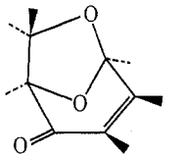
anhydro-2,3-dideoxy-Δ^2-β-D-pyranosen-4-one (isolevoglucosenone), a structural isomer of IV. A search for this compound in the pyrolysate is in progress.



V

According to the above mechanism, the transformation of I to IV does not involve a configuration change at asymmetric carbon 5. Models show that the enone of the D series is of a right-handed chirality. If IV is of this configuration, its skewed transoid α,β-unsaturated carbonyl system, which is inherently dissymmetric, should be manifested in a positive Cotton effect in the ORD spectrum.[20] The results showed this to be the case.

In addition to the elucidation of the structure of IV, two further questions require discussion. (1) Is IV a direct product of the pyrolysis process or a secondary compound formed during purification? (2) Is IV the same product isolated by the two other groups?

With respect to question 1, it is exceedingly unlikely that the mild conditions used during the work-up process before injection into the gc would alter a compound formed during the severe pyrolysis process. Isolation of the same compound using two different column packings and operating conditions strongly indicate that the product was not formed in the gc. Furthermore, a pmr spectrum obtained on the methylene chloride extract indicated that the identified end product is the major component of the tar mixture.

With respect to question 2, the principal preparation procedure of all three groups was quite similar. Although no direct comparison of the products was possible, our pmr spectrum and the comparable (*i.e.*, major) peaks of the mass spectrum corresponded closely to those observed for II[21] and III.[22] Furthermore, although we did not see the ir spectrum for III, that of II was fundamentally equivalent to that for IV. Finally, a sample of our material injected into the gc used by Lipska showed a retention time consistent with that found for III. Thus it is unlikely that more than one compound is involved.

Registry No.—I, 498-07-7; II, 25073-23-8; III, 37112-30-4; IV, 37112-31-5.

Acknowledgment.—We thank Dr. A. S. Newton, Lawrence Berkeley Laboratory, for the mass spectrometry analysis; Dr. R. E. Lundin, U. S. Agricultural Research Service, Albany, Calif., for the 100-MHz pmr spectra; Professor G. A. Olah and Dr. P. Westerman, Case Western Reserve University, and Dr. D. M. Wilson, University of California, Berkeley, for the cmr spectra. We gratefully acknowledge the financial support of Y. Halpern and R. Riffer under Grants AP00568 from the Environmental Protection Agency and GP-34494 from the National Science Foundation to the University of California Statewide Air Pollution Research Center. The contents of this paper do not necessarily reflect the views and policies of those agencies, nor does mention of trade names or commercial products constitute endorsement or recommendation for use by them or the U. S. Department of Agriculture.

(20) P. Crabbé, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Holden-Day, San Francisco, Calif., 1965, p 194.

(21) D. P. C. Fung, personal communication.

(22) A. E. Lipska, personal communication.

Terpenoids. LXVIII.[1] 23ξ-Acetoxy-17-deoxy-7,8-dihydroholothurinogenin, a New Triterpenoid Sapogenin from a Sea Cucumber[2]

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Received August 2, 1972

A new triterpenoid sapogenin was isolated and found to be 3β,20ξ-dihydroxy-23ξ-acetoxylanost-9(11)-ene-18-carboxylic acid lactone (18 → 20) (**5**). The functionality at C-23 is unprecedented in sapogenins from the sea cucumber.

Sapogenins from sea cucumbers have been very actively investigated in recent years. Structure proof of many of these compounds has been carried out.[1,5-12] All of these sapogenins have been found to be triterpenoids with a lanostane skeleton. These have included 22,25-oxidoholothurinogenin (**1a**) and its deoxy analog **1b** from *Actinopyga agassizi*[6] obtained by rigorous acid cleavage of saponins obtained from the Cuvier glands. Milder hydrolytic conditions[7] led to the isolation of 12β-methoxy-7,8-dihydroholothurinogenins of which **2** is an example. Enzymatic hydrolysis has led to a 12α-hydroxy analog. Using vigorous acid hydrolysis of the saponins from other sea cucumbers our group and others have found lanostane derivatives

(1) For part LXVII see P. Roller, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **35**, 2585 (1970).

(2) Financial assistance from the National Institutes of Health (Grant No. GM-06840) and a fellowship from the Rutgers University Research Council to I. R. is gratefully acknowledged.

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(5) For a recent review see J. S. Grossert, *Chem. Soc. Rev.*, **1**, 1 (1972).

(6) J. D. Chanley, T. Mezzetti, and H. Sobotka, *Tetrahedron*, **22**, 1857 (1966).

(7) J. D. Chanley and C. Rossi, *ibid.*, **25**, 1897, 1911 (1969).

(8) B. Tursch, I. S. de Souza Guimaraes, B. Gilbert, R. T. Aplin, A. M. Duffield, and C. Djerassi, *ibid.*, **23**, 761 (1967).

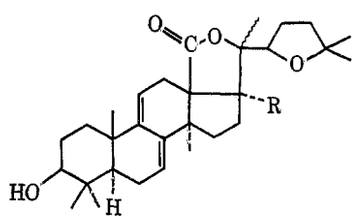
(9) G. Habermehl and G. Volkwein, *Justus Liebigs Ann. Chem.*, **731**, 53 (1970).

(10) P. Roller, C. Djerassi, R. Cloetens, and B. Tursch, *J. Amer. Chem. Soc.*, **91**, 4918 (1969).

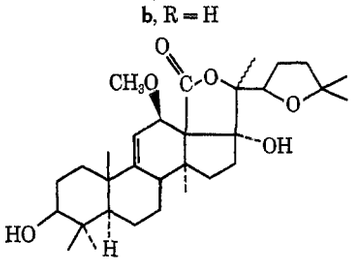
(11) B. Tursch, R. Cloetens, and C. Djerassi, *Tetrahedron Lett.*, 467 (1967).

(12) G. B. Elyakov, T. A. Kuznetsova, and Yu. N. Elkin, *ibid.*, 1151 (1969).

