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# Saponin and Sapogenol. XIV.[1] Antifungal Glycosides from the Sea Cucumber *Stichopus japonicus* Selenka. (1). Structure of Stichopogenin $A_4$, the Genuine Aglycone of Holotoxin A

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Three antifugal glycosides named holotoxin A (major), B, and C have been isolated from the Far Eastern sea cucumber *Stichopus japonicus* Selenka. Aqueous acid hydrolysis of holotoxin A furnished genin-1 (a mixture of stichopogenin $A_2$ (**3b**) and the isomer) and stichopogenin $A_4$ (=genin-2) (**3a**) in addition to xylose, quinovose, 3-O-methyl-glucose, and glucose, while mild methanolic acid hydrolysis of holotoxin A gave **3a** and another new aglycone named 25-O-methyl-stichopogenin $A_4$ (=genin-3) (**3c**).

On the basis of chemical and physicochemical evidence, the structure of stichopogenin $A_4$, the genuine aglycone of holotoxin A, has been elucidated to be expressed as **3a** rather than previously proposed **2**, and the structure of 25-O-methyl-stichopogenin $A_4$ as **3c**.

The various species of the starfish (*Asteroidea*) and the sea cucumber (*Holothuroidea*) have been known to contain saponins as the toxic principles and in this respect, these two classes are unique in *Echinodermata*.[3] It is of interest to mention that the aglycones hitherto elucidated from the starfish saponins are steroids,[4] while those from the sea cucumber are lanostane-type triterpenoids.[5]

As for the chemical constituents other than saponins of the Far Eastern sea cucumber *Stichopus japonicus* Selenka (Japanese name: ma-namako), several kinds of substances such as carotenoids,[6a] lanosterol, cycloartenol, sterols, and xyloside of $\Delta^5$-sterol[6b,c] have been elucidated. In 1968, Elyakov, *et al.* reported the isolation of three triterpenoid glycosides (saponins) designated as stichoposide A, $A_1$, and C,[7a] among which A and C were the sulfate-containing glycosides while $A_1$ was the desulfated product of A formed during the chromatographic purification procedure.[7b] They reported also the carbohydrate ingredients of stichoposide A and C as xylose, quinovose, 3-O-methyl-glucose, and glucose,[7b] and proposed the struc-

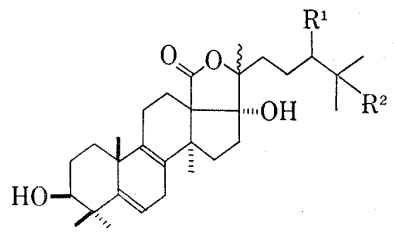
1) Part XIII: I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 121 (1976).
2) Location: *a) 133-1, Yamada-kami, Suita, Osaka, 565, Japan*; *b) Fukushima-ku, Osaka, 553, Japan.*
3) *a)* J.S. Grossert, *Chem. Soc. Rev.*, **1**, 1 (1972); *b)* Y. Yasumoto, M. Tanaka, and Y. Hashimoto, *Bull. Jap. Soc. Sci. Fish.*, **32**, 673 (1966); *c)* G.D. Ruggieri, R.F. Nigrelli, and M.F. Stempien, *Toxicon*, **8**, 149 (1970).
4) *a)* Y.M. Sheikh, B. Tursch, and C. Djerassi, *J. Am. Chem. Soc.*, **94**, 3278 (1972); *idem, Tetrahedron Letters*, **1972**, 3721; *b)* Y. Shimizu, *J. Am. Chem. Soc.*, **94**, 4051 (1972); *c)* Y.M. Sheikh and C. Djerassi, *Tetrahedron Letters*, **1973**, 2927; *d)* S. Ikegami, Y. Kamiya, and S. Tamura, *Tetrahedron*, **29**, 1807 (1973); *e)* D.S. H. Smith, A.M. Mackie, and A.B. Turner, *J. Chem. Soc, Perkin I*, **1973**, 1745; *f)* J.W. Apsimon, J.A. Buccini, and S. Badripersend, *Can. J. Chem.*, **51**, 850 (1973); *g)* I. Kitagawa, M. Kobayashi, T. Sugawara, and I. Yosioka, *Tetrahedron Letters*, **1975**, 967.
5) *a)* P.J. Scheuer, *Fortschr. Chem. Org. Naturstoffe*, **27**, 322 (1969); *b)* E. Premuzic, *ibid.*, **29**, 27 (1971); *c)* P.J. Scheuer, *Naturwissenschaften*, **58**, 549 (1971).
6) *a)* T. Matsuno and T. Ito, *Experientia*, **27**, 509 (1971); *b)* T. Nomura, Y. Tsuchiya, D. Andre, and M. Barbier, *Bull. Jap. Soc. Sci. Fish.*, **35**, 293 (1969); *c)* M. Kobayashi, R. Tsuru, K. Toda, and H. Mitsuhashi, *Tetrahedron*, **29**, 1193 (1973).
7) *a)* G.B. Elyakov, T.A. Kuzunetsova, and V.E. Vaskovskii, *Khim. Prir. Soedin*, **4**, 253 (1968) [*C. A.*, **70**, 45173 (1969)]; *b)* M.M. Anisimov, E.B. Fronert, T.A. Kuzunetsova, and G.B. Elyakov, *Toxicon*, **11**, 109 (1973); *c)* G.B. Elyakov, T.A. Kuzunetsova, A.K. Dzizenko, and Y.N. Elkin, *Tetrahedron Letters*, **1969**, 1151.

tures 1 and 2 for two aglycones, which were liberated by acid hydrolysis of stichoposide A and were designated as stichopogenin $A_2$ and $A_4$, respectively.[7c]

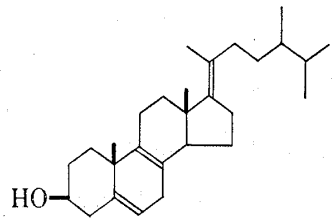
On the other hand in 1969, Shimada isolated a crystalline glycoside named holotoxin from the body wall of the same species of sea cucumber and reported that holotoxin did not possess the sulfate group and showed the significant growth inhibitory activity against several pathogenic fungi.[8] Later on, Scheuer suggested[5c] the identity of Shimada's holotoxin with one of stichoposides isolated by Elyakov, *et al.* However, the structures of these glycosides have not yet been clarified.

