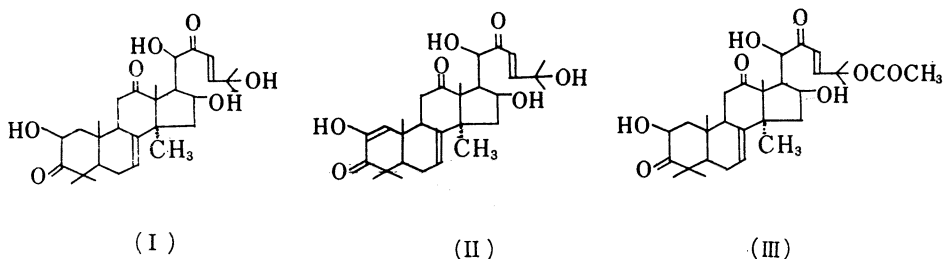


134. Hiromu Mori, Vipichandra S. Gandhi, and Erwin Schwenk :
Preparations of Some Steroidal Diosphenols.¹⁾

(Worcester Foundation for Experimental Biology*¹⁾)

The usefulness of the cheekpouch test in the Golden Hamster (*Mesocricetus Aureus*) for experiments in tumor chemotherapy has been demonstrated in this laboratory.²⁾ In the course of an investigation of the tumor growth inhibiting properties of a number of natural substances employing this test the kindness of Prof. David Lavie in Tel-Aviv made it possible to test substances isolated from Cucurbitaceae, the chemistry of which his school so excellently has studied.³⁾ It was found that elatericin A (I) and B (II), as well as elaterin (III) showed good inhibition of tumor growth in the cheekpouch.⁴⁾ Recently Gitter *et al.*⁵⁾ published an extensive study of the antitumor effects of these substances using other than hamster tumors for testing.



The most impressive constitutional feature in the formulae of these substances, to which their chemotherapeutic action might be ascribed, is the diosphenol structure in ring A of elatericin B and the cumulation of oxygen substitution in the remaining part of the molecule. To confirm this assumption it was considered of interest to prepare other diosphenols of the steroid series and study the influence which substitution present elsewhere in the substances might have a possibly tumor growth inhibiting effect.

A number of methods are available for the preparation of such diosphenols from 3-oxo steroids.

A) **Selenium Dioxide Oxidation**—Stiller and Rosenheim⁶⁾ obtained two enols of cholestane-2,3-dione, namely, 3-hydroxycholest-3-en-2-one and 2-hydroxycholest-1-en-3-one as the oxidation product of cholestan-3-one with selenium dioxide. When we oxidized lanosta-8,24-dien-3-one (IVa), prepared by chromic anhydride oxidation of lanosterol,⁷⁾ with selenium dioxide in aqueous ethanol solution, the diosphenol (VIa) was

*¹ Shrewsbury, Massachusetts, U.S.A. (森 弘, ビピチャンドラ・エス・ガンジ, アーヴィン・シュベング).

1) This work was supported by Grant No. 4550 of the U.S. Public Health Service, an Institutional Grant No. EIN-56 of the American Cancer Society and a Grant from the Massachusetts Division of the American Cancer Society.

2) D. F. Stevens, E. Schwenk : *Naturwissenschaften*, **45**, 218 (1958).

3) D. Lavie, Y. Shvo : *J. Am. Chem. Soc.*, **82**, 966 (1960). D. Lavie, D. Willner : *Ibid.*, **82**, 1668 (1960). D. Lavie, Y. Shvo : *Chem. & Ind. (London)*, **1960**, 403. D. Lavie, Y. Shvo, O. R. Gottlieb : *Tetrahedron Letters*, No. 22, 23 (1960). C. R. Noller, A. Melera, M. Gut, J. N. Shoolery, Li. F. Johnson : *Ibid.*, No. 15, 15 (1960).

4) D. Lavie, D. Willner : *J. Am. Chem. Soc.*, **80**, 710 (1958).

5) S. Gitter, R. Gallily, B. Shohat, D. Lavie : *Cancer Res.*, **21**, 516 (1961).

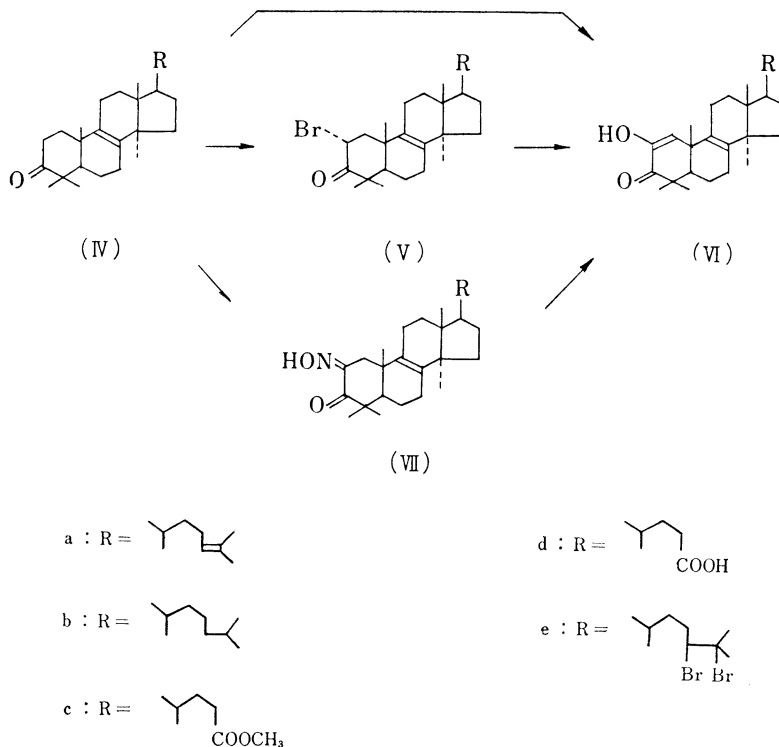
6) E. T. Stiller, O. Rosenheim : *J. Chem. Soc.*, **1938**, 353.

7) L. Ruzicka, R. Denss, O. Jeger : *Helv. Chim. Acta*, **28**, 759 (1945); **29**, 204 (1946)

isolated in 19% yield. The assignment of the product as the diosphenol (VIa) is based upon the following evidence. The product was converted into the sparingly soluble potassium salt by 40% potassium hydroxide, and it gave a positive ferric chloride test.⁹⁾ In the ultraviolet absorption spectrum a maximum was found at 270 m μ , which is characteristic for the diosphenol structure (VIa).⁹⁾ The infrared spectrum showed a hydroxyl band (3410cm⁻¹) and an α,β -unsaturated carbonyl band(1664 cm⁻¹); moreover, NMR analysis confirmed that the product has =C-H at C-1 (τ , 3.483) and C-OH at C-2 (τ , 4.025).

The diosphenol (VIa) was also obtained by the selenium dioxide oxidation of 24,25-dibromolanost-8-en-3-one (IVe) and the debromination of the resulting diosphenol (VIe) with zinc dust and acetic acid, but the yield was not better than in the first experiment.