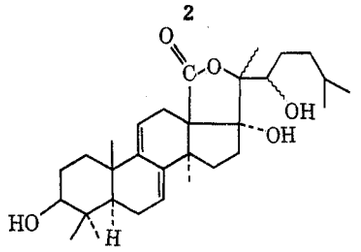
with a heteroannular diene system with variations in the side chain of which griseogenin[3] (**3**) is a representative example. Elyakov[12] has reported the isolation of two sapogenins containing homoannular diene systems (**4**).



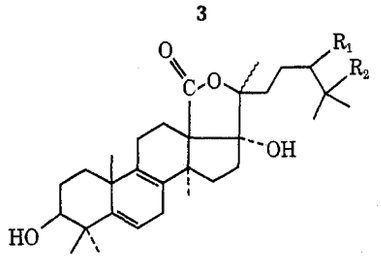
1a, R = OH
b, R = H



2



3

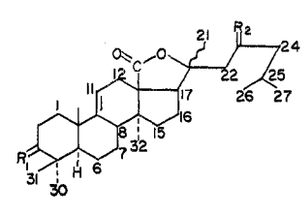


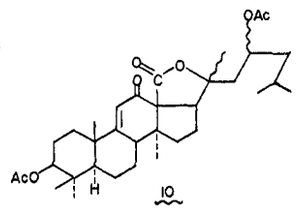
	R_1	R_2
4a,	=	
4b,	H	OH

We report here the isolation and structure proof of a new sapogenin, 23ξ-acetoxy-17-deoxy-7,8-dihydroholothurinogenin (**5**), isolated from the dried skins of *Stichopus chloronotus* Brandt found in the bay of Telukdalam, Nias Island, Indonesia. This sapogenin is highly unusual in having an acetoxy group at the 23 position and a double bond at the 9(11) position without a 12 alkoxy or hydroxy group.

High-resolution mass spectrometry established the empirical formula $C_{32}H_{50}O_5$ for **5**, and the ir spectrum showed absorption at 1760 cm^{-1} characteristic of a five-membered lactone.[1,6-11] The presence of acetate was demonstrated by an ir absorption band at 1735 cm^{-1}, a methyl peak at δ 2.02 in the nmr, and by the loss of acetic acid in the mass spectrum of **5**. There is essentially no uv absorption of **5**, thus showing the absence of a heteroannular diene system.

Treatment of **5** with acetic anhydride in pyridine yielded diacetate **6**. Oxidation of **5** with Jones reagent led to 23ξ-acetoxy-17-deoxy-7,8-dihydro-3-holothurinogenone (**7**). Hydrolysis of keto acetate **7** with hydroxide in methanol led to the keto alcohol **8** and hydolysis of (**5**) itself gave 23ξ-hydroxy-17-deoxy-7,8-dihydroholothurinogenin (**9**).





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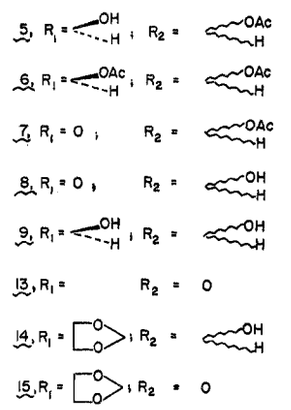
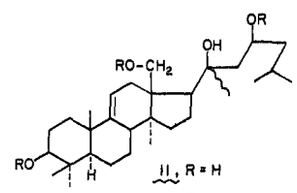




11, R = H
12, R = Ac

The nmr spectrum of **5** showed the presence of seven methyl groups in addition to an acetate methyl. The number and overall similarity of the position of the methyl absorptions to previously reported work[6-11] suggests the presence of a lanostane skeleton.

The β configuration of the C-3 hydroxyl group is indicated by the position of the nmr absorption at δ 3.19 in compound **5** and 3.20 in compound **9**. The 3α-proton signal in a large number of 3β-lanostane alcohols is known to occur at δ 3.18–3.30,[1,8,9,13-15] whereas the 3β proton in 3α alcohols is downfield from this.

Reduction of ketone **7** with sodium borohydride regenerated **5**. It has been reported previously that reduction of the 3-ketone function in lanostane derivatives with sodium borohydride leads to the 3β alcohol.[6] The location of the hydroxyl group is also very strongly indicated by the properties of the ketones **7**, **8**, and **13**. The nmr spectra of **7** and **8** show that the methyl groups at C-30 and -31 have been deshielded by the adjacent carbonyl when compared to the corresponding alcohols **5** and **9**. The C-30 and -31 methyls appear at δ 1.06 as a singlet in **7** and at 1.08 as a singlet in **8** and **13** whereas in the parent alcohols the 30 and 31 methyl groups appear upfield from δ 1.0 and appear as a doublet of methyl groups. This feature has been reported previously for 19-nor-4,4-dimethyl-5α-androstan-17β-ol-3-one.[16] The CD spectrum of **7**, $[\theta]_{302}$ −1552 (Figure 1), is essentially identical in appearance and amplitude with that of $\Delta^{9(11)}$-lanosten-3-one, $[\theta]_{302}$ −1556 (measured in our laboratory). The CD spec-

(13) N. Entwistle and A. D. Pratt, *Tetrahedron*, **24**, 3949 (1968).
(14) J. Fried, P. Grabowich, E. F. Sabo, and A. I. Cohen, *ibid.*, 2297 (1964).
(15) For a comparison of some 3α- and 3β-lanostane derivatives see H. K. Adam, T. A. Bryce, I. M. Campbell, and N. J. McCorkindale, *Tetrahedron Lett.*, 1461 (1967).
(16) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, p 167.

trum indicates also the 5α configuration which has been found in all other sea cucumber sapogenins.