As a continuation of the chemical studies on saponin and sapogenol,[1] we have been working on the glycosides of the sea cucumber *Stichopus japonicus* Selenka, and have isolated three antifungal glycosides named holotoxin A (**4**) (major), B, and C. Based on the chemical and physicochemical evidence, we have elucidated[9] that the genuine aglycone of holotoxin A is identical with stichopogenin $A_4$, whose structure is now formulated as **3a** rather than **2** which was previously proposed by Elyakov, *et al.*[7c] In addition, another new aglycone designated as 25-O-methyl-stichopogenin $A_4$ (=genin-3), which was concomitantly obtained along with stichopogenin $A_4$ upon methanolic acid hydrolysis of holotoxin A, has been elucidated as **3c**. The present paper provides with the full account on the structure elucidation of these aglycones.[9b]

The fraction containing holotoxin, which was isolated from the MeOH extractive of the whole body through the procedure shown in Chart 2, was subjected to droplet countercurrent chromatography (D.C.C.C.)[10] to give three glycosides named holotoxin A (major), B, and C. Since holotoxin isolated here was obtained from the same species of the sea cucumber as Shimada's holotoxin and both holotoxins showed the identical behavior on thin-layer chromatogram (TLC) (giving three spots) and similar antifungal properties,[11] our holotoxin has been considered to be identical with Shimada's holotoxin.[9a]




1 : $R^1$, $R^2=\Delta^{24}$
2 : $R^1$=H, $R^2$=OH
(by Elyakov, *et al.*[7c])




5

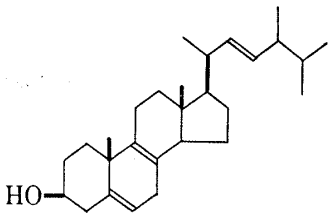



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Chart 1

Holotoxin A (**4**), mp 248—252°, shows no ultraviolet (UV) absorption maximum above 210 nm, but shows the infrared (IR) absorption bands characteristic to the glycosides (3400 (br) and 1070 (br) $cm^{-1}$) along with the absorption band at 1750 (br) cm $^{1}$ due to $\gamma$-lactone.[12] Holotoxin A was negative to the potassium rhodizonate reagent[13a] and to the $PdCl_2$ reagent,[13b] thus the presence of sulfate group being ruled out. Acid hydrolysis of holotoxin A with aq.

8) S. Shimada, *Science*, **163**, 1462 (1969).
9) Preliminary communications: *a*) I. Kitagawa, T. Sugawara, and I. Yosioka, *Tetrahedron Letters*, **1974**, 4111; *b*) I. Kitagawa, T. Sugawara, I. Yosioka, and K. Kuriyama, *ibid.*, **1975**, 963.
10) T. Tanimura, J.J. Pisano, Y. Ito, and R.L. Bowman, *Science*, **169**, 54 (1970).
11) Reported in the following paper.
12) This broad absorption band has been disclosed later to be the overlap of two absorption bands due to $\gamma$-lactone and five-membered ring CO.
13) *a*) D.P. Burma, *Anal. Chim. Acta*, **9**, 513 (1953); *b*) E. Stahl, "Thin-layer chromatography," Springer-Verlag, Berlin, 1969, p. 892.

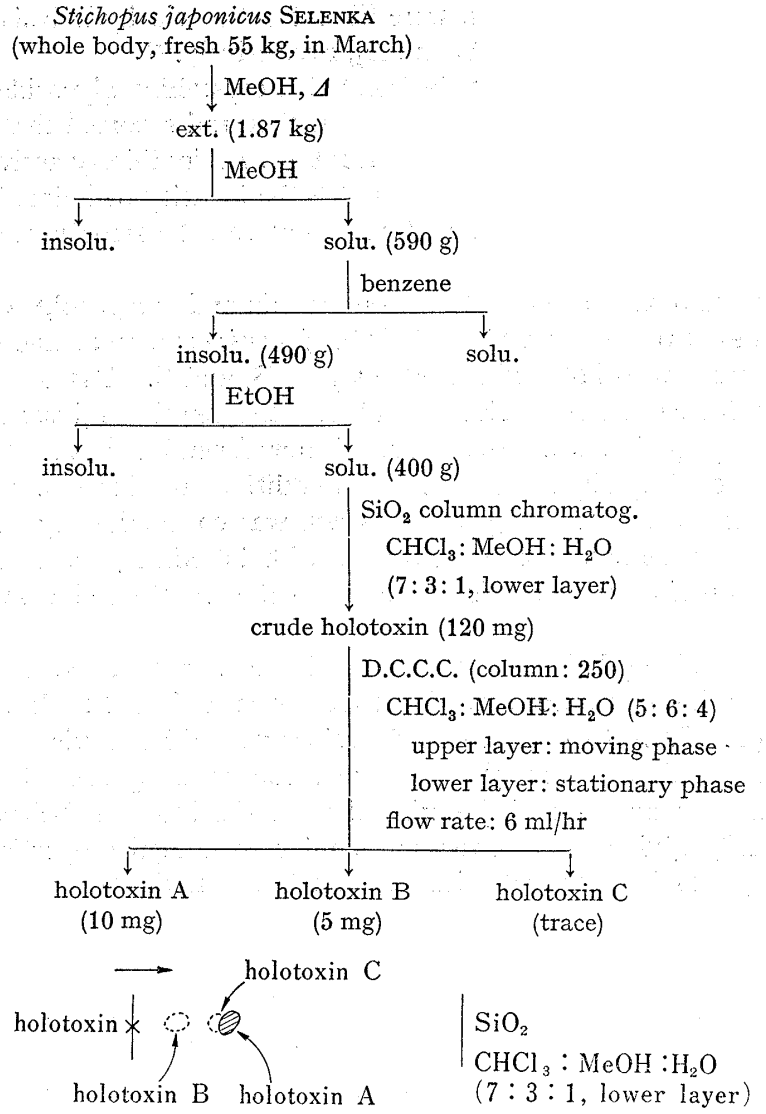


Chart 2

2N HCl furnished two aglycones (genin-1 and genin-2) in addition to xylose, quinovose, 3-O-methyl-glucose, and glucose.