Similarly lanost-8-en-3-one (IVb)¹⁰⁾ was oxidized to the diosphenol (VIb). The diosphenol (VIb) had already been prepared from lanosta-1,8-dien-3-one by McGhie, Palmer, and Rosemberger,¹¹⁾ but no directions for its preparation were given. Methyl 3-oxo-4,4,14 α -trimethyl- Δ^8 -cholenoate (IVc)¹²⁾ was also treated with selenium dioxide to give the diosphenol (VIc), but an analytically pure sample could not be obtained.



B) Method via 2-Bromo Compound—It has been reported in the classical paper of Windaus and Stein¹³⁾ that 3 β -hydroxycholestan-6-one acetate (VIII) (or -7-one (XI)) is transformed into the 6,7-dioxo compound (X) by bromination and treatment of the resulting 7-bromo-6-oxo compound (or 6-bromo-7-oxo compound) with silver nitrate in pyridine.

8) N. D. Cheronis, J. B. Entrikin : "Semimicro Qualitative Organic Analysis" 228 (1957) Interscience Publishers Ltd.

9) L. Dorfman : Chem. Rev., 53, 81 (1953).

10) L. Ruzicka, R. Denss, O. Jeger : Helv. Chim. Acta, 28, 759 (1945).

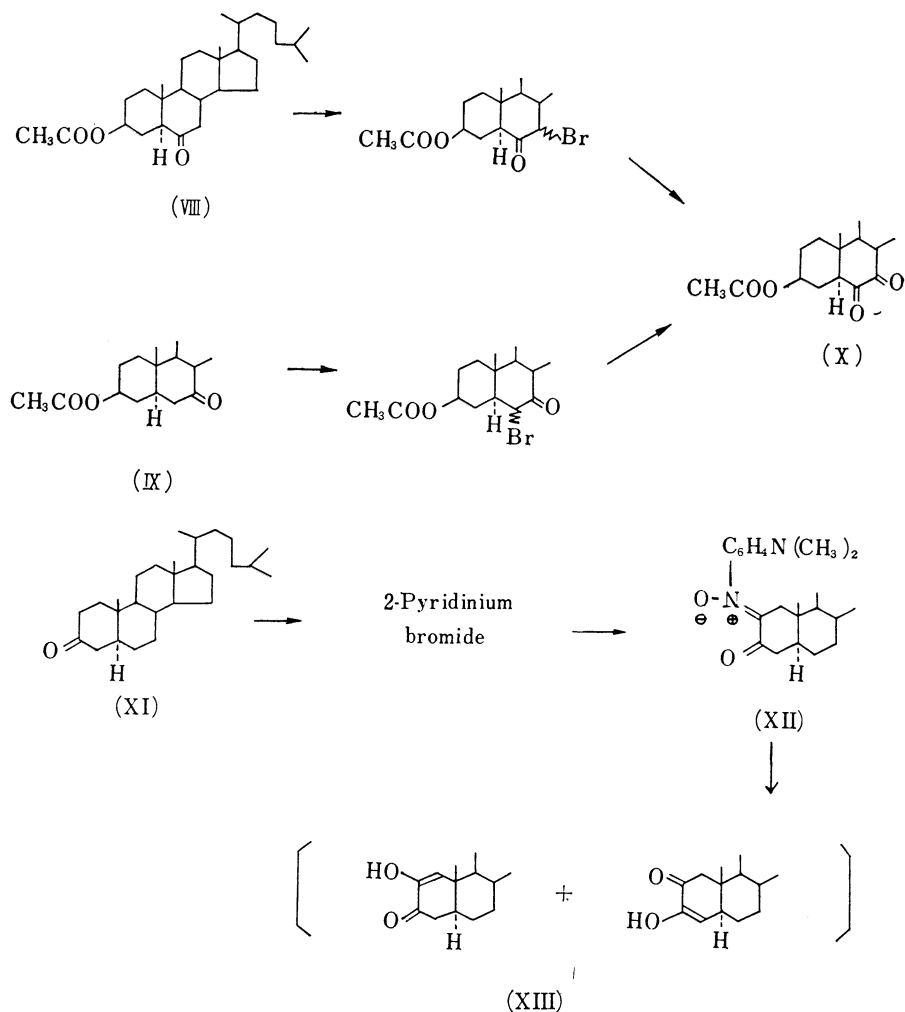
11) J. F. McGhie, P. J. Palmer, M. Rosemberger : Chem. & Ind. (London), 1959, 1221.

12) R. G. Curtis, H. Silberman : J. Chem. Soc., 1952, 1187.

13) A. Windaus, G. Stein : Ber., 37, 3699 (1904).

Lanost-8-en-3-one (IVb) was brominated to give the known 2α -bromo compound (Vb),¹⁴ which was treated with silver nitrate in pyridine solution to afford the diosphenol (VIb) in poor yield.

However, Ruzicka, Plattner and Furrer¹⁵ have reported that a mixture of the two enols of cholestan-2,3-dione is obtained by Kröhnke's method from cholestan-3-one (XI) via the 2-bromocholestan-3-one by its conversion to the 2-pyridinium bromide and condensation of this substance with *p*-nitroso-*N,N*-dimethylaniline to the nitrone (XII), which



on acid hydrolysis gave the mixture of the diosphenols (XIII). We tried to prepare the diosphenol by this method, but the pyridinium salt from the bromo-ketone (Vb) could not be obtained.

C) **Method via 2-oximino compound**—Huffman, *et al.*¹⁶ and Butenandt and Schäffler¹⁷ have reported that 16-oximinoestrone 3-methyl ether, prepared by oximation of

14) D.H.R. Barton, D.A. Lewis, J.F. McGhie : J. Chem. Soc., 1957, 2907.

15) L. Ruzicka, Pl. A. Plattner, M. Furrer : Helv. Chim. Acta, 27, 524 (1944).

16) M.N. Huffmann : J. Biol. Chem., 167, 273 (1947); 169, 167 (1947).

17) A. Butenandt, E.L. Schäffler : Z. Naturforsch., 1, 82 (1946).

estrone 3-methyl ether with isoamyl nitrite, is converted to the 16,17-diketone, 16-oxo-estrone 3-methyl ether by hydrolysis using sodium sulfite in aqueous acetic acid. It has been also reported by Sheehan and Erman¹⁸⁾ that the condensation of cholestan-3-one with isoamyl nitrite gave a 2-oximino compound, which on treatment with pyruvic acid in aqueous acetic acid afforded cholestane-2,3-dione.

Accordingly lanosta-8,24-dien-3-one (IVa) was condensed with isoamyl nitrite in the presence of potassium *t*-butoxide in *t*-butyl alcohol to give the 2-oximino compound (VIIa) in good yield, but we were unable to hydrolyze it to the diosphenol by Huffman's method. The diosphenol, however, was obtained in 60% yield by hydrolysis of the 2-oximino compound (VIIa) with pyruvic acid in aqueous acetic acid. The ester (IVc) was treated by the same method, and diosphenol (VIId) was obtained through the oximino compound (VIIId).

