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Saponin and Sapogenol. XXV.[1] Steroidal Saponins from the Starfish *Acanthaster planci* L. (Crown of Thorns). (1). Structures of Two Genuine Sapogenols, Thornasterol A and Thornasterol B, and Their Sulfates

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The saponin mixture has been isolated from the whole body of starfish *Acanthaster planci* L. (crown of thorns). By use of the glycosidase mixture from the large marine gastropod *Charonia lampas*, which is known as a natural enemy of *A. planci*, two genuine sapogenols named thornasterol A (**8**) (major) and thornasterol B (**9**) (minor) have been isolated as their diacetates from the saponin mixture. On the basis of chemical reactions and physicochemical evidence, the structures of diacetyl-thornasterol A and diacetyl-thornasterol B have been elucidated to be 3β,6α-diacetoxy-5α-cholest-9(11)-en-20-ol-23-one (**8a**) and 24ξ-methyl-3β,6α-diacetoxy-5α-cholest-9(11)-en-20-ol-23-one (**9a**), respectively. Furthermore, the sulfate group contained in the parent saponins of thornasterol A (**8**) and B (**9**) has been shown to attach to 3β-OH of the respective sapogenols. It has been also suggested that the pregnane-type sapogenol (**1**), which has been hitherto isolated not only from *A. planci* but also from many other species of *Asteroidea*, may be an artifact sapogenol.

Keywords——starfish; crown of thorns; *Acanthaster planci*; glycosidase from *Charonia lampas*; thornasterol A; thornasterol B; steroidal sapogenol sulfate; MS; CD

Two classes *Asteroidea* (starfish) and *Holothuroidea* (sea cucumber) have been characterized in the phylum *Echinodermata* by including various species which contain saponins as the toxic constituents.[3] Among these species, only a limited number of saponins have been chemically elucidated up to this time. For example, in the class Holothuroidea, holothurin A[4,5] and B[5] were isolated from *Actinopyga agassizi*,[4] *Holothuria leucospilota* (=*H. vagabunda*),[5] and *H. lubrica*[5] and their structures have been investigated. Recently, we have elucidated the total structure of holotoxin A, which is an antifungal oligoglycoside obtained from *Stichopus japonicus*.[6] On the other hand, the structural studies of thelothurin A and B, obtained from *Thelonota ananas*, have been reported.[7] All of these saponins comprise the lanostane-type triterpenoid sapogenols. Furthermore, several biogenetic studies on the sea cucumber saponins have been reported recently.[7a,8]

1) Part XXIV: I. Kitagawa, K. Shirakawa, and M. Yoshikawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 1100 (1978).
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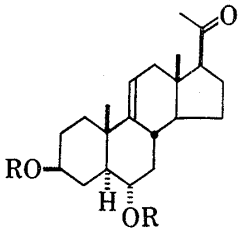
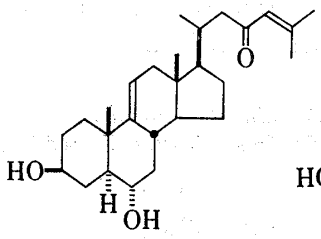
3) *a*) J.S. Grossert, *Chem. Soc. Rev.*, **1**, 1 (1972); *b*) T. Yasumoto, M. Tanaka, and Y. Hashimoto, *Bull. Jap. Soc. Sci. Fish.*, **32**, 673 (1966); *c*) G.D. Ruggieri, R.F. Nigrelli, and M.F. Stempien, *Toxicon*, **8**, 149 (1970).
4) *a*) J.D. Chanley, J. Perlstein, R.F. Nigrelli, and H. Sobotka, *Ann. N. Y. Acad. Sci.*, **90**, 902 (1960); *b*) J.D. Chanley and C. Rossi, *Tetrahedron*, **25**, 1911 (1969).
5) *a*) T. Matsuno and J. Iba, *Yakugaku Zasshi*, **86**, 637 (1966); *b*) T. Yasumoto, K. Nakamura, and Y. Hashimoto, *Agr. Biol. Chem.*, **31**, 7 (1967).
6) *a*) I. Kitagawa, T. Sugawara, I. Yosioka, and K. Kuriyama, *Tetrahedron Lett.*, **1975**, 963; *b*) *Idem, Chem. Pharm. Bull.* (Tokyo), **24**, 266 (1976); *c*) I. Kitagawa, T. Sugawara, and I. Yosioka, *Tetrahedron Lett.*, **1974**, 4111; *d*) *Idem, Chem. Pharm. Bull.* (Tokyo), **24**, 275 (1976).
7) *a*) A. Kelecom, D. Daloze, and B. Tursch, *Tetrahedron*, **32**, 2353 (1976); *b*) A. Kelecom, B. Tursch, and M. Vanhaelen, *Bull. Soc. Chim. Belg.*, **85**, 277 (1976).
8) *a*) G.B. Elyakov, V.A. Stonik, E.V. Levina, and V.S. Levin, *Comp. Biochem. Physiol.*, **52B**, 321 (1975); *b*) Y.M. Sheikh and C. Djerassi, *J. Chem. Soc. Chem. Comm.*, **1976**, 1057.