Genin-1, mp 217—220°, possesses no UV absorption maximum above 210 nm but shows the strong IR absorption band at 1760 (br) $cm^{-1}$ similarly as holotoxin A. The proton magnetic resonance (PMR) spectrum of genin-1 exhibits the presence of five tertiary Me's, two olefinic Me's, and one carbinyl H (Table I). It shows also the signals ascribable to olefinic H's at $\delta$ 4.72 (m), 4.96 (m), and 5.33 (m). Since these three signals are integrated as two H's, genin-1 has been assumed to be a mixture of the double bond isomers. Hydrogenation of genin-1 over $PtO_2$ readily afforded a single dihydro-derivative (**3d**), mp 259—261°, which gives the molecular ion peak at *m/e* 470 in its mass spectrum and shows disappearance of the above mentioned two signals at $\delta$ 4.72 and 4.96 and of two olefinic Me's and shows the generation of two secondary Me's in its PMR spectrum (Table I). Since one olefinic H is retained at $\delta$ 5.30, the hydrogenation has been presumed to occur at the side chain. On comparison of the physicochemical properties of genin-1 with those reported for stichopogenin $A_2$[7c] and based on the above evidence, it has been assumed that genin-1 is an inseparable mixture of stichopogenin $A_2$ (**3b**) and its double bond isomer (presumably $\Delta^{25}$ isomer).

Genin-2 (**3a**), mp 240—243°, also does not possess the UV absorption maximum above 210 nm but shows the IR absorption band at 1750 (br) $cm^{-1}$ as genin-1. The PMR spectrum

Table I. PMR Data ($\delta$ Values in $CDCl_3$)

| | 4-Me's | 10-Me | 14-Me | 20-Me | 25-Me's | 3α-H | 11-H |
|---|---|---|---|---|---|---|---|
| **3a** | 0.87, 0.92 | 1.24 | 1.02 | 1.43 | 1.24, 1.24 | 3.12 | 5.27 |
| **3b** | 0.83, 0.88 | 1.19 | 0.99 | 1.39 | 1.59, 1.63 | 3.12 | *a*) |
| **3c** | 0.84, 0.89 | 1.19 | 0.99 | 1.41 | 1.12, 1.12 | 3.17 | 5.29 |
| **3d**[b] | 0.88, 1.04, 1.20, 1.36, 1.38 | | | | 1.24 (6H, d, $J$=7Hz) | 3.40 | 5.30 |
| **3e** | 0.88, 0.88 | 1.24 | 0.90 | 1.43 | 1.24, 1.24 | 4.49 | 5.30 |
| **3f** | 0.88, 0.89 | 1.14 | 0.91 | 1.40 | 1.22, 1.27 | 4.47 | 5.26 |

*a*) See the text.
*b*) in $d_5$-pyridine

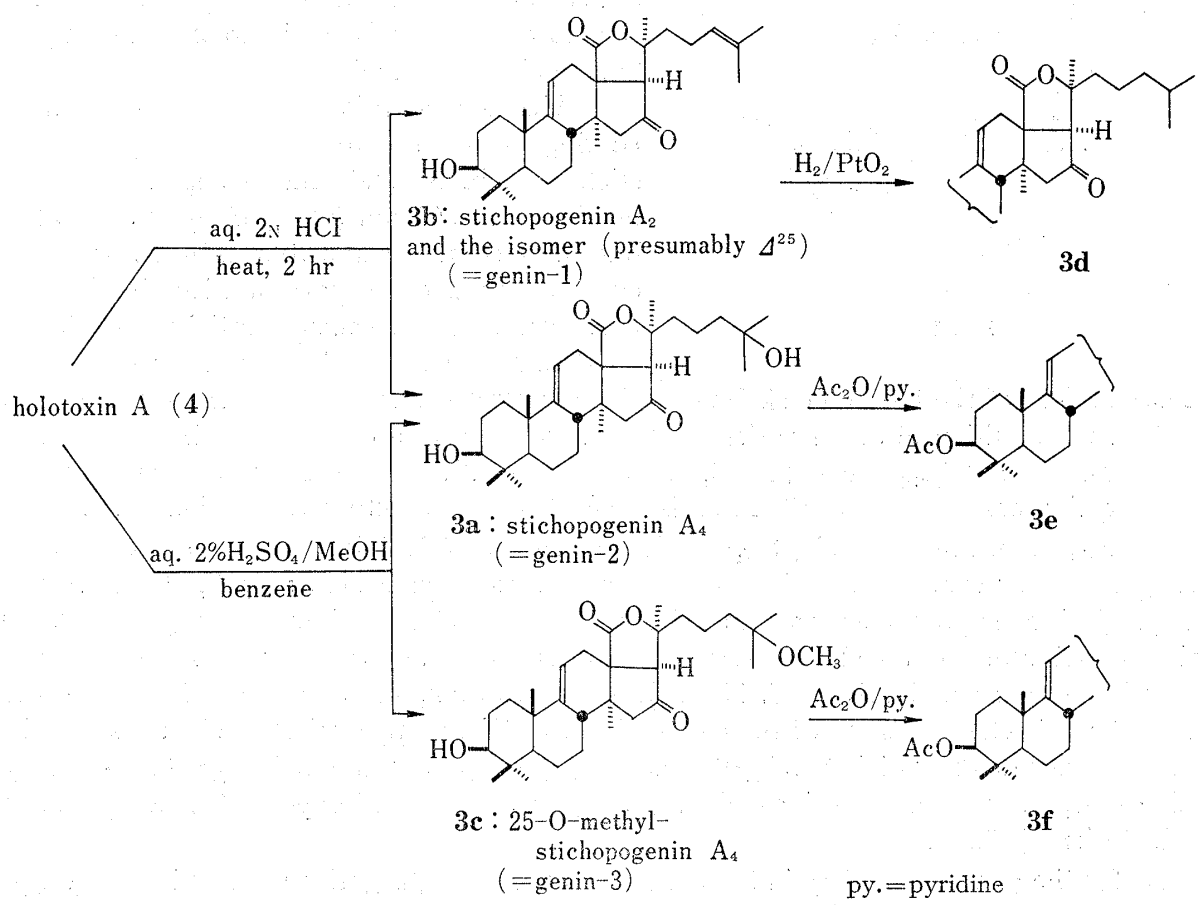


Chart 3

of genin-2 exhibits the presence of seven tertiary Me's, one carbinyl H, and one olefinic H (Table I). Acetylation of genin-2 with $Ac_2O$–pyridine furnished the monoacetate (**3e**), mp 220—222° (250—252°).[14] These chemical and physicochemical properties of **3a** and **3e** have shown the identity of genin-2 with stichopogenin $A_4$ reported by Elyakov, *et al.*[7c]