The location of the double bond was established by the following findings. There is a single olefinic proton in the nmr spectra of **8**, **9**, **10**, and **13**. The olefinic proton in **5** and **6** is masked because the proton at C-23 is superimposed upon it. There are three possible positions (Δ^5, Δ^7, or $\Delta^{9(11)}$) where the double bond could be located. From the CD spectrum (Figure 1) of **7** the 5,6 position could be excluded since the Cotton effect would be expected to be positive.[17] The 9,11 position was the most reasonable because of the very close resemblance of the CD spectrum of **7** with that of $\Delta^{9(11)}$-lanosten-3-one. The Cotton effect of Δ^7-lanosten-3-one is negative,[17] but its amplitude is different.[18] Very significant evidence for the $\Delta^{9(11)}$ position is found in the ORD spectrum of **10** prepared by chromic acid oxidation of **6** in refluxing acetic acid[19] (Figure 1). There is a very close resemblance to the spectrum of 12-oxolanost-9(11)-en-3β-yl acetate.[20] This similarity suggests a $\Delta^{9(11)}$ olefin with an 8β-hydrogen, 13β-carboalkoxy, and 14α-methyl group, since the ORD spectrum of a 6-oxo-7-ene chromophore would be expected to be opposite in sign.[21] The appearance of the olefinic proton signal in the nmr spectrum of **10** is very similar to that of the proton at C-11 in 12-oxolanost-9(11)-en-3β-yl acetate. There is a sharp doublet at δ 5.75 (J = 2 Hz) for the olefinic proton of **10** resulting from coupling with the axial 8β proton. The doublet disappears upon irradiation at δ 3.33 of the C-8 proton. This compares closely with the nmr spectra of the 12-oxo derivative of arborinol[18] and 12-oxolanost-9(11)-en-3β-yl acetate which show a sharp doublet (J = 2 Hz) for the olefinic proton resulting from coupling to the 8β proton.

The position of the acetoxy group in the side chain of **5** was established in the following manner. Both hydroxyl groups of **9** were shown to be secondary by acetylation and by nmr spectral analysis. This could also be clearly deduced by the nmr spectra of compounds **8** and **10**. Compound **9** upon treatment with acetic anhydride in pyridine yielded 23ξ-acetoxy-17-deoxy-7,8-dihydroholothurinogenin 3β-acetate (**6**), which was reduced with lithium aluminum hydride to the tetraol **11**. Tetraol **11** upon treatment with acetic anhydride–pyridine yielded a triacetate **12**, one of the hydroxyl groups not being acetylated because it is tertiary. The nmr spectrum of the triacetate **12** shows for the 18-CH_2OAc an AB quartet (J = 11 Hz, geminal coupling) which has been reported previously.[1,6] Treatment of the tetraol **11** with lead tetraacetate yields only starting material indicating that the acetoxy group in **5** is not at position 2 or 22.

Oxidation of **9** with Jones reagent led to the dione **13**, whose ir spectrum showed carbonyl absorption at 1755 (lactone) and at 1710 cm^{-1}. The carbonyl absorption at 1710 was larger than the lactone carbonyl absorption whereas in the mono ketone **8** the lactone carbonyl was slightly larger suggesting that the 1710-cm^{-1} band was being enhanced[22] by a new carbonyl group which is located in a six-membered ring or in the side chain. Dione **13** in neutral ethanol had essentially no uv spectrum but when the solution was made 0.01 M in potassium hydroxide an absorption appeared (λ_{max} 252 nm (ε 8300)). A 1,3 diketone was considered as a possible structure but was eliminated for the following reasons. 1,3-Diketolanostane derivatives are known[23] and have λ_{max} 256 nm (ε 11,000) in neutral ethanol and λ_{max} 286 nm (ε 24,000) in ethanol made 0.01 M in sodium hydroxide. Lanostane-1,3-dione and lanost-8-ene-1,3-dione readily form 3-acetoxylanost-2-en-1-one derivatives upon treatment with acetic anhydride in pyridine, whereas **13** did not form such a derivative. The strongest evidence that the acetoxy group is not in ring A comes from an examination of the properties of the 3-ethylene ketal **15**. The base peak in the mass spectrum of **15** is at m/e 99 indicating ring A is not substituted at position 1 or 2.[24] The ir spectrum of **15** shows carbonyl absorption at 1760 (lactone C=O) and at 1710 cm^{-1} indicating more clearly than could be seen in the spectrum of **13** that the carbonyl group is in the side chain or in a six-membered ring. Compound **15** showed essentially no uv absorption in neutral ethanol, but when the solu-

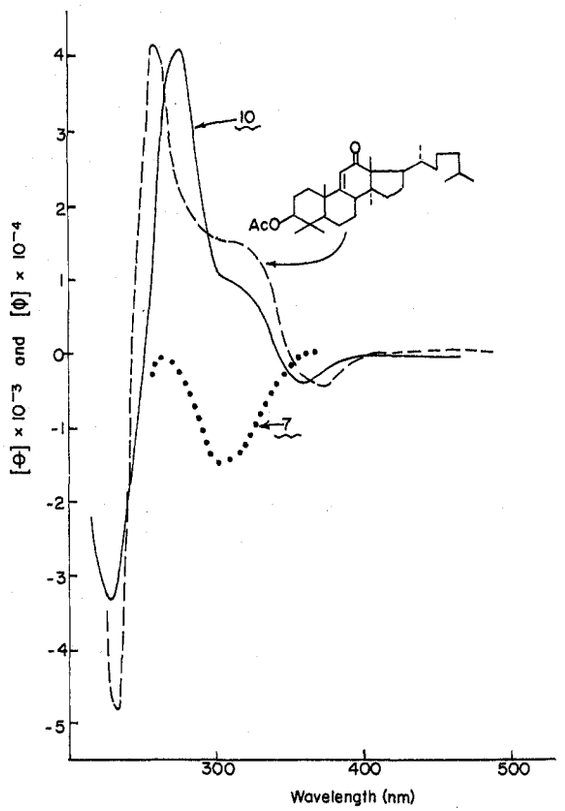


Figure 1.—CD spectrum of **7**, [θ]; ORD spectrum of **10** and 12-oxolanost-9(11)-en-3β-yl acetate, [φ].

(17) C. Djerassi, O. H. Halpern, V. Halpern, and B. Riniker, *J. Amer. Chem. Soc.*, **80**, 4001 (1958).

(18) For the ORD spectrum of $\Delta^{9(11)}$-lanosten-3-one see H. Vorbrüggen, S. C. Pakrashi, and C. Djerassi, *Justus Liebigs Ann. Chem.*, **668**, 57 (1963).

(19) H. R. Bentley, J. A. Henry, D. S. Irvine, and F. S. Spring, *J. Chem. Soc.*, 3673 (1953).

(20) We wish to thank Dr. Richard Muccino of this laboratory for a sample of this compound.