As for saponins from the species of *Asteroidea*, most of the reported works have been concerned with the structure elucidation of the sapogenols except the structural study of asterosaponin A isolated from *Asterias amurensis*.[9] It is interestingly noted that, in contrast with the sapogenols of *Holothuroidea*, all of the sapogenols hitherto obtained from *Asteroidea* are steroidal: *e.g.* 5α-pregn-9(11)-ene-3β,6α-diol-20-one (**1**) from *Asterias rubens*[10] and *A. forbesi*,[11a] **1**, 5α-cholesta-9(11), 24-diene-3β,6α-diol-23-one (**2**), and 5α-cholest-9(11)-ene-3β, 6α-diol-23-one (**3**) from *Marthasterias glacialis*,[11] and **1**, **2** (minor), and 5α-cholest-9(11)-ene-3β, 6α,23ξ-triol (**4**)(minor) from *Asterias amurensis*.[12]

The starfish *Acanthaster planci* L. is called "crown of thorns" (oni-hitode in Japanese) and has been known as a natural enemy for the coral reefs. Several years ago, they have encountered considerable threat of the starfish against the coral reefs especially in Okinawa prefeture of Japan. In regard to the chemical constituents of *Acanthaster planci*, sterols,[13] spawning pheromones,[14] and sapogenols[15,16] have been investigated. Among them, the structures of sapogenols, which were obtained by acid hydrolysis of the total saponin mixture, have been elucidated as **1** (major), 5α-cholesta-9(11), 20(22)-diene-3β,6α-diol-23-one (**5**)(major), 5α-cholesta-9(11), 17(20), 24-triene-3β,6α-diol (**6**)(minor), and 24ξ-methyl-5α-cholesta-9(11),



1 : R=H (genin-1)
1a : R=Ac (genin-1a)



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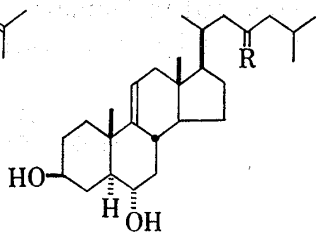
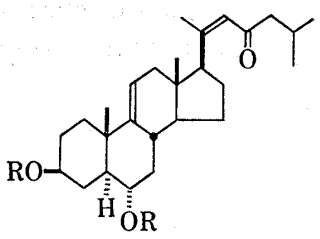
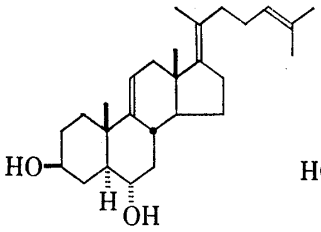


3 : R=O
4 : R=H, OH



5 : R=H (genin-2)
5a : R=Ac (genin-2a)



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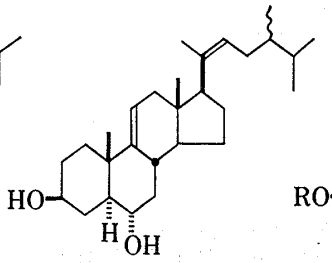

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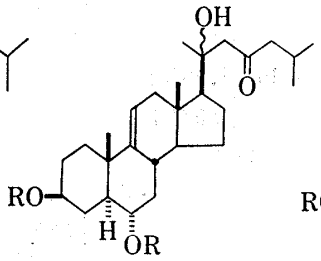
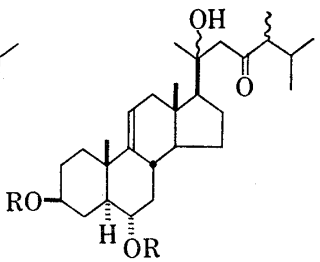


8 : R=H thornasterol A
8a : R=Ac



9 : R=H thornasterol B
9a : R=Ac

Chart 1

9) *a*) S. Ikegami, Y. Hirose, Y. Kamiya, and S. Tamura, *Agr. Biol. Chem.*, **36**, 1843 (1972); *b*) *Idem, ibid.*, **36**, 2453 (1972).
10) J.W. Apsimon, J.A. Buccini, and S. Badripersand, *Can. J. Chem.*, **51**, 850 (1973).
11) *a*) D.S.H. Smith and A.B. Turner, *Tetrahedron Lett.*, **1972**, 5263; *b*) D.S.H. Smith, A.B. Turner, and A.M. Mackie, *J. Chem. Soc. Perkin I*, **1973**, 1745; *c*) S.H. Nicholson and A.B. Turner, *ibid.*, **1976**, 1357.
12) *a*) S. Ikegami, Y. Kamiya, and S. Tamura, *Tetrahedron Lett.*, **1972**, 1601; *b*) *Idem, ibid.*, **1972**, 3725.
13) *a*) Y.M. Sheikh and C. Djerassi, *J. Chem. Soc. Perkin I*, **1974**, 909; *b*) K.C. Gupta and P.J. Scheuer, *Tetrahedron*, **24**, 5831 (1967); *c*) A. Kanazawa, S. Teshima, S. Tomita, and T. Ando, *Bull. Jap. Soc. Sci. Fish.*, **40**, 1077 (1974).
14) D.H. Beach, N.J. Hanscomb, and R.F.G. Ormond, *Nature*, **254**, 135 (1975).
15) *a*) Y. Shimizu, *Experientia*, **27**, 1188 (1971); *b*) Y. Shimizu, *J. Am. Chem. Soc.*, **94**, 4051 (1972).
16) *a*) Y.M. Sheikh, B.M. Tursch, and C. Djerassi, *J. Am. Chem. Soc.*, **94**, 3278 (1972); *b*) *Idem, Tetrahedron Lett.*, **1972**, 3721; *c*) Y.M. Sheikh and C. Djerassi, *ibid.*, **1973**, 2927.

20(22)-diene-3β,6α-diol (**7**)(minor) by Shimizu[15] and Djerassi, *et al.*[16] The structural features of these sapogenols are: i) to possess a steroidal skeleton with a double bond at 9(11), ii) to carry two equatorial hydroxyls at 3β and 6α, and iii) to vary in the side chain structure. However, the genuineness of these sapogenols has not yet been verified.