The structures, **1** and **2**, which were initially proposed to stichopogenin $A_2$ and $A_4$ by Elyakov, *et al.*,[7c] are characteristic by the possession of an unconjugated $\Delta^{5,8}$-diene moiety, which has so far been known to be present in few examples of free sterols such as ergosta-5,8,17(20)-trien-3$\beta$-ol (**5**)[15] and lichesterol (**6**)[16] and which has never been known to occur in the aglycone. It has been considered that i) since the $\Delta^{5,8}$-diene system seems to be labile

14) Double melting-point.
15) N. Sugiyama, M. Yamamoto, and K. Yamada, *Nippon Kagaku Zasshi*, **89**, 710 (1968).
16) T.R. Lenton, L.J. Goad, and T.W. Goodwin, *Phytochemistry*, **12**, 1135 (1973).

to acid treatment, the survival of the system during the acid hydrolysis of the parent glycoside is remarkable, and ii) the $\Delta^{5,8}$-diene system, if being present in stichopogenin $A_2$ or $A_4$, would give the circular dichroism (CD) curve due to the olefin $\pi\rightarrow\pi^*$ transition which shows a red shift caused by the interaction of two $\pi\rightarrow\pi^*$ transitions.[17] Furthermore, in order to elucidate the genuine aglycone of holotoxin A, we have examined the CD spectra of holotoxin A and its aglycone stichopogenin $A_4$. As shown in Fig. 1, both spectra have disclosed that holotoxin A and stichopogenin $A_4$ possess the similar chiral chromophores such as the isolated olefin, $\gamma$-lactone, and CO in their molecules, especially the unexpected presence of the latter CO chromophore being inconsistent with the formulation **2** for stichopogenin $A_4$.

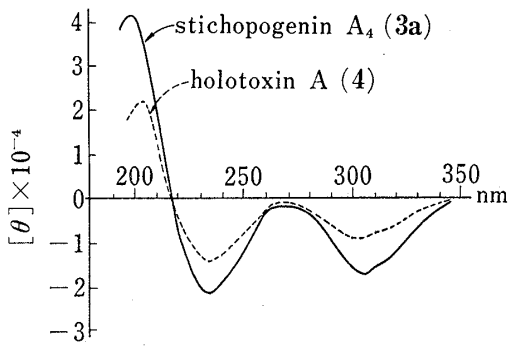


Fig. 1

On the basis of the following evidence, we have reached the conclusion that stichopogenin $A_4$ is expressed as **3a** carrying the "en-one" structure which is isomeric to **2** possessing the "dien-ol" structure.[9b]

Since no absorption band due to six-membered ring CO is observed in the IR spectrum of stichopogenin $A_4$ (**3a**), the strong negative CD maximum at 305 nm ([$\theta$]−16800) of **3a** is attributable to the n$\rightarrow\pi^*$ transition of the five-membered ring CO and comparison with the CD data (the sign and molecular ellipticity) of the related compounds (**7**—**11**)[18] has led us to locate the CO at C-16 of the lanostane skeleton. The assignment has been further supported by a fact that the addition of alkali varies the CD curve of **3a** to a curve ascribable to an enone chromophore (**16**) ([$\theta$]$_{270}$−22400 (max.), [$\theta$]$_{330}$−5300 (sh.)).[19] It follows therefore that the IR absorption band due to 16-CO in **3a** is masked by the $\gamma$-lactone absorption band.

A one-proton broad signal ($W_{h/2}$=6 Hz) observed at $\delta$ 5.27 in the PMR spectrum of **3a** is assignable to an H attached to the isolated trisubstituted double bond: $\Delta^5$, $\Delta^7$, $\Delta^{9(11)}$. Among them, $\Delta^{9(11)}$ is most probable for **3a**, since the strong positive CD maximum of **3a** ([$\theta$]$_{200}$+41000), which is attributable to the olefin $\pi\rightarrow\pi^*$ transition, is in good accord with the CD data reported for $\Delta^{9(11)}$-lanostene derivatives (*cf.* **12**—**15**).[20] In addition, the above mentioned chemical shift ($\delta$ 5.27) is close to those of the olefinic H in $\Delta^{9(11)}$-lanostene triterpenoids: grandisolide ($\delta$ 5.30),[21a] the methoxydiol ($\delta$ 5.25),[21b] lanost-9(11)-en-3$\beta$-yl acetate ($\delta$ 5.19),[21a] 23$\xi$-acetoxy-17-desoxy-7,8-dihydro-holothurinogenin ($\delta$ 5.17),[19a] and 3$\beta$-methoxy-24,24-dimethyl-lanost-9(11),25-diene ($\delta$ 5.23).[21c]

As for the $\gamma$-lactone moiety in **3a**, is observed the strong negative CD maximum ([$\theta$]$_{233}$−21000) due to the n$\rightarrow\pi^*$ transition of the lactone CO, the sign of which is well explained by the Beecham's empirical rule[22] as depicted partially in Fig. 2 where C-17 locates beneath the plane of –CO–O–. The bathochromic and hyperchromic shift of the curve as compared with

17) G. Snatzke, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Heyden & Son, Ltd., London, 1967, p. 153.

18) *a*) P. Crabbe, "ORD and CD in Chemistry and Biochemistry," Academic Press, New York, 1972, p. 35; *b*) L. Velluz and M. Legrand, *Angew. Chem.*, **73**, 603 (1961); *c*) K. Kuriyama, unpublished data.

19) *a*) I. Rothberg, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **38**, 209 (1973); *b*) C. Djerassi, R. Records, E. Bunnenberg, K. Mislow, and A. Moscowitz, *J. Am. Chem. Soc.*, **84**, 870 (1962).

20) *a*) H. Irie, S. Uyeo, and K. Kuriyama, *Tetrahedron Letters*, **1971**, 3467; *b*) A.I. Scott and A.D. Wrixon, *Tetrahedron*, **26**, 3695 (1970).