(21) C. Djerassi, R. Riniker, and B. Riniker, *J. Amer. Chem. Soc.*, **78**, 6377 (1956); J. A. Beisler and Y. Sato, *J. Org. Chem.*, **36**, 3946 (1971).

(22) R. N. Jones, D. A. Ramsay, D. S. Keir, and K. Dobriner, *J. Amer. Chem. Soc.*, **74**, 80 (1952).

(23) D. H. R. Barton, P. J. L. Daniels, J. F. McGhie, and P. J. Palmer, *J. Chem. Soc.*, 3675 (1963).

(24) (a) Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, **86**, 3722 (1964); (b) H. Audier, J. Bottin, A. Diara, M. Fétizon, P. Foy, M. Golfier, and W. Vetter, *Bull. Soc. Chim. Fr.*, 2292 (1964).

tion was made 0.01 M in potassium hydroxide an absorption appeared at λ 252 nm (ϵ 8300). Clearly the chromophore is not a lanostene-1,3-dione since the 3 position is tied up as an ethylene ketal and hence cannot be implicated.

Substitution at the 7 position could be excluded in the following manner. Ketone **15** and diketone **13** were dissolved in ethanol and made 0.01 M in potassium hydroxide. Each was then recovered and the ir spectrum taken. In each case no conjugated carbonyl absorption was present. The ir spectrum was essentially identical with the starting material ir. This excludes an 8-en-7-one.[23] A 6-one should not have uv absorption.

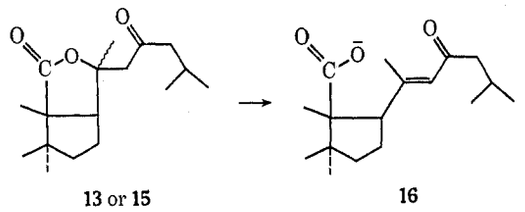
Ring D can be excluded for several reasons. The ir carbonyl absorption of **15** and **13** indicates that there is no carbonyl group in a five-membered ring. Furthermore the lack of chemical shift for the 32-methyl group downfield from δ 0.88 in **13** is indicative of the absence of a 15 ketone.[14,25] If the acetoxy group in **5** were in the 16 position, it would have to possess the α orientation. This can be seen from a comparison (Table I) of the molecular rotations of **5** *vs.* **9** and from

TABLE I
MOLECULAR ROTATIONS OF ACETATES AND ALCOHOLS

Compound	$[M]_D$ ($CHCl_3$)	$[M]_{acetate} - [M]_{alcohol}$
5	−102	−96
9	−6.40	
7	−189	−114
8	−75	

7 *vs.* **8**. The more negative value of the rotation of the acetates would indicate a 16α substituent.[26-28] The chemical shift for the 32-methyl group of **5** and all of its derivatives is upfield from δ 1.0. This is inconsistent with the 1–3 interaction of a 16α-oxygen and 32-methyl group.[6,7,29]

The location of the acetoxy group of **5** is thus limited to either the 23 or 24 positions. The 24 position can be excluded from the nmr spectrum of **13**. The nmr spectrum of 3β-acetoxylanost-8-en-24-one has been reported[30] with the 26- and 27-methyl group having signals at δ 1.03 and 1.14, respectively. This is inconsistent with the spectrum of **13**, in which the 26- and 27-methyl groups display a doublet centered at δ 0.93. Confirmation that the acetoxy group is at the 23 position is provided by the uv spectrum of **13** and **15** in basic ethanol and by the mass spectrum of some of the derivatives of **5**. The uv spectrum of **13** and **15**



13 or 15 16

in basic ethanol can be readily rationalized in terms of a base-catalyzed β elimination as shown above. The λ_{max} 252 nm of **16** is in reasonable agreement with the reported value of 248 nm for 5α-cholesta-9(11),20(22)-diene-3β,6α-diol-23-one.[31]

The mass spectal fragmentations shown in Table II

TABLE II
DIAGNOSTIC PEAKS IN THE MASS SPECTRA OF TRITERPENOID LACTONES

	Compound				
	5	**7**	**8**	**9**	**13**
M^+	514.36133	512	470.34131	472.352539	468
$M - C_4H_9$			413.26929	415.282227	411
$M - C_4H_9 + CO$					383
M − side chain	353.28829[a]	369	369.24365	371.260254	325[b]

[a] Loss of side chain and loss of water. [b] Loss of side chain and loss of CO_2.

are readily rationalized by structure **5**. Compounds **8** and **9** show loss of C_4H_9 and loss of the side chain ($C_6H_{13}O$). Both of these fragments would be expected to be the typical products[32] of α fission of a C-23 alcohol. In **5** and **7** there is a loss of $C_8H_{15}O_2$ which represents loss of the side chain containing an acetoxy group. The diketone **13** shows loss of C_4H_9 and $C_4H_9 + CO$. This can be represented as α fission at the carbonyl followed by loss of CO, typical fragments that would be expected from a C-23 ketone.[32]

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. All optical rotations were determined using chloroform as solvent. Infrared spectra were obtained using a Perkin-Elmer Model 421 grating spectrophotometer. Ultraviolet spectra were measured in 95% ethanol and in the cases mentioned in 95% ethanol made 0.01 M in potassium hydroxide on a Cary 14 spectrophotometer. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian HA-100 or XL-100 spectrometer using deuteriochloroform as solvent. Tetramethylsilane was used as internal reference and line positions are given in the δ scale. Microanalyses were carried out by Messrs. E. Meier and J. Consul. Low-resolution mass spectra (70 eV) were carried out on AEI MS-9, Atlas CH-4, and Varian MAT 711 instruments with direct inlet systems. High-resolution spectra were determined on the MS-9 and MAT-711 instruments.

Gas–liquid chromatography (glpc) was carried out on a Hewlett-Packard 402 high efficiency instrument with glass columns packed with 3% of OV-25 on Gas-Chrom Q (100–120 mesh) from Applied Science Laboratories, Inc. Column chromatography was carried out using Davison 50–200 mesh activated silica gel and E. Merck neutral, activity grade II, aluminum oxide. Analytical thin layer chromatography (tlc) was carried out on 5 × 20 cm, 250-μ silica gel HF_{254} plates. When necessary, substances were made visible by exposure to iodine vapors or by spraying with ceric sulfate solution (2% in 1 M sulfuric acid) followed by heating on a hot plate. Preparative-scale tlc was carried out or 20 × 20 cm, 1000-μ silica gel HF_{254} plates.