As a continuation of our chemical study on the sea cucumber saponins,[6] we have been working on the structure elucidation of saponins which are isolated from the whole body of *Acanthaster planci*. By use of the glycosidase mixture of the large marine gastropod *Charonia lampas*, which is known as a natural enemy for *Acanthaster planci*, we have been able to isolate two genuine sapogenols named thornasterol A (**8**)(major) and thornaterol B (**9**) (minor) (as their diacetates) from the total saponin mixture. This paper provides the full account on the structure elucidation of these sapogenols.[17]

The methanol extractive of fresh starfish (crown of thorns), which was collected at Kushimoto in Wakayama prefecture in April, was defatted successively with benzene and ethyl acetate and treated with methanol to remove salt. The methanol soluble portion was purified by silica gel column chromatography and droplet countercurrent chromatography (D.C.C.)[6,18] to afford a saponin mixture (*ca.* 0.07% from the fresh whole body)(Chart 2). Afterwards, we have modified the isolation procedure to a more simplified one starting with the same kind of starfish collected in Okinawa prefecture in May (see Experimental). The sapogenol constituents of both origins were nearly identical.

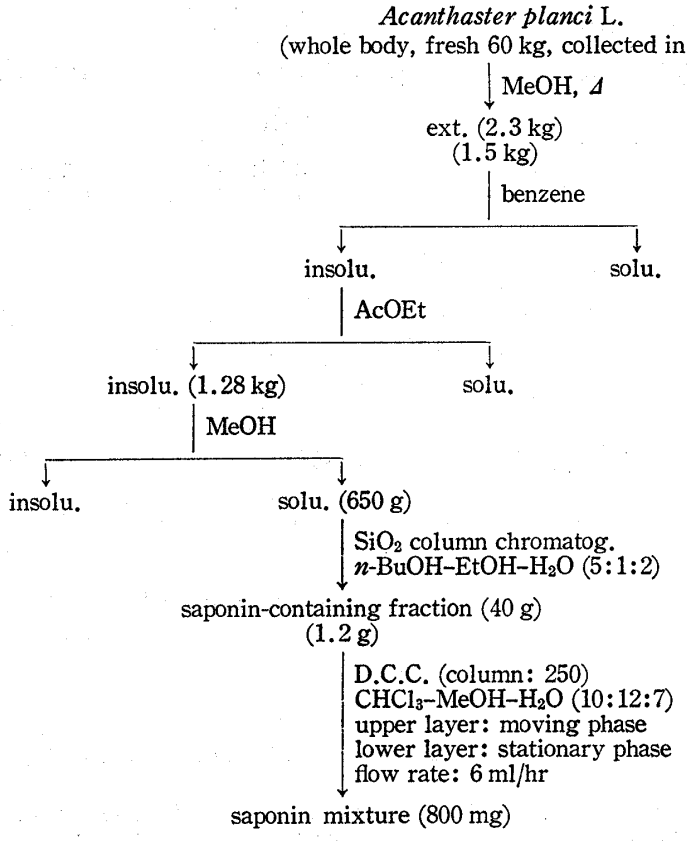


Chart 2

The infrared (IR) spectrum of the saponin mixture shows the strong absorption bands at 3450 (br) cm^{-1} and 1060 (br) cm^{-1} which are reminiscent of the glycosidic structure. The

17) I. Kitagawa, M. Kobayashi, T. Sugawara, and I. Yosioka, *Tetrahedron Lett.*, **1975**, 967 (preliminary report).
18) T. Tanimura, J.J. Pisano, Y. Ito, and R.L. Bowman, *Science*, **169**, 54 (1970).

presence of sulfate function in the saponin mixture has been suggested by a positive test for the potassium rhodizonate reagent[19] and the palladium chloride reagent.[20] Hydrolysis of the saponin mixture with aqueous 2N hydrochloric acid under reflux furnished a sapogenol mixture from which, after acetylation and thin-layer chromatographic (TLC) purification, was obtained an acetate of the major sapogenol (genin-la). The physical properties of genin-1, a deacetylation product of genin-la, were identical with those reported for **1** by Shimizu[15b] and Djerassi, *et al.*[16a]

In order to isolate the genuine sapogenol of the saponin mixture, we have initially examined the hydrolysis using various kinds of enzymes. Finally, it has been found that the mixed glycosidase of *Charonia lampas* (horagai in Japanese) effects the desired hydrolysis leading to a sapogenol sulfate mixture. The resulting sulfate mixture was positive for the potassium rhodizonate reagent and was hydrolysable with sulfatase to yield the sapogenol mixture. The IR spectrum of the sulfate mixture shows the absorption band due to the S–O bond stretching vibration (1230 cm^{-1})[21] while the proton magnetic resonance (PMR) spectrum (in pentadeutero(d_5)-pyridine) exhibits a multiplet of totally one-proton intensity between δ 4.6 and δ 4.9 which is ascribable to a methine-proton attached to a carbon bearing a sulfate group.[22]

After treatment with pyridine, the sapogenol sulfate mixture was subjected to solvolysis with dioxane containing potassium carbonate to furnish a sapogenol mixture.[23] Acetylation followed by preparative TLC separation of the sapogenol mixture gave two acetates of new sapogenols: diacetyl-thornasterol A (**8a**, 40%) and diacetyl-thornasterol B (**9a**, 13%), in addition to genin-la (**1a**, 8%) and genin-2a (**5a**, 36%).[24] The physical properties of genin-2a was identical with those reported for **5a** by Djerassi, *et al.*[16a,25]

The major acetate, diacetyl-thornasterol A (**8a**), mp 158.5—159.5°, lacks the significant ultraviolet (UV) absorption maximum above 210 nm. The IR spectrum shows the presence of acetoxyl (1742 cm^{-1}), carbonyl (1705 cm^{-1}), and free hydroxyl (3500 cm^{-1}) which resists the ordinary acetylation. In the circular dichroism (CD) spectrum of **8a**, are observed a negative maximum ($[\theta]_{292}$—2500) due to n→π^* transition of a carbonyl and a positive maximum ($[\theta]_{197}$+45000) due to $\pi_x \rightarrow \pi_x^*$ transition of $\Delta^{9(11)}$.[6c,d,26]