D) Autoxidation in the presence of potassium *t*-butoxide—Barton, *et al.*¹⁹⁾ have found that some oxo compounds when submitted to autoxidation in the presence of potassium *t*-butoxide yield diosphenols. This elegant method proved indeed very useful for our purpose. Lanosta-8,24-dien-3-one (IVa), when shaken in an oxygen atmosphere in the presence of potassium *t*-butoxide in *t*-butyl alcohol, was converted to the diosphenol (VIa) in good yield.

For the preparation of diosphenols from 4,4-dimethyl-5-en-3-oxo steroids, we prepared the starting materials by the method introduced by Woodward, *et al.*²⁰⁾ For the preparation of diosphenols from these compounds, only methods C and D were used in these experiments because of the superior yields obtained.

The condensation of 4,4-dimethyl-17 β -hydroxyandrost-5-en-3-one (XIVa)²¹⁾ with isoamyl nitrite gave smoothly the 2-oximino compound, which was hydrolyzed into the diosphenol (XVIIb) by pyruvic acid in aqueous acetic acid. It is interesting that simultaneously with the hydrolysis of the oximino group the esterification of the 17 β -hydroxyl group with pyruvic acid took place. Hydrolysis of (XVIIb) with sodium hydroxide at room temperature gave the diosphenol (XVIIa) and a small amount of the rearrangement product (XVII). It is well known that diosphenols, when treated with alkali in ethanol at refluxing temperature, undergo benzylic acid rearrangement to give a hydroxy acid.²²⁾

22 α -Spirost-4-en-3-one²³⁾ was methylated by the method of Woodward, *et al.*²⁰⁾ to give the 4,4-dimethyl compound (XIVc), which was transformed into the diosphenol (XVIIc) through the oximino compound (XVc) with satisfactory results.

It seemed especially interesting to prepare diosphenols by oxygen substitution in ring C, because both elatericin A and B have an oxygen atom in this ring. Desoxycholic acid seemed a satisfactory starting material for our purpose. At first, we prepared 12 α -hydroxy-3-oxo- Δ^4 -cholenic acid (XVIII) and 3,12-dioxo- Δ^4 -cholenic acid (XXII) according to the known methods.^{24,25)} The acid (XVIII) was methylated to give the 4,4-dimethyl compound (XIX), which was transformed into the diosphenol (XXIb) through the oximino compound (XX). The diosphenol (XXIa) was obtained from (XIX) by autoxidation.

The methylation of the acid (XXII) gave a nonconjugated diketone (XXIIIa). Undoub-

18) J. C. Sheehan, W. F. Erman : J. Am. Chem. Soc., **79**, 6050 (1957).

19) D. H. R. Barton, S. K. Pradhan, S. Sternhell, J. F. Templeton : J. Chem. Soc., **1961**, 255. D. Arigoni, D. H. R. Barton, E. J. Corey, O. Jeger : *Experientia*, **16**, 41 (1960).

20) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. I. Ives, R. B. Kelly : J. Chem. Soc., **1957**, 1131.

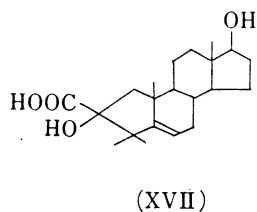
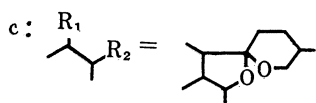
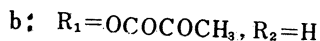
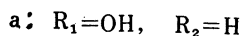
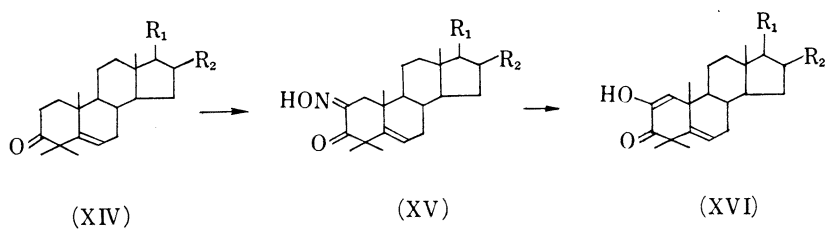
21) H. J. Ringold, G. Rosenkranz : J. Org. Chem., **22**, 602 (1957).

22) D. Lavie, S. Szinai : J. Am. Chem. Soc., **80**, 707 (1958).

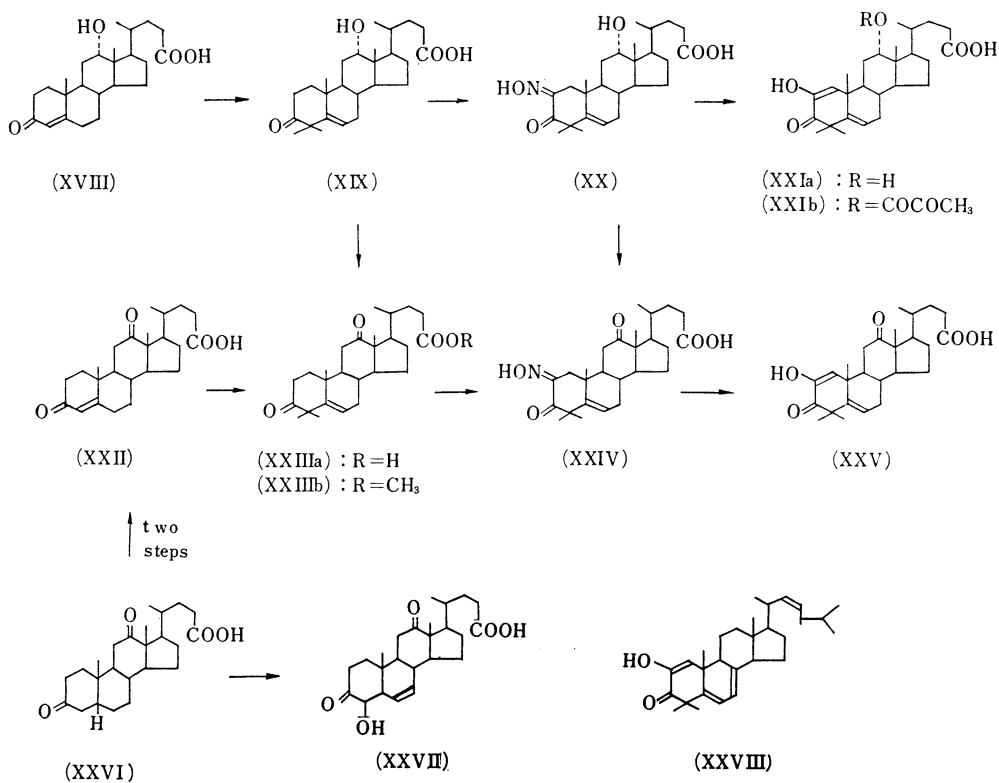
23) R. E. Marker, T. Tsukamoto, D. L. Turner : *Ibid.*, **62**, 2525 (1940).

24) V. Burckhardt, T. Reichstein : *Helv. Chim. Acta*, **25**, 821 (1942).