We thank Dr. L. J. Durham for the nmr spectra, Mr. R. Ross, Mr. R. Conover, and Miss A. Wegeman for the mass spectra, and Mrs. R. Records for the ORD spectra.

Isolation of the Saponin from *Stichopus chloronotus*.[33]—Dried skins (500 g) from *Stichopus chloronatus* were stirred in a blender

(25) A. I. Cohen, D. Rosenthal, G. W. Krakower, and J. Fried, *Tetrahedron*, **21**, 3171 (1965).
(26) F. C. Chang and C. K. Chiang, *Chem. Commun.*, 1156 (1968).
(27) A. Bowers, T. G. Halsall, and G. C. Sayer, *J. Chem. Soc.*, 3070 (1954).
(28) W. Klyne and W. M. Stokes, *ibid.*, 1979 (1954).
(29) For the interaction of a 16α-hydroxy or acetoxy group on the 32-methyl substituent in cucurbitanes see D. Lavie, B. S. Benjaminov, and Y. Shvo, *Tetrahedron*, **20**, 2585 (1964).
(30) D. H. R. Barton, D. M. Harrison, G. P. Moss, and D. A. Widdowson, *J. Chem. Soc. C*, 775 (1970).
(31) Y. M. Sheikh, B. M. Tursch, and C. Djerassi, *J. Amer. Chem. Soc.*, **94**, 3278 (1972).
(32) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967.
(33) This experiment was carried out by Dr. R. J. Liedtke.

with 2 l. of 75% ethanol and allowed to stand overnight. This was filtered through Celite and then extracted a second time with 2 l. of 50% ethanol and filtered. The combined filtrates were evaporated at reduced pressure on a rotary evaporator. The residue was dissolved in 1 l. of water and carefully washed with benzene. The aqueous layer was extracted with 1-butanol (1 l.). The 1-butanol was evaporated, and the residue was dissolved in water (500 ml) and washed with ethyl ether. The saponin was removed from the aqueous layer by extraction into butanol. After evaporation of the butanol there was obtained 22 g of crude saponin. This was found to be toxic to guppies.

Isolation of 23ξ-Acetoxy-17-deoxy-7,8-dihydroholothurinogenin (5).—Saponin (21 g) was dissolved in 1 l. of 2.5 *N* hydrochloric acid and heated on a steam bath for 3 hr. After cooling the mixture was extracted with chloroform. The chloroform was washed with water and sodium bicarbonate, dried (magnesium sulfate), and evaporated to give 15 g of semisolid. Chromatography on silica gel (700 g) using gradient elution with benzene–ether and several recrystallizations gave 1.1 g of **5** in greater than 90% purity by glpc. Nonhomogeneous materials showed a single spot by tlc identical with pure genin **5**: mp 223–224° (from methanol); $[\alpha]^{20}D$ −20° (*c* 0.74); ir (KBr) 3430 (broad), 1760 (lactone C=O), 1735 (ester C=O), 1450, 1370, 1240, 1170, 1030, 940 cm^{-1}; essentially no uv absorption above 210 nm; nmr δ 0.83 (3, s, CH_3-32), 0.87 (3, s, CH_3-31), 0.91 (6, d, *J* = 6 Hz, CH_3-26, 27), 0.98 (CH_3-30), 1.15 (3, s, CH_3-19), 1.40 (3, s, CH_3-21), 2.03 (3, s, $OCOCH_3$), 2.95 (1, broad, CH-8), 3.19 (1, broad, CH-3), 5.17 (2, broad, CH-11 and CH-23); mass spectrum *m/e* (rel intensity) 514 (23, M^+), 512 (3), 499 (3, M − CH_3), 496 (2, M − H_2O), 481 (2, M − CH_3 + H_2O), 454.34204 (4, M − CH_3COOH requires 454.34448), 439.31958 (8, M − CH_3COOH + CH_3 requires 439.32104), 421.31128 (9, M − CH_3COOH + H_2O + CH_3 requires 421.31055), 395.32910 (25, M − CO_2 + CH_3COOH + CH_3 requires 395.33130), 353.24829 (3, M − side chain ($C_8H_{15}O_2$) + H_2O requires 353.24780), 95 (16), 81 (24), 69 (28), 55 (25), 43 (100).

Anal. Calcd for $C_{32}H_{50}O_5$: C, 74.65; H, 9.80; mol wt, 514.36572. Found: C, 74.69; H, 9.60; mol wt (mass spectrometry), 514.36133.

Preparation of 23ξ-Acetoxy-17-deoxy-7,8-dihydroholothurinogenin 3β-Acetate (6).—Compound **5** (0.650 g) was treated with 1:1 pyridine–acetic anhydride at room temperature and worked up in the usual way. The crude reaction product was chromatographed on silica gel and recrystallized from methanol–water to give 0.602 g of **6**: mp 192–194°; $[\alpha]^{20}D$ −3.28 (*c* 0.6); ir (KBr) 1760 (lactone C=O), 1735 (ester C=O), 1460, 1370, 1240, 1170, 1130, 1030 cm^{-1}; essentially no uv absorption above 210 nm; nmr δ 0.85. (6, s, CH_3-31, 32), 0.90 (3, s, CH_3-30), 0.91 (6, d, *J* = 6 Hz, CH_3-26, 27), 1.17 (s, CH_3-19), 1.40 (s, CH_3-21), 2.02 (6, s, $OCOCH_3$), 2.95 (1, broad, CH-8), 4.50 (1, M, CH-3), 5.18 (2, broad, CH-11 and CH-23); mass spectrum *m/e* (rel intensity) 556 (71, M^+), 554 (7), 541 (7, M − CH_3), 496 (23, M − AcOH), 481 (24, M − AcOH + CH_3), 437 (64, M − AcOH + CO_2 + CH_3), 436 (7, M − 2 AcOH), 421 (51, M − 2 AcOH + CH_3), 353 (7, M − side chain + CH_3COOH), 325 (18), 127 (37), 109 (62), 81 (42), 69 (50), 55 (97), 43 (100).