The PMR spectrum of **8a** shows the signals due to two tertiary methyls at C-10 (δ 0.99) and C-13 (δ 0.74), two secondary methyls at C-25 (6H, d, δ 0.87, J=6 Hz), two secondary acetoxyls (6H, s, δ 2.00 and 2H, m, δ 4.55—4.90), one olefinic proton at C-11 (1H, m, δ 5.30), one tertiary methyl (3H, s, δ 1.30) attached to a carbon (C-20) bearing a hydroxyl, and a methylene (C-22)(2H, br. s, δ 2.51) located between a carbonyl and a tertiary carbon. Among the functions elucidated by the PMR spectrum, latter two are in significant contrast with those contained in genin-2a (**5a**).

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19) *a*) J.J. Schneider and M.L. Lewbart, *J. Biol. Chem.*, **222**, 787 (1966); *b*) D.P. Burma, *Anal. Chim. Acta*, **9**, 513 (1953).

20) E. Stahl (ed.), "Thin-Layer Chromatography, a Laboratory Handbook," 2nd ed., Springer Verlag, Berlin, 1969, p. 892.

21) J.R. Turvey, *Advan. Carbohyd. Chem.*, **20**, 183 (1965).

22) S. Ikegami, Y. Kamiya, and S. Tamura, *Tetrahedron*, **29**, 1807 (1973).

23) The initial treatment with pyridine was undertaken for converting the sulfate group to the pyridinium salt. Potassium carbonate was added in order to avoid the reaction medium to become acidic during the solvolysis. Solvolysis of the sulfate group with dioxane only: *cf.* J. McKenna and J.K. Norymberski, *J. Chem. Soc.*, **1957**, 3889.

24) The yields of respective sapogenol acetates are calculated from the total mixture of sapogenol acetates.

25) As for geometry of $\Delta^{20(22)}$, further examination seems to be needed. The structures (**5**, **5a**) given here follow the literature.[16a]

26) *a*) Y. Yogev and Y. Mazur, *Chem. Comm.*, **1965**, 552; *b*) A.I. Scott and A.D. Wrixon, *Tetrahedron*, **26**, 3695 (1970); *c*) *Idem*, *ibid.*, **27**, 4787 (1971); *d*) J. Hudec and D.N. Kirk, *Tetrahedron*, **32**, 2475 (1976).

The mass spectrum (MS) of **8a** provides the further information on the structure (Chart 3).[27] The base peak observed at *m/e* 281 (iii) is formally derived from the molecular ion through a McLafferty-type fragmentation of a β-hydroxy-carbonyl moiety[28] in the side chain of **8a** (giving *m/e* 416) which is followed by elimination of two acetic acid units and one methyl (giving the base peak). This finding, in conjunction with the foregoing PMR evidence, strongly supports the presence of a 20-hydroxy-23-carbonyl structure in **8a**. Two fragment ion peaks at *m/e* 441 (vi)(weak) and *m/e* 85 (viia), which are formed through α-cleavage of the carbonyl, also support the presence of 23-carbonyl in the side chain. In addition, a prominent fragment ion peak of *m/e* 211 is presumed to be formed by splitting at the D ring of the steroidal skeleton.[29]