21) *a*) J.P. Kutney, D.S. Grierson, G.D. Knowlis, and N.D. Westcott, *Tetrahedron*, **29**, 13 (1973); *b*) J.P. Kutney, G. Eigendorf, R.B. Swingle, G.D. Knowlis, J.W. Rowe, and B.A. Nagasampagi, *Tetrahedron Letters*, **1973**, 3315; *c*) W.S. Chan and W.H. Hui, *J. Chem. Soc., Perkin I*, **1973**, 490.

22) A.F. Beecham, *Tetrahedron Letters*, **1968**, 2355, 3591.

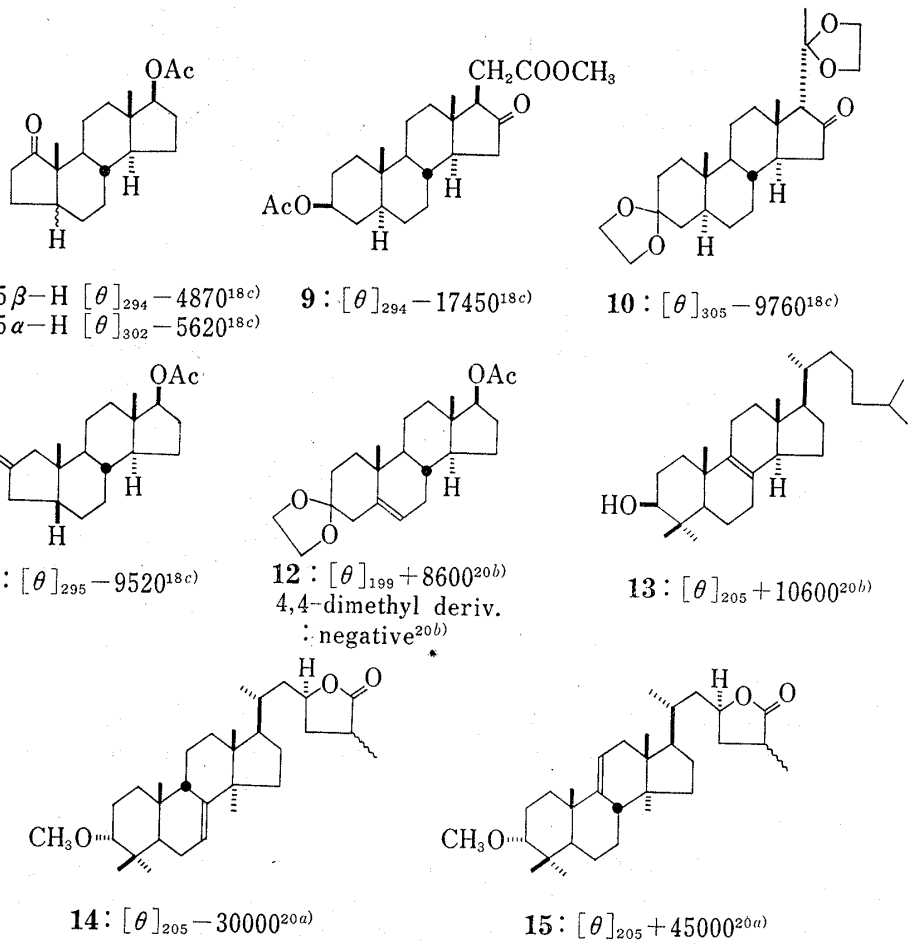


Chart 4

the CD curves of the ordinary lactonic compounds[23] is presumed to be caused by the interaction between the spacially proximate lactone CO and $\Delta^{9(11)}$ double bond.[24] In addition, the partial overlap with the slope of strong positive CD curve due to $\Delta^{9(11)}$ double bond is presumed to result in the apparent red shift of the CD maximum due to the γ-lactone. The presumption has been verified by a fact that 9α,11α-epoxystichopogenin $A_4$ (**17**), prepared from **3a** by *m*-chloroperbenzoic acid oxidation, shows the negative CD maximum ([θ]228 −5400) due to the n→π* transition of the lactone CO with the ordinary value.[23]

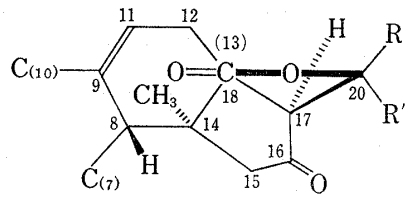


Fig. 2. Partial Stereostructure of **3a**

In the structure study by Elyakov, *et al.* who proposed **1** and **2** for stichopogenin $A_2$ and $A_4$, the presence of 17α-OH was based on the PMR analysis: *i.e.* the chemical shift of 14-Me in stichopogenin $A_2$ (δ 1.24) or $A_4$ (δ 1.25)[7c] is quite alike to that (δ 1.22) of 22,25-oxido-holothurinogenin (**18**) having 17α-OH.[25] However, we have clarified the absence of 17α-OH in stichopogenin $A_4$ on the following basis. Mild methanolic acid hydrolysis (aq. 2% $H_2SO_4$–

23) ref. 18*a*, p. 50.
24) *a*) H. Labhart and G. Wagniere, *Helv. Chim. Acta*, **42**, 2219 (1959); *b*) R.C. Cookson and S. Mackengie, *Proc. Chem. Soc.*, **1961**, 423; *c*) A. Moscowitz, K. Mislow, M.A.W. Glass, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1945 (1962).
25) J.D. Chanley, J. Mezzetti, and H. Sobotka, *Tetrahedron*, **22**, 1857 (1966).