Anal. Calcd for $C_{34}H_{52}O_6$: C, 73.33; H, 9.42. Found: C, 73.60; H, 9.23.

23ξ-Acetoxy-17-deoxy-7,8-dihydro-3-holothurinogenone (7).—Compound **5** (20 mg) was dissolved in 15 ml of acetone and cooled to 0°. Jones reagent (CrO_3, 10 g, and sulfuric acid, 8.0 g, diluted to 37 ml with water) was added slowly with stirring until an orange color persisted. The excess oxidizing agent was destroyed by adding 2-propanol and work-up in the usual manner, and recrystallization from methanol–water gave 15 mg of **7**: mp 217–218°; $[\alpha]^{20}D$ −37° (*c* 0.4); CD (dioxane) $[\theta]_{302}$ −1552; ir (KBr) 1760 (lactone C=O), 1735 (ester C=O), 1710 (C=O in six-membered ring), 1460, 1435 (methylene adjacent to C=O in a six-membered ring), 1375, 1280, 1245, 1165, 1140, 1110, 1010, 935 cm^{-1}; nmr δ 0.86 (3, s, CH_3-32), 0.93 (6, d, *J* = 6 Hz, CH_3-26, 27), 1.06 (6, s, CH_3-30, 31), 1.34 (3, s, CH_3-19), 1.40 (3, s, CH_3-21), 3.0 (1, m, CH-8), 5.22 (2, broad, CH-11 and CH-23); mass spectrum *m/e* (rel intensity) 512 (82, M^+), 510 (9), 497 (4, M − CH_3), 452 (49, M − AcOH), 437 (34, M − AcOH + CH_3), 407 (24), 393 (100, M − AcOH + CO_2 + CH_3), 369 (7, M − side chain ($C_8H_{15}O_2$)), 323 (19), 295 (29), 281 (27), 269 (24), 255 (15), 171 (13), 157 (12), 145 (18), 127 (32), 109 (35), 95 (27), 81 (35), 69 (37), 55 (40), 43 (61).

Anal. Calcd for $C_{32}H_{48}O_5$: C, 74.95; H, 9.44. Found: C, 74.90; H, 9.17.

Reduction of Ketone 7.—Ketone **7** (10 mg) in 6 ml of dioxane and 0.4 ml of water was allowed to react with 15 mg of sodium borohydride at room temperature for 4 hr. Work-up in the usual manner, gradient elution chromatography on alumina (benzene–ether), and recrystallization gave 3 mg of material identical with **5** by ir, mass spectrum, mp, mmp, and tlc.

23ξ-Hydroxy-17-deoxy-7,8-dihydro-3-holothurinogenone (8).—Keto acetate **7** (25 mg) was hydrolyzed by refluxing with 5% potassium hydroxide in methanol, worked up in the usual way to give 20 mg of crude product, and recrystallized (methanol–water) to give pure **8**: mp 174–176°; $[\alpha]^{20}D$ −16° (*c* 0.3); ir (KBr) 3450, 1755 (lactone C=O), 1705 (C=O at C_3), 1460, 1380, 1260, 1160, 1110, 1030, 940, 800 cm^{-1}, shows essentially no uv absorption above 210 nm; nmr δ 0.88 (3, s, CH_3-32), 0.92 (6, d, *J* = 6 Hz, CH_3-26, 27), 1.08 (6, s, CH_3-30, 31), 1.34 (3, s, CH_3-19), 1.53 (3, s, CH_3-21), 3.98 (1, m, CH-23), 5.27 (1, m, CH-11); mass spectrum *m/e* (rel intensity) 470 (100, M^+), 468 (19), 455 (7, M − CH_3), 452 (4, M − H_2O), 437 (7, M − CH_3 + H_2O), 413.26929 (12, M − C_4H_9 by high-resolution mass spectrum), 407 (8), 393.31665 (20, M − CH_3 + CO_2 + H_2O), 384.26538 (23, M − $C_5H_{10}O$ (ring A cleavage[34] or side chain cleavage), 369.24365 (35, M − $C_6H_{13}O$ (side chain)), 325.25024 (14, M − $C_6H_{13}O$ + CO_2), 69 (43), 57 (50), 55 (45), 43 (40).

Anal. Calcd for $C_{30}H_{46}O_4$: mol wt, 470.33961. Found: mol wt (mass spectrometry), 470.34131.

Oxidation of 23ξ-Acetoxy-17-deoxy-7,8-dihydroholothurinogenin 3β-Acetate (6).—Compound **6** (200 mg) was dissolved in 25 ml of acetic acid, heated to reflux, and stirred. Over the course of 1 hr chromic acid (100 mg) in 25 ml of acetic acid was added. After addition was complete the reaction mixture was refluxed for 1 hr. The acetic acid was then largely evaporated at reduced pressure. The residue was worked up in the usual manner, chromatographed on 20 g of alumina using gradient elution with benzene–ether, and recrystallized from methanol–water to give 65 mg of pure **10**: mp 259–260°; ORD (dioxane, *c* 0.27) $[\phi]_{361}$ −3615, $[\phi]_{350}$ −3081, $[\phi]_{273}$ +41,300, $[\phi]_{230}$ −33,044; ir (KBr) 1760 (lactone C=O), 1735 (ester C=O), 1675 (conjugated C=O), 1470, 1370, 1240, 1170, 1130, 1095, 1020 cm^{-1}; uv λ_{max} 251 nm (ε 10,565), position and ε not changed when made 0.01 *M* in potassium hydroxide; nmr δ 0.86 (3, s, CH_3-31), 0.88 (3, s, CH_3-32), 0.91 (6, d, *J* = 6 Hz, CH_3-26, 27), 0.94 (3, s, CH_3-30), 1.34 (3, s, CH_3-19), 1.44 (3, s, CH_3-21), 2.04 (6, s, $OCOCH_3$), 2.96 (1, t, *J* = 6 Hz, CH-17), 3.33 (1, broad m, CH-8), 4.50 (1, broad m, CH-3), 5.20 (1, broad m, CH-23), 5.75 (1, d, *J* = 2 Hz); mass spectrum *m/e* (rel intensity) 570 (16, M^+), 510 (100, M − AcOH), 495 (6, M − AcOH + CH_3), 451 (30, M − AcOH + CO_2 + CH_3), 427 (8, M − side chain), 367 (3, M − side chain + AcOH), 359 (30), 341 (18), 269 (43), 69 (30), 55 (35), 43 (66).