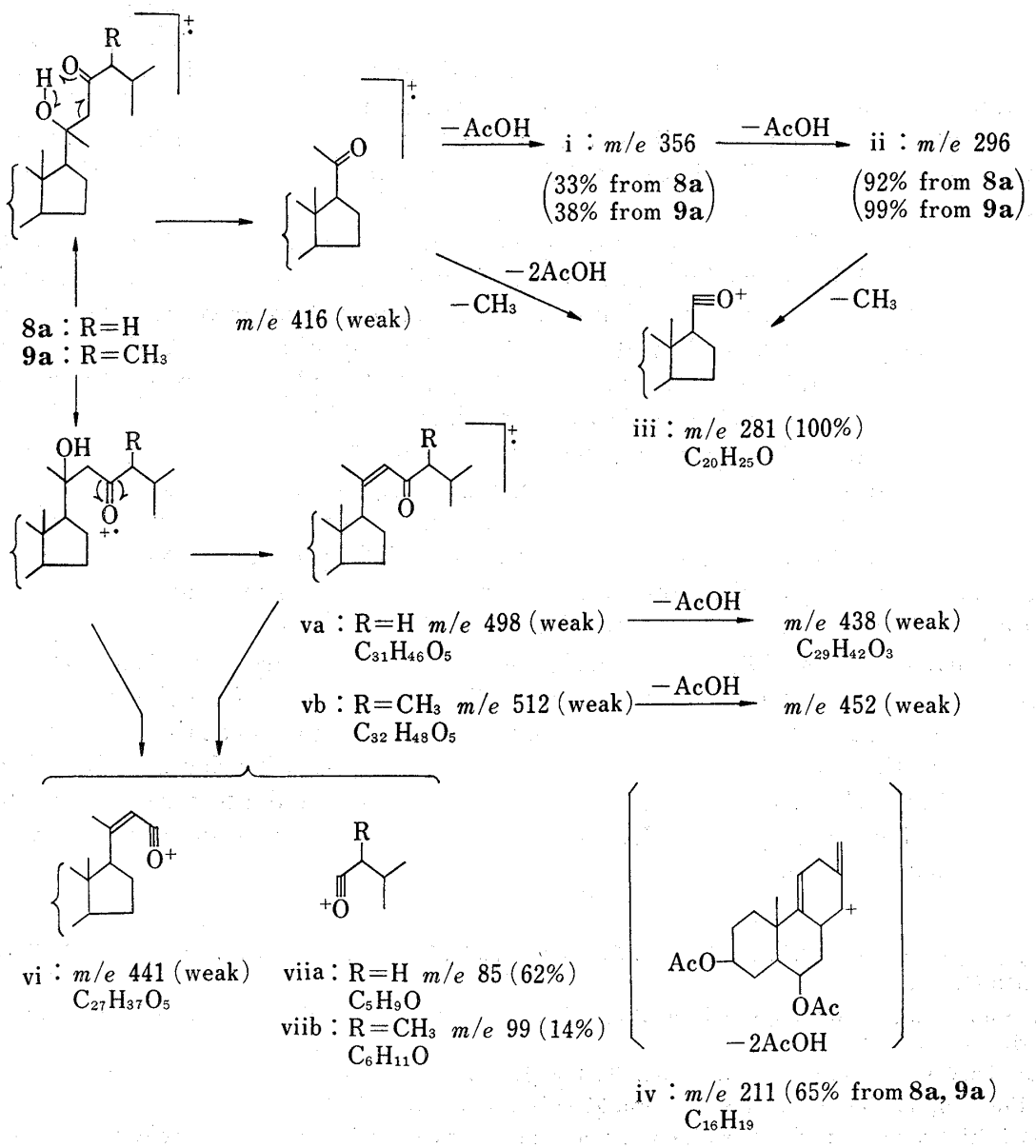


Chart 3

27) The percentages given in the parentheses indicate relative abundance of the ion peaks to the base peak (*m/e* 281). The elemental compositions of the fragment ions given with the chemical formulae were determined by high resolution MS.

28) The similar type fragmentation in methyl α-substituted β-hydroxy-carboxylate: *cf.* A.H. Etemadi, *Bull. Soc. Chim. France*, **1964**, 1537.

29) L. Tökes, G. Jones, and C. Djerassi, *J. Am. Chem. Soc.*, **25**, 5465 (1968).

Finally, treatment of **8a** with aqueous 2N hydrochloric acid in benzene under reflux gave rise to genin-1a (**1a**)(minor) and genin-2a (**5a**)(major). On the basis of the accumulated evidence mentioned above, the structure of diacetyl-thornasterol A has been elucidated to be 3β,6α-diacetoxy-5α-cholest-9(11)-en-20-ol-23-one (**8a**).

The minor acetate, diacetyl-thornasterol B (**9a**), mp 147—148°, shows the similar spectral properties as **8a**: lack of significant UV absorption maximum above 210 nm and the IR absorption bands at 3500 cm^{-1} (hydroxyl), 1743 cm^{-1} (acetoxyl), and 1700 cm^{-1} (carbonyl). The CD spectrum of **9a** shows a positive maximum ($[\theta]_{305}$+1000) due to n→π^* transition of a carbonyl and a positive maximum ($[\theta]_{197}$+38000) due to π_x—π_x^* transition of $\Delta^{9(11)}$ as observed for **8a**.[6c,d,26]

The PMR spectrum of **9a** is very alike to that of **8a** except that the former shows an additional signal being ascribable to a secondary methyl at C-24. It shows the signals due to two tertiary methyls at C-10 (δ 1.00) and C-13 (δ 0.76), two secondary methyls at C-24 and C-25 (9H, δ 0.79—0.94), two secondary acetoxyls (6H, s, δ 2.00, and 2H, m, δ 4.50—4.85), one olefinic proton at C-11 (1H, m, δ 5.28), one tertiary methyl (3H, s, δ 1.32) at C-20 bearing a hydroxyl, and a methylene at C-22 (2H, br. s, δ 2.56) which is adjacent to a carbonyl and a tertiary carbon. Therefore, the presence of a 20-hydroxy-23-carbonyl moiety in the side chain of **9a** has been suggested.

The mass spectrum of **9a** gives the same fragment ion peaks as found for **8a** (Chart 3): *i.e.* i, ii, iii, and iv, indicating that **9a** possesses the same steroidal nucleus as **8a**. Here again, the base peak is observed at *m/e* 281 (iii), thus suggesting the occurrence of McLafferty-type fragmentation of the molecular ion and, in consequence, indicating the presence of a 20-hydroxy-23-carbonyl moiety in the side chain of **9a**. Two fragment ion peaks at *m/e* 441 (vi, weak) and *m/e* 99 (viib), which are derived through α-cleavage at 23-carbonyl, suggest the location of the additional secondary methyl to be at C-24.

Based on the above described evidence, the structure of diacetyl-thornasterol B has been formulated as 24ξ-methyl-3β,6α-diacetoxy-5α-cholest-9(11)-en-20-ol-23-one (**9a**). The configurations at C-20 of **8a** and **9a** and at C-24 of **9a** are not yet defined.

Four sapogenol acetates mentioned above (**1a**. **5a**, **8a**, and **9a**) have been obtained by enzymatic hydrolysis of the total saponin mixture followed by solvolysis (for removing the sulfate group) and acetylation. In order to study the genuineness of these sapogenols, their CD spectra have been examined in comparison with the CD spectrum of the parent saponin mixture as were undertaken in the studies of holotoxin A and its genuine aglycone.[6] As shown in Table I and Fig. 1a, the CD spectrum of saponin mixture exhibits a strong positive maximum at *ca.* 200 nm (due to $\Delta^{9(11)}$ in the sapogenol portion[26]), a weak negative maximum

Table I. Yields and CD Data of **1a**, **5a**, **8a**, and **9a** in Comparison with CD Data of Saponin Mixture

	Yield (%)[a]	CD data
Saponin mixture		$[\theta]_{200}$ (pos. max.)[b]
		$[\theta]_{248}$ (neg. max.)
		$[\theta]_{293}$ (neg. max.)
Diacetyl-thornasterol A (**8a**)	40	$[\theta]_{197}$ +45000 (pos. max.)
		$[\theta]_{292}$ −2500 (neg. max.)
Diacetyl-thornasterol B (**9a**)	13	$[\theta]_{197}$ +38000 (pos. max.)
		$[\theta]_{305}$ +1000 (pos. max.)
Genin-1a (**1a**)	8	$[\theta]_{287}$ +6400 (neg. max.)[c]
Genin-2a (**5a**)	36	$[\theta]_{246}$ −10600 (neg. max.)[c]

a) The yields are calculated from the total acetylation product of sapogenol mixture prior to TLC separation.
b) The wave length of this maximum is an approximate value.
c) The CD spectra below 210 nm of **1a** and **5a** were not measured.

