 ml of MeOH) for 1 hr at room temperature. Ethyl acetate was added; then most of the solvent was removed in a stream of N_2 and the residue chromatographed with chloroform–methanol (15:1) to give 17 mg of the erythro isomer of antheridiol. About 6 mg of the $\Delta^{3,5}$-dien-7-one was also obtained. This resulted from elimination of the 3β substituent.

Oxidation of the Acetate of 7-Deoxy-7-dihydroantheridiol (13). Jones reagent[29] (4 ml) was gradually added to a stirred solution of **13** (22*R*,23*S*) (1 g) in 150 ml of acetone cooled to 0°. The mixture was kept at 0° for 1 hr; then methanol was added to destroy any excess reagent. Most of the solvent was removed *in vacuo*, and cold water added to precipitate the crystalline ketone **16** (0.98 g): mp 154–158°; uv max (MeOH) 212 nm (ϵ 7000), 225 sh (6000), 321 (1900), 363 (650). The intensity of the 321- and 363-nm maxima depended on the concentration of the solution, being more intense at lower concentrations; uv (MeOH/drop of dilute NaOH solution) 363 nm (ϵ 30,000); uv (MeOH/dilute HCl) 212 nm (ϵ 6200), 225 sh (5900), 321 (2100); ir 1802, 1770, 1733, 1724 cm^{-1}; nmr δ 0.67 (18 H), 1.02 (19 H), 1.18 (d, J = 7 Hz, 21 H), 1.21, 1.23 (pair of d, J = 7 Hz, 26 and 27 H), 2.03 (acetate), 5.33 (d, J = 2 Hz, 23 H; the signal partly overlapped that at 5.4 due to 6 H), 5.92 (t, J = 1 Hz, 28 H); mass spectrum *m/e* 436 (M − 60), 421, 371, 328, 279; CD max $[\theta]_{301}$ −61,400°.

Oxidation of 55 mg of **13** (22*R*,23*R*) in the same way with Jones reagent gave 52 mg of the ketone **17**: mp 156–160°; uv same as that of **16**; ir 1802, 1770, 1733, 1724 sh cm^{-1}; nmr δ 0.73 (18 H), 0.98 (d, J = 7 Hz, 21 H), 1.03 (19 H), 1.21, 1.23 (pair of d, J = 7 Hz, 26 and 27 H), 2.03 (acetate), 5.23 (d, J = 2 Hz, 23 H), 5.4 (m, 6 H), 5.88 (t, J = 1 Hz, 28 H); CD max $[\theta]_{302}$ +46,000°.

Autoxidation of Ketone 16. A solution of 500 mg of **16** in 150 ml of tetrahydrofuran–methanol (2:1) was stirred with 20 g of silica gel-G for 24 hr. The uv spectrum (NaOH–MeOH) then showed that no starting material remained. The solution was filtered and the solvent removed leaving a white solid which was crystallized from ethyl acetate–petroleum ether to give 200 mg of the 23-hydroxy ketone (**18**). Chromatography of the residue from the mother liquor gave 175 mg more of **18**: mp 214–217°; ir 3340, 1779, 1730, 1709 cm^{-1}; nmr δ 0.67 (18 H), 1.01 (19 H), 2.02 (acetate), 5.4 (m, 6 H), 6.11 (d, J = 1.5 Hz, 28 H); mass spectrum *m/e* 452 (M − 60), 408, 283 (base peak).

Anal. Calcd for $C_{31}H_{44}O_6$: C, 72.63; H, 8.65; O, 18.72. Found: C, 72.44; H, 8.59; O, 18.94.

Later fractions from the chromatography gave 70 mg of a pure compound which was identified as 3β-acetoxy-22,23-bisnor-Δ^5-cholenic acid by comparison with an authentic sample. Several experiments were performed in which the concentration of **16**, the nature of the solvent, and the amount of silica were varied. The conditions described above gave the best yield of **18**.

Sodium Borohydride Reduction of 18. Sodium borohydride (15 mg) was added to a solution of 30 mg of **18** in 3 ml of tetrahydrofuran–ethanol (3:1) and the mixture allowed to stand at 10° for 20 hr. The solvent was removed in a stream of N_2 and water followed by a few drops of dilute HCl was added to the residue. The white insoluble solid was filtered, washed (H_2O), dried, and chromatographed to give 6 mg of **13** (22*S*,23*R*), 16 mg of **13** (22*R*,23*S*), and 5 mg of a mixture of the threo isomers of **13**. The reduction was repeated with different solvents, *e.g.*, tetrahydrofuran–methanol, 2-propanol, and dioxane–water, but the proportion of the desired isomer **13** (22*S*,23*R*) was not as good as that obtained above. When ethanol alone was used as solvent, an almost quantitative yield of diols **14** was obtained.