Anal. Calcd for $C_{34}H_{50}O_7$: C, 71.53; H, 8.83. Found: C, 71.69; H, 8.55.

Lithium Aluminum Hydride Reduction of Diacetate 6.—Diacetate **6** (20 mg) was allowed to react with lithium aluminum hydride in refluxing tetrahydrofuran for 5 hr, discharged with ethyl acetate, and worked up with saturated sodium sulfate in the usual way. Recrystallization (tetrahydrofuran–hexane) gave 17 mg of **11**: mp 223–226°; ir (KBr) 3400, 1460, 1370, 1180, 1100, 1050, 1030, 970, 860, 790 cm^{-1}; mass spectrum *m/e* (rel intensity) 476 (3, M^+), 458 (10, M − H_2O), 443 (4, M − H_2O + CH_3), 440 (18, M − H_2O + H_2O), 428.364258 (13, M − CH_2 + H_2O requires 428.365234), 425.339355 (10, M − CH_3 + H_2O + H_2O requires 425.341797), 413,342773 (43, M − CH_2O + CH_3 + H_2O requires 413.341797), 357.277100 (71, M − $C_6H_{13}O$ (cleavage between C_{20} and C_{22}) + H_2O), 357.279297, 299.237061 (55, $C_{21}H_{31}O$ requires 299.237305), 145 (92), 85 (100), 43 (90).

Anal. Calcd for $C_{30}H_{52}O_4$: mol wt, 476.38656. Found: mol wt (mass spectrometry), 476.38647.

Acetylation of the Tetraol 11.—Tetraol **11** (220 mg) was acetylated by heating with acetic anhydride–pyridine (1:1) on a steam bath for 2 hr and worked up in the usual manner. Chromatography on alumina using gradient elution (benzene–ether) gave 190 mg of crude triacetate as an oil and recrystallization (hexane) gave **12** as a white solid: mp 137–139°; $[\alpha]^{20}D$ + 59° (*c* 0.5); ir (KBr) 1735 (ester C=O), 1460, 1370, 1240, 1025, 980 cm^{-1}; nmr δ 0.88 (6, s, CH_3-31, 32), 0.90 (3, s, CH_3-30), 0.94 (6, d, *J* = 6 Hz, CH_3-26, 27), 1.13 (3, s, CH_3-19), 1.35 (3, s, CH_3-21), 2.05 (3, s, $OCOCH_3$), 2.06 (6, s, $OCOCH_3$), AB quartet at 3.90 and at 4.46 (1 each, *J* = 11 Hz, CH_2-18), 4.50 (1, m,

(34) R. H. Shapiro and C. Djerassi, *Tetrahedron*, **20**, 1987 (1964).

CH-3), 5.22 (2, m, CH-11 and CH-23); mass spectrum *m/e* (rel intensity) 602 (2, M^+), 584 (10, M − H_2O), 527 (4, M − CH_3COOH + CH_3), 524 (60, M − CH_3COOH + H_2O), 511 (4, M − CH_2OAc + H_2O), 464 (40, M − CH_3COOH + CH_3CO-OH + H_2O), 459 (5, M − $C_8H_{15}O_2$ (cleavage between C_{20} and C_{22}), 451 (100, M − CH_2OAc + CH_3COOH + H_2O), 449 (60, M − CH_3COOH + CH_3COOH + CH_3 + H_2O), 399 (12, M − $C_8H_{15}O_2$ (cleavage between C_{20} and C_{22}) + CH_3COOH), 225 (65), 109 (50), 69 (48), 43 (67).

Anal. Calcd for $C_{36}H_{58}O_7$: C, 71.71; H, 9.70. Found: C, 72.03; H, 9.72.

Hydrolysis of Triacetate 12.—The triacetate **12** (20 mg) was hydrolyzed by refluxing with 5% potassium hydroxide in methanol, worked up in the usual way, and recrystallized (hexane) to give 15 mg of material identical (tlc, ir, mp and mmp) with tetraol **11**.

Attempted Cleavage of Tetraol 11 with Lead Tetraacetate.—Tetraol **11** (20 mg) dissolved in 5 ml of acetic acid to which 40 mg of lead tetracetate was added, was allowed to react at room temperature for 24 hr. The acetic acid was lyophilized, water added (30 ml), and the water lyophilized. The residue was extracted with dichloromethane and the dichloromethane washed with water, dried, and evaporated. The ir spectrum of the residue shows no carbonyl absorption and tlc shows only starting material. Chromatography on alumina was carried out to give 14 mg of material which after recrystallization (tetrahydrofuran–hexane) was identical with starting tetraol **11** by tlc, mp, mmp, and ir spectra.

23ξ-Hydroxy-17-deoxy-7,8-dihydroholothurinogenin (9).—Compound **5** (20 mg) was refluxed with 5% potassium hydroxide in methanol for 30 min, worked up in the usual way, and recrystallized (methanol–water) to give 11 mg of diol **9**: mp 233–236°; $[\alpha]^{20}D$ − 1.35 (*c* 0.4); ir (KBr) 3450, 1760 (lactone C=O), 1460, 1370, 1260, 1090, 1020, 940, 800 cm^{-1}; nmr δ 0.82 (3, s, CH_3-32), 0.87 (3, s, CH_3-31), 0.91 (6, d, *J* = 6 Hz, CH_3-26, 27), 0.98 (3, s, CH_3-30), 1.15 (3, s, CH_3-19), 1.50 (3, s, CH_3-21), 2.95 (1, m, CH-8), 3.20 (1, m, CH-3), 5.17 (1, m, CH-11); mass spectrum *m/e* (rel intensity) 472 (100, M^+), 470 (15), 457 (12, M − CH_3), 454 (5, M − H_2O), 439 (13, M − CH_3 + H_2O), 421.307129 (10, M − H_2O + 2H + CH_3 requires 421.310547), 415.282227 (10, M − C_4H_9 (cleavage between C_{23} and C_{24}) requires 415.284668), 413.339111 (8, M − CO_2 + CH_3 requires 413.341797), 411.323730 (7, M − CO + CH_3 + H_2O requires 411.326172), 395.331543 (13, M − CO_2 + CH_3 + H_2O requires 395.331299), 386.281738 (9 ($C_{25}H_{38}O_3$), cleavage between C_{22} and C_{23} with loss of one hydrogen requires 386.281982), 371.260254 (17, M − loss of side chain ($C_6H_{13}O$) requires 371.258545), 353.248291 (31, M − loss of side chain ($C_6H_{13}O$) + H_2O requires 353.247803), 309.256348 (8, M − loss of side chain ($C_6H_{13}O$) + H_2O + CO_2 requires 309.258057), 267 (12), 95 (30), 69 (55), 55 (44), 43 (48).