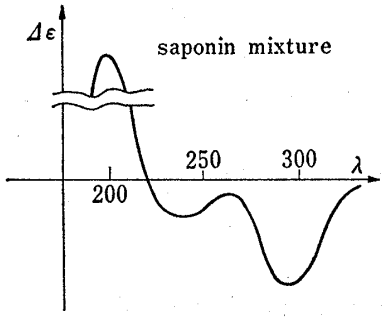


Fig. 1a

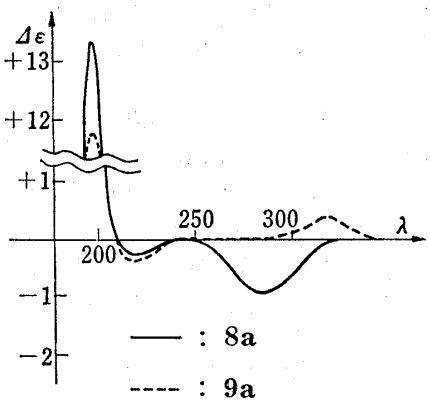


Fig. 1b

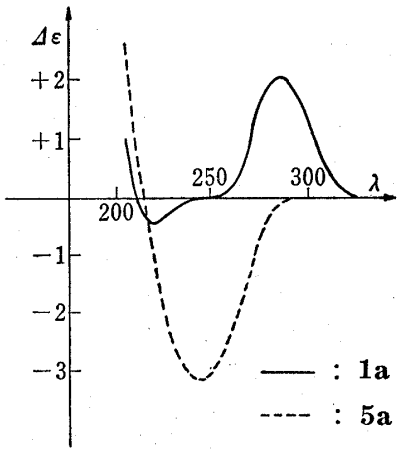
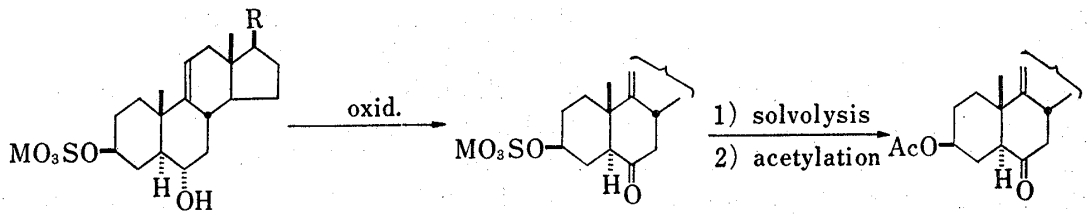
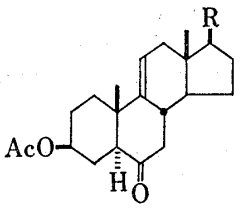


Fig. 1c



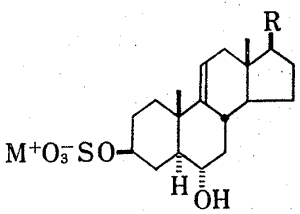
a (sapogenol sulfate mixture) **b** **c**



10 : R= (KA-1)

11 : R= (KA-2)

12 : R= (KA-3)



15 : R=

16 : R=

17 : R=

Chart 4

around 248 nm (due to $\pi\rightarrow\pi^*$ transition of an α,β-unsaturated carbonyl), and a strong negative maximum at 293 nm (due to $n\rightarrow\pi^*$ transition of 23-carbonyl).

Comparison of these CD data of the saponin mixture with those of sapogenol acetates (**1a**, **5a**, **8a**, and **9a**)(Table I, Fig. 1b, 1c) have led to a conclusion that thornasterol A (**8**) and thornasterol B (**9**)[30] are two genuine sapogenols of saponins contained in *Acanthaster planci*. Furthermore, the pregnane-type sapogenol (**1**)(genin-1), which has been obtained as a major sapogenol by acid hydrolysis of the total saponin mixture and also by acid treatment of **8a** (obtained as **1a** in this case), seems to be an artifact sapogenol. The fact, that **1a** is formed in minor quantity along with **8a**, **9a**, and **5a** upon solvolysis of the sapogenol sulfate mixture followed by acetylation, is presumably due to secondary change of **8a** and/or **9a** during the solvolysis. It is also noted that the pregnane-type compound has not yet been formed under the milder solvolytic conditions as described later. As for genuineness of **5** (genin-2), although it seems to be an artifact sapogenol to some extent as judged from $[\theta]_{246}$ of **5a** in comparison with the CD data of saponin mixture, further examinations seem to be needed, since the total saponin mixture shows a weak UV absorption maximum at 255 nm ($E^{1\%}_{1cm}$ cm $=ca.$ 15 in water).

In 1973, Turner, *et al.*[11b] suggested a compound, having a 20-hydroxy-23-carbonyl moiety in the side chain, as a probable biogenetic precursor for **1** and **5**. However, the present work demonstrates that thornasterol A (**8**) and B (**9**) possessing the 20-hydroxy-23-carbonyl moiety are in fact genuine sapogenols rather than the speculative biogenetic precursors. In addition, the pregnane-type sapogenol (**1**), which has been obtained by acid hydrolysis of saponins isolated from many species of *Asteroidea* (*vide supra*),[10,11,12] is assumed to be an artifact sapogenol.