25) T. Sawlewicz, T. Reichstein : *Ibid.*, **20**, 992 (1937).



tedly methylation had taken place exclusively at C-4 position and not at C-11 position to give the 4,4-dimethyl compound. Not only had the new acid (XXIIIa) no characteristic absorption in the ultraviolet, but it could also be obtained by chromic anhydride oxidation of the acid (XIX) in acetone solution.²⁶⁾ Evidently the C-11 position is too much sterically hindered to be attacked by methyl iodide. Oximation of 4,4-dimethyl compound (XXIIIa) gave the compound (XXIV). Here again it was easily shown that the attack



of the reagent (isoamyl nitrite) occurred only in ring A, because the oximino compound (XX) was converted into oximino compound (XXIV) by chromic anhydride oxidation. The oximino compound (XXIV) was also prepared from the ester (XXIIIb) by oximation. The hydrolysis of (XXIV) gave the diosphenol (XXV) in good yield.

3,12-Dioxocholanic acid (XXVI) was autoxidized in the presence of potassium *t*-butoxide to give the diosphenol (XXVII). It was evident from the analysis that only one oxygen atom has entered into the starting material. Accordingly (XXVII) could be the enol form of one of the three diketones (2,3-, 3,4-, or 11,12-). From the ultraviolet spectrum²⁷⁾ it is deduced that the formula of an enol of the 3,4-diketone is the right one for (XXVII). 4,4-Dimethylergosta-5,7,22-trien-3-one²⁸⁾ was converted by the autoxidation into the diosphenol (XXVIII).

Only compound (VIIa) and (VIIb) have been tested by us in the cheekpouch test. The first substance showed an inhibition of tumor growth of 40%, while the effect of the second was questionable. It is interesting in this connection that also 2-hydroxycholesterol-1-en-3-one prepared by selenium dioxide reaction with cholestan-3-one has slight tumor growth inhibiting properties in the cheekpouch test. All these observations suggest that in these substances the biological action is closely related to the diosphenol structure.

Experimental*2

2-Oximinolanosta-8,24-dien-3-one (VIIa)—K metal (12.5 g.) was dissolved in *t*-BuOH (500 ml.), and lanosta-8,24-dien-3-one (IVa, 10.0 g.) was added. Isoamyl nitrite (10.0 ml.) was dropped into the stirred solution under a N₂ atmosphere, and stirring continued at room temperature for 1 hr. The solution was poured into H₂O, and acidified with HCl. The precipitate was filtered, washed with H₂O, and dried. Recrystallization from benzene-MeOH mixture gave the oximino compound (VIIa, 9.16 g.), m.p. 196~199°. An analytical sample was obtained as colorless needles by several recrystallizations from the same solvent, m.p. 206~208°, $[\alpha]_D^{20} + 182^\circ$ (c=0.61), IR: ν_{\max}^{KBr} cm⁻¹ 3309 (-OH), 1716 (C=O), 1616 (C=N). *Anal.* Calcd. for C₃₀H₄₇NO₂: C, 79.41; H, 10.44; N, 3.09. Found: C, 79.55; H, 10.61; N, 3.21.

2-Hydroxy-24,25-dibromolanosta-1,8-dien-3-one (VIe)—24,25-Dibromolanosta-8-en-3-one (IVe, 5.0 g.) was dissolved in boiling EtOH (100 ml.) and was added to a hot solution of SeO₂ (40 g.) in H₂O (22.5 ml.) and EtOH (100 ml.). On refluxing the mixture turned yellow and a red precipitate of Se was formed, which was removed by filtration after 15 min. of refluxing, and thoroughly washed with hot EtOH. The filtrate deposited a flocculent yellow precipitate on cooling which was discarded. The remaining clear solution was diluted with Et₂O, and washed with saturated salt solution, H₂O, and 5% NaHCO₃. The product was extracted with 40% KOH from the ethereal layer under cooling, and the alkali extract was acidified with HCl while cooling. The diosphenol was extracted with Et₂O, washed with H₂O, and dried over Na₂SO₄. Removal of Et₂O gave a solid residue (1.2 g.). It was chromatographed on silica gel (75 g.), and elution with benzene-petroleum ether (80 : 20) gave the diosphenol (VIe, 0.685 g.) as a pure material on recrystallization from a CHCl₃-MeOH mixture, m.p. 198~200°, $[\alpha]_D^{15} + 38^\circ$ (c=0.98), IR: ν_{\max}^{KBr} cm⁻¹ 3420 (-OH), 1680 (C=O). *Anal.* Calcd. for C₃₀H₄₆O₂Br₂: C, 60.20; H, 7.74; Br, 26.70. Found: C, 60.21; H, 7.90; Br, 26.81.

2-Hydroxylanosta-1,8,24-trien-3-one (VIa)—A) By SeO₂ oxidation of (IVa): Lanosta-8,24-dien-3-one (IVa, 5.0 g.) was treated with SeO₂ as described above. After the resulting Se was removed by filtration, Et₂O was added to the filtrate. The solution was washed with 5% NaHCO₃, 10% HCl, and H₂O, and dried over Na₂SO₄. The removal of the solvent gave a yellow solid (2.5 g.), which was chromatographed on silica gel (125 g.) Elution with benzene-petroleum ether (75 : 25) gave the crude diosphenol (IVa), which was recrystallized from a CHCl₃-MeOH mixture to give the pure diosphenol (VIa, 1.0 g.), m.p. 161~163°, $[\alpha]_D^{18} + 52^\circ$ (c=0.86), UV: $\lambda_{\max}^{\text{EtOH}}$ m μ (ϵ) 270 (9,000), IR: ν_{\max}^{KBr} cm⁻¹ 3410 (-OH), 1664 (C=O). *Anal.* Calcd. for C₃₀H₄₆O₂: C, 82.14; H, 10.57. Found: C, 82.38; H, 10.84.

B) By debromination of (VIe): 24,25-Dibromo-2-hydroxylanosta-1,8-dien-3-one (VIe, 0.10 g.) and Zn dust (0.20 g.) were mixed thoroughly and AcOH (7.0 ml.) was added. It was heated on a steam bath for 2 min. with stirring. After cooling, Et₂O and H₂O were added, and the Et₂O layer was

*2 All optical rotations were measured in CHCl₃ solution unless otherwise described. All melting points were uncorrected.

27) L. Dorfman: Chem. Rev., 53, 80 (1953).

28) G. Cooley, B. Ellis, V. Petrow: J. Chem. Soc., 1955, 2998.

decanted. It was washed with H₂O, 2% NaOH and H₂O and then dried over Na₂SO₄. Evaporation of the solvent gave a crystalline residue, which on recrystallization from a CHCl₃-MeOH mixture gave the diosphenol (VIa, 0.05 g), m.p. 157~159°. It showed a negative Beilstein test and was identical with the diosphenol obtained from (IVa) in all respects.

C) By hydrolysis of (VIIa): The oximino compound (VIIa, 5.52 g.) was refluxed with AcOH (350 ml.), H₂O (90 ml.), and pyruvic acid (20 ml.) overnight. The solution was poured into H₂O, and the product was extracted with Et₂O, washed with 5% K₂CO₃ and H₂O, and dried over Na₂SO₄. The solvent was evaporated to leave a yellow solid, which on recrystallization from a CHCl₃-MeOH mixture gave the diosphenol (VIa, 3.58 g.), m.p. 147~152°. Several recrystallizations from the same solvent afforded an analytical sample, m.p. 160~162.5°, which was identical in all respects with the diosphenol obtained from (IVa).