Condensation of Aldehyde 3 with α-Methylbut-2-enolide.[18] The butenolide (1.33 g) and the aldehyde (4.0 g) were condensed in the same way as described earlier for the preparation of **13**. The crude product (8.3 g) on chromatography with ethyl acetate–petroleum ether gave first the adduct **20** (2.3 g): mp 221–222° (ethyl acetate), uv max (EtOH) 199 nm (ϵ 72,400), 245 (687), 258 (730), 265 (653), 271 (425); ir 1767, 1600, 1496 cm^{-1}; nmr δ 1.40 (d, J = 7.5 Hz, methyl), 2.58 (d of q, J = 8 Hz, J = 2.5 Hz, methine H), 3.8–4.8 (m, methine H + 2H α to oxygen), 7.25 (s, 15 aromatic H); mass spectrum *m/e* 342 (M$^+$), 243 ($C_{19}H_{15}^+$, base peak), 165 ($C_{13}H_9^+$).

Anal. Calcd for $C_{24}H_{22}O_2$: C, 84.18; H, 6.48. Found: C, 84.34; H, 6.46.

Later fractions from the chromatography contained an isomeric mixture of **19** (1.85 g). The main component was obtained pure by recrystallization from ethyl acetate–petroleum ether: mp 220–224°; ir 1767 sh, 1733 cm^{-1}; nmr δ 0.72 (18 H), 1.03 (19 H), 1.95 (t, J = 1.5 Hz, 27 H), 2.03 (acetate), 3.53 (d, J = 7 Hz, 22 H), 4.93 (m, 23 H) 5.4 (m, 6 H), 7.03 (t, J = 1.5 Hz, 24 H); mass spectrum *m/e* 410 (M − 60), 312 (M − 98 − 60).

Anal. Calcd for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 73.79; H, 9.11.

The other components of the isomeric mixture were present in smaller amount and were well resolved on tlc, but were not examined in detail.

Condensation of Aldehyde 3 with But-2-enolide.[18] But-2-enolide (1.83 g) and the aldehyde (6.1 g) were condensed as described above and the crude product (15.4 g) was recrystallized from ethyl acetate (5.1 g). A portion of this material (200 mg) was resolved into two components by preparative tlc with $CHCl_3$–MeOH (100:3). One component, **22** (81 mg), had mp 168–174°, 236–238° (MeOH): ir 1780 sh, 1761, 1739, 1603, 1497, 1475, 754, 745, 705 cm^{-1}; nmr δ 0.65 (18 H), 0.83 (d, J = 7 Hz, 21 H), 1.00 (19 H), 2.03 (acetate), 2.78 (d, J = 10 Hz, 23 H), 5.4 (m, 6 H), 7.30 (s, 15 aromatic H); mass spectrum *m/e* 312, 243, 165.

Anal. Calcd for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 73.79; C, 79.74; H, 8.21.

The other component, **22** (95 mg), had mp 241–244° (EtOAc): ir 1773 sh, 1745, 1600, 1498, 1475, 762, 748, 705 cm^{-1}; nmr δ 0.73 (18 H), 0.73 (d, J = 6 Hz, 21 H), 1.02 (19 H), 2.02 (acetate), 2.68 (d, J = 5.5 Hz, 23 H), 5.40 (m, 6 H), 7.26 (s, 15 aromatic H); mass spectrum *m/e* 397 (M − 243 − 60), 312 (M − 243 − 60 − 85), 243 ($C_{19}H_{15}^+$ base peak), 165 ($C_{13}H_9^+$).

Anal. Calcd for $C_{47}H_{56}O_5$: C, 80.53; H, 8.05. Found: C, 80.26; H, 7.90.

The material (10.3 g) in the mother liquor from recrystallization of **22** was chromatographed with ethyl acetate–petroleum ether to give the adduct **21** (0.84 g) contaminated with steroidal material **22**). It was freed from the contaminant by acid hydrolysis which deacetylated the steroid and then chromatography with $CHCl_3$–MeOH (50:1): mp 207–209° (ethyl acetate–petroleum ether); uv max 199 nm (ϵ 31,100), 253 (561), 259 (630), 264 (596), 270 (365); ir 1764, 1600 cm^{-1}; nmr δ 2.47–2.83 (m, methylene H), 4.2–4.6 (m, methine H + methylene H α to oxygen); 7.26 (s, 15 aromatic H); mass spectrum *m/e* 328 (M$^+$), 243 ($C_{19}H_{15}^+$, base peak), 165 ($C_{13}H_9^+$).

Anal. Calcd for $C_{23}H_{20}O_2$: C, 84.12; H, 6.14. Found: C, 84.09; H, 6.10.