As mentioned above, the saponins of *Acanthaster planci* contain the sulfate group in their molecules. Our attention has been next focused to elucidation of the location of the sulfate group in saponins. The object has been effected *via* the following scheme (**a**→**b**→**c**, Chart 4). The sapogenol sulfate mixture (**a**) was successively subjected to oxidation with chromium trioxide in pyridine (giving **b**), solvolysis with dioxane–pyridine,[31] and acetylation (giving **c**) (TLC diagrams of **a**, **b**, and **c** in Fig. 2). Preparative TLC separation of the final product (**c**) furnished three acetates: KA-1 (**10**), KA-2 (**11**), and KA-3 (**12**) (in the order of polarity on TLC), in the yields as shown in Table II.

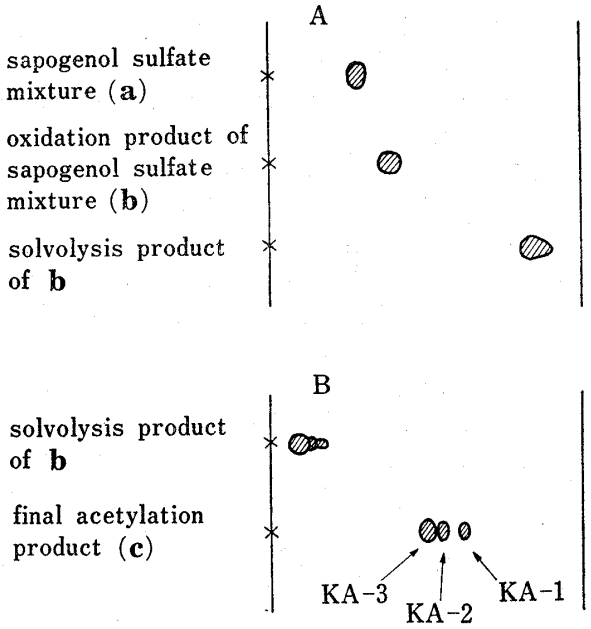


Fig. 2. TLC Diagrams of Reaction Products Starting from Sapogenol Sulfate Mixture (**a**→**b**→**c**)

Adsorbent: Kieselgel 60 F_{254} (Merck).
Detection: 1% $Ce(SO_4)_2$–10% H_2SO_4.
A) solvent: $CHCl_3$–MeOH–H_2O (7:3:1, lower layer).
B) solvent: benzene–acetone (10:1) (developing twice).

30) Since thornasterol B (**9**) is a minor sapogenol and the molar ellipticity due to $n\rightarrow\pi^*$ transition of 23-carbonyl is small, contribution of **9** in the CD spectrum of the total saponin mixture is considered to be small.

31) The method applied here is a modified one from the above described procedure using K_2CO_3–dioxane. Under the milder solvolytic conditions, no formation of any pregnane-type derivative (*e.g.* **1**) has been observed.

Table II. Yields and CD Data of **10**, **11**, and **12** in Comparison with ORD Data of **13** and **14**

	Yield (%)[a]	CD or ORD data
KA-1 (**10**)	14	$[\theta]_{244}$ −12000 (neg. max.) $[\theta]_{292}$ −6100 (neg. max.)
KA-2 (**11**)	18	$[\theta]_{293}$ −6600 (neg. max.)
KA-3 (**12**)	60	$[\theta]_{293}$ −7420 (neg. max.)
5α-Cholestan-3-one (**13**)[32]		$[\Phi]_{265}$ −2380 (trough) $[\Phi]_{307.5}$ +3050 (peak)
3β-Acetoxy-5α-cholestan-6-one (**14**)[32]		$[\Phi]_{270}$ +4050 (trough) $[\Phi]_{306}$ −3580 (peak)

a) The yields are calculated from the total acetylation product prior to TLC separation.

KA-1 (**10**), mp 169—170°, shows a strong UV absorption maximum at 248 nm and the IR absorption bands due to acetoxyl (1741 cm^{-1}), saturated carbonyl (1722 cm^{-1}), and α,β-unsaturated carbonyl (1687, 1606 cm^{-1}). The PMR spectrum of **10** resembles that of genin-2a (**5a**) except the signals due to only one secondary acetoxyl (3H, s, δ 2.03, and 1H, m, δ 4.50—4.75) observed in the former while two in **5a**. The mass spectrum of **10** also shows a similar fragmentation pattern as that of **5a** except that the molecular ion of **10** is observed at *m/e* 454. Therefore, KA-1 has been presumed to be a monoacetate of either a 3-carbonyl or a 6-carbonyl derivative of genin-2 (**5**). The structure (**10**) has been finally assigned to KA-1 on the basis of the CD spectra in comparison with the optical rotatory dispersion (ORD) data of 5α-cholestan-3-one (**13**)[32] and 3β-acetoxy-5α-cholestan-6-one (**14**)[32] (Table II). The CD spectrum of **10** shows a negative maximum ($[\theta]_{292}$—6100) which is ascribable to $n\rightarrow\pi^*$ transition of 6-carbonyl together with a negative maximum ($[\theta]_{244}$—12000) due to an enone in the side chain. Consequently, it has become clear that the location of a sulfate group in the parent saponin of **5**, if any, is 3β–OH.