D) By autoxidation of (IVa): Lanosta-8,24-dien-3-one (IVa, 2.5 g.) was dissolved in *N* potassium *t*-butoxide in *t*-BuOH (200 ml.). The solution was shaken in an O₂ atmosphere (10 p.s.i. pressure) for 2 hr. and then poured into H₂O. The product was extracted with Et₂O, washed with H₂O, and dried over Na₂SO₄. The solvent was evaporated to leave a yellow solid, which on recrystallization from a CHCl₃-MeOH mixture gave the diosphenol (VIa, 1.74 g.), m.p. 152~154°. Further recrystallizations from the same solvent gave an analytical sample, which was identical in all respects with the diosphenol obtained by SeO₂ oxidation of (IVa).

2-Hydroxylanosta-1,8-dien-3-one (VIb)—A) By SeO₂ oxidation of (IVb): Lanosta-8-en-3-one (IVb, 5.0 g.) was treated with SeO₂ as described above. After removal of Se by filtration, the filtrate was extracted with Et₂O, and the solution was washed with H₂O, and 5% NaHCO₃, and shaken with 20% KOH under cooling. A pale yellow K-salt appeared at the interface. The aqueous layer was discarded and the Et₂O layer was decanted off from the K-salt. The decanted Et₂O layer was once more treated with 20% KOH as before. The combined K-salt was suspended in Et₂O, and shaken with 10% HCl. The new Et₂O layer was washed with H₂O and dried over Na₂SO₄. Removal of the solvent left a crystalline solid (1.3 g.), which was chromatographed on silica gel (50 g.). Elution with benzene gave a colorless crystalline material, which was recrystallized from CHCl₃-MeOH to give the diosphenol (VIb), m.p. 162~164°, $[\alpha]_D^{18} + 54^\circ (c=0.79)$, IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3400 (-OH), 1670 (C=O). *Anal.* Calcd. for C₃₀H₄₈O₂: C, 81.76; H, 10.98. Found: C, 81.63; H, 11.01.

B) By the method via the 2 α -bromo compound (Vb): Lanost-8-en-3-one (IVb, 4.26 g.) in AcOH (70 ml.) was treated under efficient stirring at 20° during 5 min. with a solution of Br₂ (0.55 ml.) in AcOH (30 ml.). The solution was allowed to stand overnight, and the resulting precipitate was filtered to give the crude 2 α -bromo compound, m.p. 122~128°.

The 2 α -bromo compound (Vb) obtained above (1.0 g.) was refluxed with pyridine (20 ml.) and AgNO₃ (2.0 g.) for 2 hr. Et₂O was added to the cold solution and the mixture was washed with 5% H₂SO₄ and H₂O. After drying over Na₂SO₄, the solvent was evaporated. The residue was recrystallized from a CHCl₃-MeOH mixture to give the diosphenol (VIb), m.p. 162~163°, which was identical with the diosphenol obtained from (IVb) by SeO₂ oxidation.

Methyl 2-Hydroxy-3-oxo-4,4,14 α -trimethyl- Δ^8 -5 α -choladienoate (VIc)—Methyl 3-oxo-4,4,14 α -trimethyl- Δ^8 -5 α -cholenoate (IVc, 0.75 g.) was treated with SeO₂ (6.0 g.) as described above. After removal of Se by filtration, Et₂O was added to the filtrate, and washed with half saturated salt solution, H₂O and 5% NaHCO₃. The ethereal layer was shaken with 40% KOH. After the K-salt had precipitated, the Et₂O layer was removed by decantation. The alkaline extract was acidified with HCl under cooling, and the product was extracted with Et₂O, and washed with H₂O. After drying over Na₂SO₄, the solvent was evaporated to leave a crystalline solid, which was chromatographed on silica gel (15 g.). Elution with benzene-AcOEt (95:5) gave the diosphenol (VIc), which on recrystallization from a CHCl₃-MeOH mixture gave the diosphenol as needles, m.p. 199~200°, $[\alpha]_D^{18} + 77^\circ (c=0.83)$, IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3500 (-OH), 1740 (C=O, at ester group), 1655~1660 (C=O, at C-3). Although no satisfactory analysis could be obtained of this substance, there can be no doubt about its constitution, because hydrolysis with ethanolic alkali at room temperature gave the diosphenol (VIb).

2-Oximino-3-oxo-4,4,14 α -trimethyl- Δ^8 -5 α -cholenic Acid (VIId)—Methyl 3-oxo-4,4,14 α -trimethyl- Δ^8 -5 α -cholenoate (IVc, 1.0 g.) was oximated as described above: K (1.35 g.), *t*-BuOH (50 ml.), and iso-amyl nitrate (2.0 ml.) were used. The solution was poured into H₂O, and acidified with AcOH. The precipitate was filtered, washed with H₂O, dissolved in MeOH (100 ml.) and *N* NaOH (200 ml.), and the solution was allowed to stand overnight. It was then acidified with HCl, and the product was extracted with Et₂O, washed with H₂O, and dried over Na₂SO₄. After evaporation of the solvent there remained a yellow solid, which on recrystallization from benzene gave the oximino compound (VIId, 0.6 g.), m.p. 213~215° (decomp.). Two recrystallizations from the same solvent gave an analytical sample. Colorless needles, m.p. 218~219° (decomp.), $[\alpha]_D^{19} + 144^\circ (c=0.66, \text{dioxane})$, IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3332, 3150 (-OH), 1739 (C=O, at carboxyl group), 1717 (C=O, at C-3), 1610 (C=N). *Anal.* Calcd. for C₂₇H₄₁NO₄: C, 73.10; H, 9.32; N, 3.16. Found: C, 73.48; H, 9.34; N, 3.67.

2-Hydroxy-3-oxo-4,4,14 α -trimethyl- Δ^8 -5 α -choladienic Acid (VIId)—The oximino compound (VIId, 0.43 g.) was refluxed with AcOH (10 ml.), H₂O (5.0 ml.), and pyruvic acid (1.5 ml.) overnight. The

solution was poured into H₂O, and the precipitate was filtered, washed with H₂O, and dried. Recrystallization from Me₂CO gave the diosphenol (VId, 0.34 g.), m.p. 219~223°. Two recrystallizations from Me₂CO gave an analytical sample as colorless needles, m.p. 222~224°, $[\alpha]_D^{20} + 17^\circ$ (c=0.39), UV : $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ) 270 (9000), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3443 (-OH), 1722 (C=O, at barboxyl group), 1676 (C=O, at C-3). *Anal.* Calcd. for C₂₇H₄₀O₄ : C, 75.66; H, 9.41. Found : C, 75.67; H, 9.42.