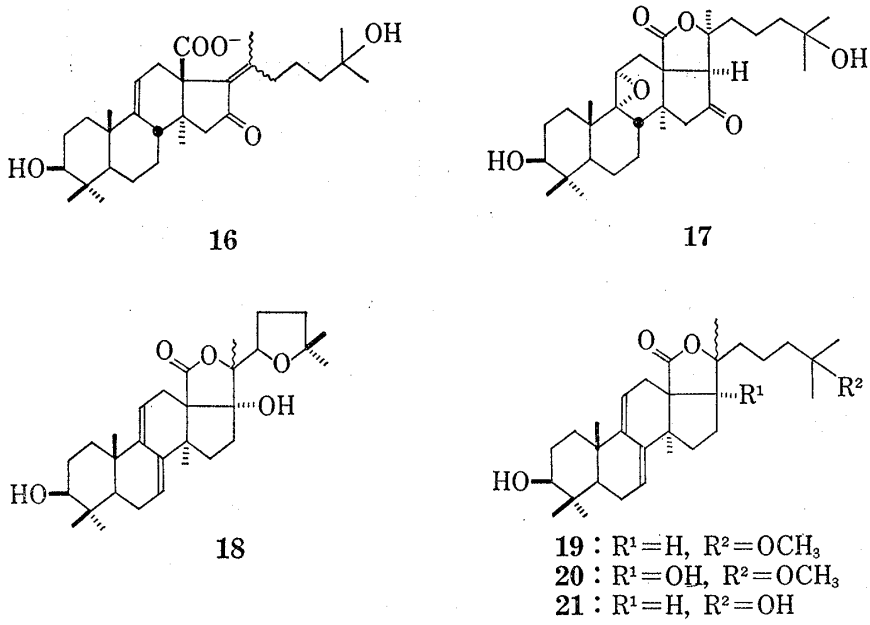


Chart 5

MeOH (1: 1)/benzene) of holotoxin A (4) furnished stichopogenin $A_4$ (**3a**) as the major product in addition to another minor aglycone designated as 25-O-methyl-stichopogenin $A_4$ (=genin-3). Genin-1 was not detected in the total hydrolysate in this case.

Genin-3 (**3c**), mp 240—243°, shows no UV absorption maximum above 210 nm but gives the similar CD spectrum as stichopogenin $A_4$ (**3a**), thus the broad IR absorption band of **3c** at 1747 $cm^{-1}$ being assigned to both $\gamma$-lactone and five-membered ring CO. In the PMR spectrum of **3c** (Table I), is observed a three-proton singlet due to OMe attached to a tertiary carbon along with the signals due to seven tertiary Me's, one carbinyl H, and one olefinic H. The mass spectrum of **3c** exhibits the molecular ion peak at *m/e* 500, the prominent ion peak at *m/e* 468 ($M^+$—MeOH), and the base peak at *m/e* 73 assignable to $(CH_3)_2C{=}\overset{+}{O}CH_3$, which has been known to occur as the base peak in the mass spectra of ternaygenin (**19**)[26a,27] and praslinogenin (**20**).[26b,27]

Therefore, genin-3 (**3c**) has been formulated as the 25-O-methyl derivative of stichopogenin $A_4$, the formation of which is reasoned analogously as in the conversion of koellikerigenin (**21**) to ternaygenin (**19**) on HCl–MeOH treatment.[26a] Ordinary acetylation of genin-3 (**3c**) with $Ac_2O$–pyridine furnished the monoacetate (**3f**), mp 238—241°, which exhibits no OH absorption band in its IR spectrum but the bands at 1768 (sh.), 1751, and 1742 (sh.) $cm^{-1}$ ascribable to $\gamma$-lactone, five-membered ring CO, and OAc. The finding here is not in accord with the structure having 17$\alpha$-OH, and the structures of stichopogenin $A_4$ (**3a**) and 25-O-methyl-stichopogenin $A_4$ (**3c**) have been elucidated except the configuration at C-20.[9]

Furthermore for instance, since the signals due to 10-Me in the $\Delta^{9(11)}$-lanostene derivatives have been known to appear at the similar position as the signal due to 14-Me in **18**,[19a,28] the formulations with 17$\alpha$-OH (**1** and **2**), which were mainly based on the Me chemical shift assignment, could not be conclusive.

After completion of our work, we have become aware of the recent publication[29] which discloses the full structure including the configuration at C-20 of stichopogenin $A_4$ (**3a**) on the

26) *a*) R. Roller, C. Djerassi, R. Cloetens, and B. Tursch, *J. Am. Chem. Soc.*, **91**, 4918 (1969); *b*) B. Tursch, R. Cloetens, and C. Djerassi, *Tetrahedron Letters*, **1970**, 467.
27) G. Habermehl and G. Volkwein, *Ann.*, **731**, 53 (1970).
28) A.I. Cohen, D. Rosenthal, G.W. Krakower, and J. Fried, *Tetrahedron*, **21**, 3171 (1965).
29) W.L. Tan and C. Djerassi, *J. Org. Chem.*, **40**, 466 (1975).

basis of X-ray analysis of **3c** and which supports our proposal.[9b] It follows therefore that the full structures of stichopogenin $A_4$ and all of its derivatives are formulated as given above.[30]

Stichopogenin $A_4$ (**3a**) is considered to be the genuine aglycone of holotoxin A (**4**),[11] since i) the mild acid hydrolysis of holotoxin A liberated **3a** as the major product, and ii) the CD spectra of **3a** and **4** disclose the presence of the similar chiral chromophore in both (Fig. 1) although the molecular ellipticity of **4** is slightly smaller than that of **3a**, the reason of which will be a subject of further investigation.

## Experimental[31]

**Isolation of Holotoxin A, B, and C**——As shown in Chart 2, the whole body of the fresh sea cucumber (cut, 55 kg, collected in March) was extracted three times with MeOH (100 liters each) under reflux and the extract was concentrated under reduced pressure to give a residue (1.87 kg), which was treated with MeOH (total 14 liters) at room temperature. The MeOH soluble portion, after evaporation of the solvent under reduced pressure, gave a residue (590 g), which was washed with benzene (total 12 liters) and the soluble portion was removed by decantation. The benzene insoluble portion (490 g) was treated twice with 95% EtOH (*ca.* 10 times by volume) under reflux for 30 min, and the combined EtOH solution was evaporated under reduced pressure to give a residue (400 g), which was chromatographed on silica gel (1 kg) eluting with $CHCl_3$–MeOH–$H_2O$ (7: 3: 1, lower layer). The fraction (1.7 g) containing holotoxin was chromatographed again on silica gel (150 g) to furnish crude holotoxin (120 mg), which showed three spots on TLC ($CHCl_3$–MeOH–$H_2O$=7: 3: 1, lower layer, Chart 2). Crude holotoxin (120 mg) was then subjected to D.C.C.C. using $CHCl_3$–MeOH–$H_2O$ (5: 6: 4) mixture (upper layer=moving phase; lower layer=stationary phase): flow rate=6 ml/hr, one fraction=2 ml, fr. 19—20 gave holotoxin B (5 mg), fr. 21—22 gave holotoxin C (trace), and fr. 50—70 gave a residue (15 mg), which was recrystallized from $CHCl_3$–MeOH–$H_2O$ mixture to furnish holotoxin A (10 mg). Holotoxin A (**4**), mp 248—252°, $[\alpha]_D^{24}$ −53° ($c$=0.3, pyridine). *Anal.* Calcd. for $C_{59}H_{94}O_{27}\cdot 2H_2O$: C, 56.46; H, 7.81. Found: C, 56.30; H, 7.93. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (br), 1750 (br), 1070 (br). UV $\lambda_{max}^{EtOH}$ nm: transparent above 210 nm. CD ($c=1.24\times10^{-4}$, MeOH): $[\theta]_{340}$ 0, $[\theta]_{305}$ −9900 (neg. max.), $[\theta]_{263}$ −550 (neg. min.), $[\theta]_{233}$ −13700 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{205}$ +22000 (pos. max.).