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Registry No.—(22*S*,23*R*)-**1**, 22263-79-2; (22*R*,23*S*)-**1**, 22233-25-6; (22*R*,23*R*)-**1**, 49686-14-8; (22*S*,23*S*)-**1**, 49686-15-9; **3**, 10211-88-8; **4**, 40125-53-9; **5**, 686-18-2; **6**, 35457-50-2; **7**, 10547-89-4; **8**, 49686-20-6; **9**, 49686-21-7; **10**, 49686-22-8; (22*R*,23*S*)-**11**, 49686-23-9; (22*S*,23*R*)-**13**, 37926-51-5; (22*R*,23*S*)-**13**, 37926-52-6; (22*R*,23*R*)-**13**, 37926-53-7; (22*S*,23*S*)-**13**, 37926-54-8; (22*S*,23*R*)-**14**, 35878-68-3; (22*R*,23*S*)-**14**, 49686-29-5; (22*R*,23*R*)-**14**, 35878-70-7; (22*S*,23*S*)-**14**, 35878-69-4; **16**, 37926-55-9; **17**, 38672-73-0; **18**, 37926-57-1; **19**, 49686-34-2; **20**, 49686-35-3; **21**, 49686-36-4; **22**, 49686-37-5; **23**, 49686-38-6; 3-methyl-1-butene, 563-451; 1-acetoxy-3-methylbutan-2-one, 36960-07-3; brominated butenolide, 49686-40-0; 2,5-diacetoxy-3-isopropyl-2,5-dihydrofuran, 49686-41-1; 2-diazopropane, 2684-60-8.

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Alkaloids of *Cephalotaxus harringtonia* var. *drupacea*. 11-Hydroxycephalotaxine and Drupacine[1a]

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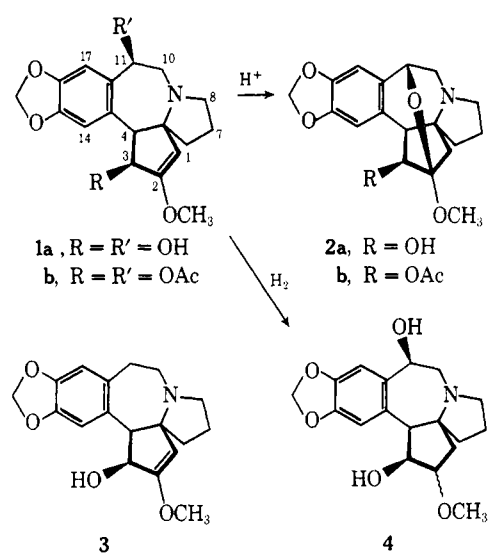
Two isomeric alkaloids, 11-hydroxycephalotaxine and drupacine, have been isolated from *Cephalotaxus harringtonia* var. *drupacea* (Sieb. + Zucc.) Koidzumi. Evidence is presented to show that these alkaloids are represented by structures **1a** and **2a**, respectively. Close proximity of the two hydroxyl functions of **1a** leads to some unusual reaction products. Nearly quantitative conversion of **1a** to ketal **2a** occurs under mild acidic conditions. Treatment of **1a** with tosyl chloride in pyridine affords cyclic ether **5**, and oxidation of **1a** under modified Oppenauer conditions results in formation of hemiketal **7**. The diacetate of **1a** is epimerized under extraordinarily mild conditions.

Initial investigations of the alkaloids of *Cephalotaxus drupacea* were carried out by Paudler, *et al.*,[2] and by McKay.[3] Although earlier listed as a member of the family Taxaceae,[4] the genus *Cephalotaxus* has now been assigned to a separate family, the Cephalotaxaceae, and the plant formerly referred to as *C. drupacea* is now considered to be *C. harringtonia* var. *drupacea*.[5] Two different structural types of *Cephalotaxus* alkaloids have been noted; the first group is based on the cephalotaxine ring system (**3**), and the second group embodies the homoerythrina ring system.[6,7] Several natural cephalotaxine esters have recently gained attention as potential tumor inhibitors.[8] This paper gives details of the structural determinations of two oxygenated cephalotaxine derivatives first noted in a seed extract of *C. harringtonia* var. *drupacea* and describes some unusual reactions of hydroxycephalotaxine. Portions of this work were described in a preliminary communication.[9]

Alkaloids **1a**, **2a**, and **3** were isolated by preparative tlc of an alkaloid concentrate from *C. harringtonia* var. *drupacea* twigs. The first of these (**1a**, $C_{18}H_{21}NO_5$, $[\alpha]^{26}D$ −139°) had a broad hydroxyl band in its ir spectrum (3500 cm^{-1}) indicative of strong intramolecular hydrogen bonding. An nmr spectrum of **1a** contained signals (Table I) that allowed assignment of the cephalotaxine (**3**) ring system to **1a** and, in addition, exhibited a signal at δ 4.78 which was assigned to a proton on a carbon bearing both hydroxyl and aryl groups (C_{11}). Preparation of a di-*O*-acetyl derivative (**1b**, $C_{22}H_{25}NO_7$) demonstrated that **1a** contained two hydroxyl groups. Signals attributed to protons on the two hydroxyl-bearing carbons (C_3 and C_{11}) were shifted markedly downfield, as expected, upon acetylation of **1a**. These observations led to the conclusion that **1a** was an 11-hydroxycephalotaxine.[9]



The second alkaloid was isomeric with **1a** (**2a**, $C_{18}H_{21}NO_5$, $[\alpha]^{26}D$ −137°), and its ir spectrum demonstrated the presence of at least one hydroxyl group (3600