2-Oximino-17 β -hydroxy-4,4-dimethylandrosta-5-en-3-one (XVa)—17 β -Hydroxy-4,4-dimethylandrosta-5-en-3-one (XIVa, 12.78 g.) was oximated as described above : K (15.0 g.), *t*-BuOH (500 ml.), and iso-amyl nitrite (36.0 ml.) were used. The solution was poured into H₂O, and after washing AcOH with Et₂O, the aqueous layer was acidified with AcOH, and allowed to stand overnight. The precipitate was filtered, washed with H₂O, and dried. Recrystallization from MeOH gave the oximino compound (XVa, 9.76 g.), m.p. 248~249.5° (decomp.). Further recrystallization from MeOH afforded an analytical sample as colorless plates, m.p. 248.5~250° (decomp.), $[\alpha]_D^{20} + 95^\circ$ (c=0.93), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3356, 3209 (-OH), 1718 (C=O), 1625 (C=N). *Anal.* Calcd. for C₂₁H₃₁NO₃ : C, 73.00; H, 9.05; N, 4.05. Found : C, 73.48; H, 9.13; N, 4.30.

2,17 β -Dihydroxy-4,4-dimethylandrosta-1,5-dien-3-one 17-Pyruvate (XVib)—The oximino compound (XVa, 1.0 g.) was refluxed with AcOH (30 ml.), H₂O (15 ml.), and pyruvic acid (5 ml.) overnight. The solution was poured into H₂O, and the product extracted with Et₂O, and washed with H₂O, 5% K₂CO₃, and H₂O. After drying over Na₂SO₄, evaporation of the solvent left a yellow solid. Recrystallization of the residue from MeOH gave the diosphenol (XVib, 0.61 g.), m.p. 196~200°. By further recrystallization from MeOH an analytical sample was obtained as colorless needles, m.p. 200~202.5°, $[\alpha]_D^{20} + 47^\circ$ (c=0.38), IR : $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹ 3462 (-OH), 1728, 1680 (C=O, at C-3 and ester group). *Anal.* Calcd. for C₂₄H₃₂O₄· $\frac{1}{2}$ CH₃OH : C, 70.65; H, 8.23. Found : C, 70.70; H, 8.52.

The Hydrolysis of (XVib)—The pyruvate (XVib, 4.2 g) was dissolved in EtOH (500 ml.) and *N* NaOH (50 ml.), and allowed to stand overnight. The solution was poured into H₂O, and acidified with HCl. The product was extracted with Et₂O, washed with 5% K₂CO₃ (see below), and H₂O, and dried over Na₂SO₄. The solvent was evaporated to give a yellow gum, which was recrystallized from MeOH to afford 2,17 β -dihydroxy-4,4-dimethylandrosta-1,5-dien-3-one (XVIa, 2.97 g.), m.p. 150~155°. Further recrystallization from MeOH gave an analytical sample as colorless needles, m.p. 155.5~157°, $[\alpha]_D^{20} + 69^\circ$ (c=0.72), UV : $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ) 270 (8100), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3445, 3345 (-OH), 1694 (C=O). *Anal.* Calcd. for C₂₁H₃₀O₃ : C, 76.32; H, 9.15. Found : C, 76.15; H, 9.34.

The 5% K₂CO₃ layer described above was acidified with HCl, and extracted with Et₂O, the extract was washed with H₂O, and dried over Na₂SO₄. The solvent was evaporated to leave a yellow solid, which on recrystallization from Me₂CO gave 2,17 β -dihydroxy-3,3-dimethyl-A-norandrosta-5-ene-2-carboxylic acid (XVII, 0.23 g.), m.p. 256~257°. Further recrystallization from Me₂CO gave an analytical sample as colorless cubes, m.p. 258~259°, $[\alpha]_D^{19} + 9^\circ$ (c=0.96, dioxane), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3427 (-OH), 1720 (C=O). *Anal.* Calcd. for C₂₁H₃₂O₄ : C, 72.38; H, 9.26. Found C, 72.57; H, 9.22.

4,4-Dimethyl-22 α -spirost-5-en-3-one (XIVc)—To K metal (0.6 g.) dissolved in *t*-BuOH (50 ml.) was added 22 α -spirost-4-en-3-one (2.0 g.). A solution of MeI (1.9 ml.) in *t*-BuOH (10 ml.) was added dropwise at room temperature over a period of 30 min., and the stirring was continued for an additional 3 hr. The resulting suspension was poured into H₂O, and the product was extracted with Et₂O, washed with H₂O and dried over Na₂SO₄. The solvent was evaporated to leave a yellow solid, which on recrystallization from Me₂CO gave the dimethyl compound (XIVc, 1.4 g.), m.p. 200~206°. Two recrystallizations from the same solvent afforded an analytical sample as colorless cubes, m.p. 211~214°, $[\alpha]_D^{15} - 80^\circ$ (c=0.85), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 1714 (C=O), 1078 (C-O-C, five membered), 1051 (C-O-C, six membered). *Anal.* Calcd. for C₂₉H₄₄O₃ : C, 79.04; H, 10.07. Found : C, 78.68; H, 10.20.

2-Oximino-4,4-dimethyl-22 α -spirost-5-en-3-one (XVc)—The dimethyl compound (XIVc, 12.5 g.) was oximated as described above : K (12.6 g.), *t*-BuOH (600 ml.) and iso-amyl nitrate (20 ml.) were used. The solution was poured into H₂O, and acidified with HCl. The precipitate was filtered, washed with H₂O, and dried. Recrystallization from MeOH gave the oximino compound (XVc, 10.29 g.), m.p. 247~250° (decomp.). Further recrystallization from MeOH gave an analytical sample as colorless cubes m.p. 256~258°, $[\alpha]_D^{15} + 5^\circ$ (c=1.94), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3309 (-OH), 1723 (C=O), 1629 (C=N), 1078 (C-O-C, five membered), 1060 (C-O-C, six membered). *Anal.* Calcd. for C₂₉H₄₃NO₄ : C, 74.16; H, 9.23; N, 2.98. Found : C, 74.31; H, 9.24; N, 3.25.

2-Hydroxy-4,4-dimethyl-22 α -spirosta-1,5-dien-3-one (XVIc)—The oximino compound (XVc, 9.35 g.) was refluxed with AcOH (500 ml.), H₂O (160 ml.), and pyruvic acid (50 ml.) overnight. The solution was poured into H₂O, and the precipitate was filtered, washed with 5% K₂CO₃ and H₂O, and dried. Recrystallization from MeOH gave the diosphenol (XVIc, 7.9 g.), m.p. 178~184°. Two recrystallizations from MeOH gave an analytical sample as colorless needles, m.p. 185~187°, $[\alpha]_D^{15} - 29^\circ$ (c=1.21), UV : $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ) 270 (8500), IR : $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3462 (-OH), 1680 (C=O), 1077 (C-O-C, five membered), 1054 (C-O-C, six membered). *Anal.* Calcd. for C₂₉H₄₂O₄ : C, 76.61; H, 9.31. Found : C, 76.82; H, 9.52.