A solution of holotoxin A (**4**) (5 mg) in aq. 2N HCl (1 ml) was refluxed for 2 hr, neutralized with dil. NaOH and evaporated to dryness under reduced pressure. The residue was subjected to PPC (Toyo Filter Paper no. 50) developing with MeOH–$H_2O$ (9: 1) mixture. After drying in the air, a solution of $BaCl_2$ (100 mg) in 70% MeOH (50 ml) was sprayed and air dried again. The paper was then sprayed with a solution of potassium rhodizonate (10 mg) in aq. 50% MeOH (50 ml) to develop the coloration: holotoxin A negative; $(NH_4)_2SO_4$ yellow; starfish saponin[4g] yellow.

**Acid Hydrolysis of Holotoxin A** (**4**)——A solution of **4** (100 mg) in aq. 2N HCl (5 ml) was heated on a boiling water-bath for 2 hr and centrifuged after cooling. The precipitate (15 mg) thus obtained was purified by preparative TLC ($CHCl_3$–MeOH=50: 1) and recrystallized from MeOH to give genin-1 (9 mg) and stichopogenin $A_4$ (=genin-2) (**3a**) (2 mg). Genin-1 (**3b**+isomer), mp 217—220°, $[\alpha]_D^{21}$ −93° ($c$=0.7, $CHCl_3$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 1760 (br). UV $\lambda_{max}^{EtOH}$ nm: transparent above 210 nm. Mass Spectrum *m/e* (%): 468 ($M^+$, 27), 453 ($M^+-CH_3$, 16), 450 ($M^+-H_2O$, 9), 435 ($M^+-CH_3-H_2O$, 22), 274 (17), 259 (20), 241 (14), 109 ($\overset{+}{C}\overbrace{H_2{=}CH{=}CH}-CH_2CH{=}C(CH_3)_2$, 40), 69 ($\overset{+}{C}H_2CH{=}C(CH_3)_2$, 100). PMR (100 MHz): as given in Table I. Stichopogenin $A_4$ (=genin-2) (**3a**), mp 240—243°, $[\alpha]_D^{23}$ −77° ($c$=1.0, $CHCl_3$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1750 (br). UV $\lambda_{max}^{MeOH}$ nm: transparent above 210 nm. CD ($c=5.41\times10^{-4}$): $[\theta]_{345}$ 0, $[\theta]_{305}$ −16800 (neg. max.), $[\theta]_{262}$ −1200 (neg. min.), $[\theta]_{233}$ −21000 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{200}$ +41000 (pos. max.); CD (MeOH+conc. KOH): $[\theta]_{387}$ 0, $[\theta]_{330}$ −5300

30) Although **3a** is designated as holotoxinogenin in ref. 29), we adopt stichopogenin $A_4$ for **3a** because of its precedency.

31) The following instruments were used for obtaining the physical data. Melting-points: Yanagimoto Micro-meltingpoint Apparatus and recorded uncorrected; Specific Rotation: Rex Photoelectric Polarimeter NEP-2 (l=1 dm); IR Spectra: Hitachi IR Spectrometer EPI-S2 or EPI-G3; UV Spectra: Shimadzu MPS-50L or Hitachi EPS-032 Spectrophotometer; Mass Spectra: Hitachi RMU-6D Spectrometer; CD Spectra: JASCO ORD/UV-5 or ORD/UV-6 Spectrometer; PMR Spectra: Hitachi R-22 (90 MHz) or Varian HA-100 NMR Spectrometer, in $CDCl_3$ and $Me_4Si$ as the internal standard. The chemical shifts are given in $\delta$ values and coupling constants (J) are in Hz (abbreviations: d=doublet, s=singlet).

Silica gel D-5 (Camag) was used for TLC and detection was made by spraying 1% $Ce(SO_4)_2$ in 10% $H_2SO_4$ followed by heating. On preparative TLC, detection was made by spraying dist. water or by keeping the developed plate in the $I_2$ chamber. For column chromatography, silica gel (Merck, 0.05—0.2 mm) was used.

(neg. max.), $[\theta]_{306}$ −4000 (neg. min.), $[\theta]_{270}$ −22400 (neg. max.), $[\theta]_{246}$ 0, $[\theta]_{225}$ +22900 (pos. max.). PMR (90 MHz): as shown in Table 1. Mass Spectrum *m/e* (%): 486 ($M^+$, 1), 468 ($M^+-H_2O$, 65), 453 ($M^+-CH_3-H_2O$, 21), 435 ($M^+-2H_2O-CH_3$, 28), 274 (35), 259 (35), 241 (16), 109 ($\overset{+}{C}H_2=CH-CH-CH_2CH=C(CH_3)_2$, 89), 69 ($\overset{+}{C}H_2-CH=C(CH_3)_2$, 100). High resolution mass spectrum: Found: 486.334, 468.323, 274.194, 259.170, 241.159, 109.066, 109.102. Calcd. for $C_{30}H_{46}O_5$=486.335, $C_{30}H_{44}O_4$=468.324, $C_{18}H_{26}O_2$=274.193, $C_{17}H_{23}O_2$=259.170, $C_{17}H_{21}O$=241.159, $C_7H_9O$=109.065, $C_8H_{13}$=109.102. The physical properties described above are coincided with those reported for stichopogenin $A_4$ (mp 238—240°).[7c]

The aqueous supernatant was neutralized with resin Dowex 44 ($OH^-$), and concentrated under reduced pressure. The residue was subjected to i) PPC (Toyo Filter Paper no. 50) developing with iso-PrOH–*n*-BuOH–$H_2O$ (7: 1: 2) for 17 hr and detected with aniline hydrogen phthalate: quinovose +3-O-methyl-glucose (*Rf*=0.50), xylose (*Rf*=0.40), and glucose (*Rf*=0.36), and ii) TLC (cellulose MN 300) developing with phenol–$H_2O$ (100: 40) and detected as above: 3-O-methyl-glucose (*Rf*=0.80), quinovose (*Rf*=0.72), xylose (*Rf*=0.50), and glucose (*Rf*=0.45), and iii) GLC (as TMS derivative using hexamethyldisilazane/$Me_3SiCl$/pyridine): 3% SE-30 on chromosorb W, 1 m×3 mm; column temp.: 180°; $N_2$ flow rate: 40 ml/min, and xylose, quinovose, 3-O-methyl-glucose, and glucose were identified.