Anal. Calcd for $C_{30}H_{48}O_4$: mol wt, 472.354980. Found: mol wt (mass spectrometry), 472.352539.

23-Oxo-17-deoxy-7,8-dihydro-3-holothurinogenone (13).—The diol **9** (15 mg) was oxidized with Jones reagent as described for **7** and the product recrystallized (methanol–water) to give 13 mg of **13**: mp 190–192°; $[\alpha]^{20}D$ −17° (*c* 0.3); CD (dioxane) $[\theta]_{300}$ − 1665; ir (KBr) 1755 (lactone C=O), 1710 (ketone at C_3 and at C_{23}), 1465, 1450, 1370, 1280, 1160, 1110, 1010, 940 cm^{-1}; uv, essentially no absorption in neutral ethanol; uv λ_{max} 252 (ε 8300) in ethanol 0.01 *M* in potassium hydroxide; nmr δ 0.88 (3, s, CH_3-32), 0.93 (6, d, *J* = 6 Hz, CH_3-26, 27), 1.08 (6, s, CH_3-30, 31), 1.36 (3, s, CH_3-19), 1.50 (3, s, CH_3-21), 2.98 (2, s, CH_2-22), 5.25 (1, m, CH-11); mass spectrum *m/e* (rel intensity) 468 (100, M^+), 453 (13, M − CH_3), 411 (3, M − C_4H_9), 407 (50), 383 (10, M − C_4H_9 + CO), 325 (30, M − side chain ($C_6H_{11}O$) + CO_2), 323 (50), 85 (50), 57 (55), 43 (63).

Anal. Calcd for $C_{30}H_{44}O_4$: C, 76.86; H, 9.47. Found: C, 76.97; H, 9.34.

Preparation of Ethylene Ketal Derivative of 7.—23-Acetoxy 3-ketone **7** (12 mg) was dissolved in 100 ml of benzene and 0.20 ml of ethylene glycol and 10 mg of *p*-toluenesulfonic acid (monohydrate) added and refluxed for 18 hr using a Dean–Stark trap. The reaction mixture was then poured into saturated potassium carbonate solution. The benzene layer was separated and washed with saturated potassium carbonate, water, dried (magnesium sulfate), and evaporated. After partial evaporation the ir spectrum (benzene) showed absorption at 1755 (lactone C=O) and at 1730 (ester C=O) but none at 1700 cm^{-1}. The residue after evaporation was dissolved in 10 ml of methanol and 500 mg of potassium hydroxide added and refluxed for 30 min. The reaction mixture was poured into water and extracted with ether. The ether was dried and evaporated, and the residue chromatographed on 2 g of basic activity grade II alumina using gradient elution chromatography with benzene–ether. Recrystallization (methanol) gave 5 mg of **14**: mp 229–233; $[\alpha]^{20}D$ −10° (*c* 0.4); ir (benzene) 3600, 1760 (lactone C=O), 1550, 1250, 1080, 800 cm^{-1}; mass spectrum *m/e* (rel intensity) 514 (30, M^+), 512 (3), 499 (6, M − CH_3), 457 (7, M − C_4H_9), 415 (65), 413 (13, M − side chain ($C_6H_{13}O$)), 397 (42), 329 (100), 99 (60).

Anal. Calcd for $C_{32}H_{50}O_5$: mol wt, 514. Found: mol wt (mass spectrometry), 514.

Oxidation of Ethylene Ketal Derivative 14.—Ethylene ketal **14** (5 mg) was dissolved in 0.5 ml of pyridine and added to 1.0 ml of pyridine to which 20 mg of chromic acid had been previously added. The reaction mixture was stirred at room temperature for 18 hr and then poured into ether and water. The water was extracted with ether and the combined ether washed with water, dried, and evaporated. The residue was chromatographed on 3 g of basic alumina (activity II) using gradient elution chromatography with benzene–ether and recrystallized (benzene–hexane) to give 2 mg of **15**: mp 233–236°; ir (KBr) 1760 (lactone C=O), 1710 (side chain C=O), 1450, 1370 (ketal 1160, 1135, 1110, 1060), 1010, 940 cm^{-1}; essentially no uv in neutral ethanol, uv λ_{max} 252 (ε 8320) when in 0.01 *M* potassium hydroxide in ethanol; mass spectrum *m/e* (rel intensity) 512 (8, M^+), 497 (3, M − CH_3), 413 (25), 329 (11), 99 (100).

Anal. Calcd for $C_{32}H_{48}O_5$: mol wt, 512. Found: mol wt (mass spectrometry) 512.

Recovery of the Uv-Absorbing Material.—3-Ethylene ketal 23-one **15** (0.5 mg) was dissolved in 5 ml of ethanol containing 0.01 *M* potassium hydroxide (uv λ_{max} 252 (ε 8300)) and the solution poured into water and extracted with chloroform. The chloroform was washed well with water, dried, and evaporated. The ir spectrum of the residue was essentially identical with starting material ir with no carbonyl absorption below 1710 cm^{-1}. The same experiment was carried out with diketone **13** to give the same results.

Registry No.—**5**, 36872-76-1; **6**, 36872-77-2; **7**, 36872-78-3; **8**, 36872-79-4; **9**, 36872-80-7; **10**, 36872-81-8; **11**, 36871-79-1; **12**, 36871-80-4; **13**, 36871-81-5; **14**, 36871-82-6; **15**, 36871-83-7.