3-Oxo-12 α -hydroxy-4,4-dimethyl- Δ^8 -cholanic Acid (XIX)—12 α -Hydroxy-3-oxo- Δ^8 -cholanic acid (XVIII, 1.1 g.) was methylated as described above : K (0.7 g.), *t*-BuOH (60 ml.), and MeI (1.2 ml.) were

used. The resulting suspension was poured into H₂O and acidified with HCl. The product was extracted with Et₂O, and the extract was washed with H₂O, and dried over Na₂SO₄. After evaporation of the solvent a yellow solid was obtained, which on recrystallization from Me₂CO gave the dimethyl compound (XIX, 0.64 g.), m.p. 220~224°. Two recrystallizations from Me₂CO gave the dimethyl compound (XIX, 0.64 g.), m.p. 220~224°. Two recrystallizations from Me₂CO gave an analytical sample as colorless needles, m.p. 222.5~224.5°, $[\alpha]_D^{19} + 8^\circ$ (c=0.94), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3518 (-OH, at C-12), 3100 (-OH, at carboxyl group), 1719 (C=O). *Anal.* Calcd. for C₂₆H₄₀O₄: C, 74.96; H, 9.68. Found: C, 75.33; H, 9.61.

2-Oximino-12 α -hydroxy-4,4-dimethyl-3-oxo- Δ^5 -cholenic Acid (XX)—The dimethyl compound (XIX, 2.0 g.) was oximated as described above: K (1.7 g.), *t*-BuOH (70 ml.), and iso-amyl nitrite (2.1 ml.) were used. The solution was poured into H₂O, and acidified with HCl. The precipitate was filtered, washed with H₂O, and dried. Recrystallization from MeOH gave the oximino compound (XX, 1.30 g.), m.p. 223~225° (decomp.). Further recrystallization from MeOH gave an analytical sample as colorless needles, m.p. 233~234.5° (decomp.), $[\alpha]_D^{19} + 79^\circ$ (c=0.84, dioxane), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3318 (-OH), 1719 (C=O, at carboxyl group), 1707 (C=O, at C-3), 1618 (C=N). *Anal.* Calcd. for C₂₆H₃₈N₂O₅: C, 70.08; H, 8.82; N, 3.14. Found: C, 70.14; H, 8.66; N, 3.22.

3-Oxo-2,12 α -dihydroxy-4,4-dimethyl- $\Delta^{1,5}$ -choladienic Acid 12-Pyruvate (XXIb)—The oximino compound (XX, 0.7 g.) was refluxed with AcOH (30 ml.), H₂O (15 ml.), and pyruvic acid (5 ml.) overnight. The solution was poured into H₂O and the precipitate was filtered, washed with H₂O, and dried. It was chromatographed on silica gel (40 g.). Elution with benzene-AcOEt (80:20) gave the crude diosphenol (XXIb, 0.203 g.), which on recrystallization from a Me₂CO-hexane mixture gave an analytical sample, m.p. 202.5~204°, $[\alpha]_D^{19} + 114^\circ$ (c=0.49), UV: $\lambda_{\max}^{\text{EtOH}} \text{ m}\mu$ (ϵ) 270 (7900), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3427 (-OH), 1736 (C=O, at ester group), 1721 (C=O, carboxyl group), 1673 (C=O, at C-3). *Anal.* Calcd. for C₂₉H₄₀O₇: C, 69.57; H, 8.05. Found: C, 69.10; H, 8.14.

3-Oxo-2,12 α -dihydroxy-4,4-dimethyl- $\Delta^{1,5}$ -choladienic Acid (XXIa)—The dimethyl compound (XIX, 1.0 g.) was dissolved in a solution of *N* potassium *t*-butoxide in *t*-BuOH (100 ml.) and shaken in an O₂ atmosphere (10 p.s.i. pressure) for 2 hr. The solution was poured into H₂O, and acidified with HCl. The precipitate was filtered, washed with H₂O, and dried. It was chromatographed on silica gel (50 g.). Elution with benzene-AcOEt (80:20) gave the diosphenol (XXIa, 0.66 g.), m.p. 186.5~188.5°. Recrystallization from Me₂CO-hexane gave an analytical sample as colorless cubes, m.p. 186~187.5°, $[\alpha]_D^{19} + 45^\circ$ (c=0.78), UV: $\lambda_{\max}^{\text{EtOH}} \text{ m}\mu$ (ϵ) 270 (7300), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3453, 3285 (-OH, at C-2 and carboxyl group), 1719 (C=O, at carboxyl group), 1683 (C=O, at C-3). *Anal.* Calcd. for C₂₆H₃₈O₅: C, 72.52; H, 8.90. Found: C, 72.21; H, 8.61.

3,12-Dioxo-4,4-dimethyl- Δ^5 -cholenic Acid (XXIIIa)—A) From the acid (XXII): The acid (XXII, 5.0 g.) was methylated as described above: K (3.0 g.), *t*-BuOH (300 ml.), and MeI (6.5 ml.) were used. The resulting suspension was poured into H₂O, and acidified with HCl. The precipitate was filtered, washed with H₂O, and dried. Recrystallization from AcOEt gave the dimethyl compound (XXIIIa, 2.11 g.), m.p. 193~198°. By further recrystallization from AcOEt an analytical sample was obtained as colorless needles, m.p. 199~201°, $[\alpha]_D^{19} + 51^\circ$ (c=0.87), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3332 (-OH), 1739 (C=O, at carbonyl group), 1713, 1708 (C=O, at C-3 and C-12). *Anal.* Calcd. for C₂₆H₃₈O₄: C, 75.32; H, 9.24. Found: C, 75.50; H, 9.40.

B) From the dimethyl compound (XIX): The dimethyl compound (XIX, 0.3 g.) was dissolved in Me₂CO (60 ml.) and to it 8*N* CrO₃ solution (0.7 ml.)²⁹ was added dropwise with stirring at 15~20°. After stirring for 10 min. at the same temperature, the resulting suspension was poured into H₂O. The product was extracted with Et₂O, washed with H₂O, and dried over Na₂SO₄. Evaporation of the solvent gave a yellow solid, which on recrystallization from AcOEt gave the dimethyl compound (XXIIIa, 0.18 g.) m.p. 195~199°. The product was identical with the dimethyl compound obtained above.

Methyl-3,12-Dioxo-4,4-dimethyl- Δ^5 -cholenoate (XXIIIb)—The acid (XXIIIa, 1.5 g.) was refluxed with MeOH (100 ml.) and H₂SO₄ (2.5 ml.) overnight. The solution was poured into H₂O, and the product was extracted with Et₂O, washed with 5% K₂CO₃ and H₂O, and dried over Na₂SO₄. The solvent was evaporated to give a yellow solid, which on recrystallization from MeOH gave the ester (XXIIIb, 1.21 g.), m.p. 160~161.5°. Further recrystallization from MeOH provided an analytical sample as colorless prisms, m.p. 161~162°, $[\alpha]_D^{19} + 46^\circ$ (c=1.09), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 1740 (C=O, at ester group), 1712 (C=O, at C-3 and C-12). *Anal.* Calcd. for C₂₇H₄₀O₄: C, 75.66; H, 9.41. Found: C, 76.06; H, 9.16.