**Mild Acid Hydrolysis of Holotoxin A (4)**——A mixture of **4** (200 mg) in aq. 2% $H_2SO_4$–MeOH (30 ml—30 ml)/benzene (50 ml) was refluxed for 50 hr. During the period, the benzene layer was replaced by fresh benzene every 5 hr. The combined benzene layer was washed with water and evaporated to dryness to give a residue (60 mg), which was subjected to preparative TLC ($CHCl_3$–MeOH=50: 1) to furnish stichopogenin $A_4$ (**3a**, 25 mg) and 25-O-methyl-stichopogenin $A_4$ (=genin-3) (**3c**, 10 mg). 25-O-Methyl-stichopogenin $A_4$ (**3c**), mp 240—243°, $[\alpha]_D^{24}$ −96° (*c*=0.2, MeOH). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1747 (br). UV $\lambda_{max}^{EtOH}$ nm: transparent above 210 nm. CD ($c=1.34\times10^{-3}$, MeOH): $[\theta]_{350}$ 0, $[\theta]_{303.5}$ −15500 (neg. max.), $[\theta]_{260.5}$ −1100 (neg. min.), $[\theta]_{233}$ −16400 (neg. max.), $[\theta]_{216.5}$ 0, $[\theta]_{200}$ +33000 (pos. max.). PMR (90 MHz): 3.17 (3H, s, OMe) and other signals as given in Table I. Mass Spectrum *m/e* (%): 500 ($M^+$, 1), 468 ($M^+-CH_3OH$, 10), 274 (5), 259 (5), 241 (1), 73 ($(CH_3)_2C=\overset{+}{O}CH_3$, 100). High resolution mass spectrum: Found: 500.350, 73.068. Calcd. for $C_{31}H_{48}O_5$=500.350, $C_4H_9O$=73.068.

**Catalytic Hydrogenation of Genin-1 giving 3d**——A solution of genin-1 (28 mg) in EtOAc (15 ml) was hydrogenated over $PtO_2$ (20 mg) at room temperature for 3 hr, filtered, and concentrated under reduced pressure to give a product (27 mg). Recrystallization of the product from MeOH furnished **3d**, mp 259—261°. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1763 (br). PMR (90 MHz): as shown in Table I. Mass Spectrum *m/e* (%): 470 ($M^+$, 4), 455 ($M^+-CH_3$, 3), 452 ($M^+-H_2O$, 2), 437 ($M^+-CH_3-H_2O$, 4), 274 (5), 259 (5), 241 (5), 69 (100).

**Stichopogenin $A_4$ Monoacetate (3e)**——A solution of **3a** (10 mg) in $Ac_2O$ (1 ml)-pyridine (1 ml) mixture was left standing at room temperature for 48 hr, poured into ice-water, and extracted with ether. The ether extract, after usual work-up, gave a product (10 mg), which was purified by preparative TLC (benzene–acetone=8: 1) and recrystallized from MeOH to give **3e** (4 mg), mp 220—222° (250—252°) (double melting-point), $[\alpha]_D^{28}$ −53° (*c*=0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1732, 1716 (sh.). PMR (90 MHz): as shown in Table I. Mass Spectrum *m/e* (%): 528 ($M^+$, 1), 510 ($M^+-H_2O$, 65), 495 ($M^+-CH_3-H_2O$, 10), 450 ($M^+-H_2O$–AcOH, 9), 435 ($M^+-CH_3-H_2O$–AcOH, 48), 316 (45), 301 (7), 241 (48), 228 (45), 109 (100), 69 ($\overset{+}{C}H_2CH=C(CH_3)_2$, 84).

**9α,11α-Epoxy-stichopogenin $A_4$ (17)**——To a solution of **3a** (3 mg) in $CHCl_3$ (2 ml, spectrograde) was added dropwise a solution of *m*-chloroperbenzoic acid (3 mg) in $CHCl_3$ (5 ml, spectrograde), and the total solution was kept stirring at room temperature for 2 hr, washed with aq. $Na_2SO_3$, aq. $NaHCO_3$, and water successively. A residue obtained by evaporation of the solvent was treated with small amount of MeOH to give 9α,11α-epoxy-stichopogenin $A_4$ (**17**) (amorphous), IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 1750 (br), 898 (epoxide). CD ($c=1.79\times10^{-4}$, MeOH): $[\theta]_{345}$ 0, $[\theta]_{305}$ −12000 (neg. max.), $[\theta]_{254}$ −400 (neg. min.), $[\theta]_{228}$ −5400 (neg. max.), $[\theta]_{212}$ 0.

**25-O-Methyl-stichopogenin $A_4$ Monoacetate (3f)**——Acetylation of **3c** (14 mg) with $Ac_2O$ (0.2 ml)-pyridine (0.2 ml) at 34° for 24 hr, followed by ordinary work-up, gave a product (12 mg). Recrystallization of the product from MeOH furnished **3f** (7 mg) of mp 238—241°, $[\alpha]_D^{28}$ −11° (*c*=0.5, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: no OH, 1768 (sh.), 1751, 1742 (sh.). PMR (90 MHz): as shown in Table I, CD ($c=1.25\times10^{-4}$, MeOH): $[\theta]_{340}$ 0, $[\theta]_{305}$ −11000 (neg. max.), $[\theta]_{260}$ −530 (neg. min.), $[\theta]_{232}$ −14800 (neg. max.), $[\theta]_{213}$ 0, $[\theta]_{210}$ +10560. High resolution mass spectrum: Found: 542.361. Calcd. for $C_{33}H_{50}O_6$=542.361.

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