2-Oximino-3,12-dioxo-4,4-dimethyl- Δ^5 -cholenic Acid (XXIV)—A) From the acid (XXIIIa): The acid (XXIIIa, 0.5 g.) was oximated as described above: K (0.8 g.), *t*-BuOH (30 ml.), and iso-amyl nitrite (1.0 ml.) were used. The solution was poured into H₂O, and after washing with Et₂O the aqueous layer was acidified with HCl. The precipitate was filtered, washed with H₂O, and dried. Recrystallization from AcOEt afforded the oximino compound (XXIV, 0.55 g.), m.p. 227~230°. Two recrystallizations from AcOEt gave an analytical sample as colorless needles, m.p. 230~231°, $[\alpha]_D^{19} + 101^\circ$ (c=0.72), IR: $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 3223, 3128 (-OH), 1720 (C=O, at carboxyl group), 1705 (C=O, C-3 and C-12). *Anal.* Calcd. for C₂₆H₃₇N₂O₅: C, 70.40; H, 8.41; N, 3.16. Found: C, 70.42; H, 8.55; N 3.45.

B) From the ester (XXIIIb): The ester (XXIIIb, 1.2 g.) was oximated as described above: K (1.0 g.) *t*-BuOH (40 ml.), and iso-amyl nitrite (1.3 ml.) were used. The solution was poured into H₂O, and allowed to stand overnight. After acidification with HCl, the precipitate was filtered, washed with H₂O, and dried. Recrystallization from a Me₂CO-hexane mixture gave the oximino compound (XXIV, 0.7 g.), which was identical with the oximino compound obtained above in all respects.

C) From the oximino compound (XX): The oximino compound (XX, 0.099 g.) was suspended in Me₂CO (10 ml.). 8N CrO₃ solution (0.2 ml.) was added dropwise with stirring at 15~20° to the suspension, and the mixture was stirred for 10 min. at the same temperature. Thereafter it was poured into H₂O, and the precipitate filtered, washed with H₂O, and dried. Recrystallization from a Me₂CO-hexane mixture gave the oximino compound (XXIV, 0.049 g.), which was identical with the oximino compound obtained above.

2-Hydroxy-3,12-dioxo-4,4-dimethyl- $\Delta^{1,5}$ -choladienic Acid (XXV)—The oximino compound (XXIV, 6.98 g.) was refluxed with AcOH (280 ml.), H₂O (140 ml.), and pyruvic acid (45 ml.) overnight. The solution was poured into H₂O, and the precipitate was filtered, washed with H₂O, and dried. Recrystallization from Me₂CO gave the diosphenol (XXV, 4.1 g.), m.p. 191~194°. Two recrystallizations from the same solvent gave an analytical sample as colorless prisms, m.p. 195~197°, $[\alpha]_D^{25} + 115^\circ$ (c=0.31), UV: $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ) 270 (7,900), IR: $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3453, 3332 (-OH), 1720 (C=O, at carboxyl group and C-12), 1689 (C=O, at C-3). *Anal.* Calcd. for C₂₆H₃₆O₅: C, 72.86; H, 8.47. Found: C, 72.90; H, 8.44.

4-Hydroxy-3,12-dioxo- Δ^4 -cholenic Acid (XXVII)—3,12-Dioxocholanic acid (XXVI, 1.5 g.) was dissolved in a solution of *N* potassium *t*-butoxide in *t*-BuOH (150 ml.), and shaken under an O₂ atmosphere (15 p.s.i. pressure) for 2 hr. The solution was poured into H₂O, and acidified with HCl. The product was extracted with Et₂O, and the extract was washed with H₂O, and dried over Na₂SO₄. After evaporation of the solvent a yellow solid was obtained, which on recrystallization from MeOH gave the diosphenol (XXVII, 0.72 g.), m.p. 215~218°. Recrystallization from Me₂CO afforded an analytical sample as colorless needles, m.p. 222~224°, $[\alpha]_D^{25} + 115^\circ$ (c=1.19), UV: $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ) 278 (12,400), IR: $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3405, 3318 (-OH), 1752 (C=O, at carboxyl group), 1706 (C=O, at C-12), 1670 (C=O, at C-3). *Anal.* Calcd. for C₂₄H₃₄O₅: C, 71.61; H, 8.15. Found: C 71.57; H, 8.53.

2-Hydroxy-4,4-dimethylergosta-1,5,7,22-tetraen-3-one (XXVIII)—4,4-Dimethyl- $\Delta^{5,7,22}$ -ergostatrien-3-one (0.6 g.) was dissolved in a solution of *N* potassium *t*-butoxide in *t*-BuOH (50 ml.), and shaken under an O₂ atmosphere (8 p.s.i. pressure) for 2 hr. The solution was poured into H₂O, and acidified with HCl. The reaction product was extracted with Et₂O, and the extract was washed with H₂O, and dried over Na₂SO₄. Evaporation of the solvent gave a yellow solid, which on recrystallization from a Me₂CO-MeOH mixture afforded the diosphenol (XXVIII, 0.31 g.), m.p. 160~162.5°. Further recrystallization from the same solvents gave an analytical sample as colorless plates, m.p. 162.5~164°, $[\alpha]_D^{25} : -8^\circ$ (c=1.36), UV: $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ) 264 (13,700), IR: $\nu_{\text{max}}^{\text{KBr}}$ 3440 (-OH), 1676 (C=O). *Anal.* Calcd. for C₃₀H₄₄O₂: C, 82.51; H, 10.16. Found: C, 82.48; H, 10.19.

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Summary

Some 4,4-dimethyl-2-hydroxy- Δ^1 -3-one type steroids (diosphenols) were prepared as potential tumor growth inhibiting chemotherapeutics modeled on the cancer inhibiting elatericin B. These substances were obtained from 4,4-dimethyl-3-oxo steroids by selenium dioxide oxidation, by a method via the 2-bromo compound, by a method via the 2-oximino compound or by autooxidation. The last two procedures were found to be the most suitable for the preparation of the desired substances.

The diosphenols we obtained were as follows: 2-hydroxylanosta-1,8,24-trien-3-one (VIa), 2-hydroxylanosta-1,8-dien-3-one (VIb), 2-hydroxy-3-oxo-4,4,14 α -trimethyl- $\Delta^{1,8}$ -5 α -choladienic acid (VIc), 2,17 β -dihydroxy-4,4-dimethylandrosta-1,5-dien-3-one (XVIa), 2-hydroxy-4,4-dimethyl-22 α -spirosta-1,5-diene-3-one (XVIc), 3-oxo-2,12 α -dihydroxy-4,4-dimethyl- $\Delta^{1,5}$ -choladienic acid (XXIa), 2-hydroxy-3,12-dioxo-4,4-dimethyl- $\Delta^{1,5}$ -choladienic acid (XXVa), and 2-hydroxy-4,4-dimethylergosta-1,5,7,22-tetraen-3-one (XXVIII). 4-Hydroxy-3,12-dioxo- Δ^4 -cholenic acid (XXVII) was also prepared.

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29) A solution of CrO₃ (26.72 g.) in H₂SO₄ (23 ml.) diluted with H₂O to a volume of 100 ml. was used.