KA-2 (**11**), mp 184—185°, exhibits the IR absorption bands due to acetoxyl (1740 cm^{-1}), carbonyl (1720, 1703 (sh.) cm^{-1}), and hydroxyl (3490 cm^{-1}) which is retained on the ordinary acetylation. The PMR and mass spectra of **11** are alike to those of diacetyl-thornaterol B (**9a**) except that **11** shows the PMR signals due only one secondary acetoxyl (3H, s, δ 2.02, and 1H, m, δ 4.53—4.77) and gives an MS ion peak at *m/e* 468 (M^+–H_2O). In addition, the CD spectrum of **11** shows a negative maximum ($[\theta]_{293}$—6600)[33] due to 6-carbonyl. The structure of KA-2 has been thus elucidated as **11**, which is a monoacetate of 6-carbonyl derivative of thornasterol B.

The major acetate, KA-3 (**12**), mp 178—179°, possesses the same functions as **11** (IR: 3500, 1742, 1720, and 1706 cm^{-1}). The PMR and mass spectra of **12** are alike to those of diacetyl-thornasterol A (**8a**), as are experienced in the relation between KA-2 (**11**) and diacetyl-thornasterol B (**9a**). The CD spectrum of **12**, showing a negative maximum ($[\theta]_{293}$—7420),[34] has finally rationalized the structure of KA-3 to be expressed as **12**. Consequently, the sulfate group in the parent saponins of thornasterol A (**8**) and thornasterol B (**9**) have been elucidated to attach to 3β–OH of the respective sapogenols.

In conclusion, the sapogenol sulfate mixture, which is obtained by glycosidase hydrolysis of the total saponin mixture, has been revealed to comprise three sulfates: **15**, **16**, and **17**. The latter two are sulfates of thornasterol B (**9**) and A (**8**), respectively. It should be noted here that any pregnane-type sulfate has not been obtained from the total sapogenol sulfate

32) C. Djerassi and W. Klyne, *J. Chem. Soc.*, **1963**, 2390.
33) The CD maximum due to 23-carbonyl of **11** is obscure because of small ellipticity (*cf.* **9a** in Table I) and overlap with the CD maximum of 6-carbonyl.
34) The large ellipticity is due to overlap with the CD curve of 23-carbonyl.

mixture. This provides an additional evidence that genuineness of the pregnane-type sapogenol (**1**) is less likely as mentioned above.

Experimental[35]

Isolation of Saponin Mixture——a) Fresh whole body of *Acanthaster planci* (crown of thorns, 60 kg, collected at Kushimoto in Wakayama prefecture in April) was extracted four times with MeOH (100 l each) under reflux. Combined MeOH extract was concentrated under reduced pressure to give the residue (2.3 kg). The residue was treated repeatedly with boiling benzene (total amount: 150 l) and then with boiling AcOEt (total amount: 40 l). The supernatants were removed by decantation to give the resinous insoluble part (solidified at room temperature: 1.28 kg), which was extracted with MeOH (totally 30 l) at room temperature. The MeOH extract, obtained by filtration, was evaporated under reduced pressure to give the residue (650 g), which was chromatographed on a silica gel column (1 kg) developing with *n*-BuOH–EtOH–H_2O (5: 1: 2) to afford the saponin-containing fraction (40 g). Purification of the saponin-containing fraction (1.2 g) with D.C.C. (moving phase=upper layer of $CHCl_3$–MeOH–H_2O (10: 12: 7); stationary phase=the lower layer; column 250; flow rate 6 m/hr; 1 fraction 2 ml) gave the saponin mixture (800 mg).

b) Recent method. Finely crushed whole body of *A. planci* (37 kg, collected in Okinawa prefecture in May and June) was extracted with aq. 50% MeOH under reflux for 5 hr. The MeOH extract (totally *ca.* 50 l) was concentrated under reduced pressure (up to 1/10 volume) to remove MeOH. The concentrated aq. solution was shaken with *n*-BuOH and the *n*-BuOH soluble portion was taken and evaporated under reduced pressure to give the residue (10 g from 4 l of 50% MeOH ext.). The residue (10 g) was then extracted with a small amount (*ca.* 150 ml) of MeOH and the MeOH extract was diluted with AcOEt (three times of volume, *ca.* 450 ml). The precipitate (3.6 g) was collected by filtration, dissolved in aq. MeOH, and treated with active charcoal with stirring. After filtration, the filtrate was concentrated under reduced pressure to furnish the saponin-containing fraction (2.2 g) as above. Saponin mixture, IR ν_{max}^{KBr} cm^{-1}: 3450 (br), 1060 (br). UV $\lambda_{max}^{H_2O}$ nm: 255 ($E_{1cm}^{1\%}$ *ca.* 15). CD ($c=1.9\times10^{-1}$, MeOH): as shown in Table I and Fig. 1a.

Detection of Sulfate Function in Saponin Mixture——A solution of saponin-mixture (1 mg) in aq. 2 N HCl (1 ml) was refluxed for 2 hr, neutralized with dil. NaOH and evaporated to dryness under reduced pressure. The residue was subjected to paper partition chromatography (PPC) (Toyo Filter Paper No. 50) developing with MeOH–H_2O (9: 1) mixture. After drying in the air, the paper was sprayed with a solution of $BaCl_2$ (100 mg) in 70% MeOH (50 ml) and dried again in the air. The paper was then sprayed with a solution of potassium rhodizonate (10 mg) in aq. 50% MeOH (50 ml) to develop the coloration: saponin mixture—positive (yellow).

Acid Hydrolysis of Saponin-Containing Fraction followed by Acetylation giving 1a——A solution of saponin-containing fraction (*cf.* Chart 2, 8.5 g) in aq. 2 N HCl (200 ml) was heated on a boiling water-bath for 2 hr. The reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ extract was neutralized with aq. $NaHCO_3$, washed with water, and dried over $MgSO_4$. Evaporation of the solvent gave the residue (480 mg), which was acetylated with Ac_2O (3 ml) and pyridine (4 ml) at 39° for 24 hr. The total reaction mixture was poured into ice-water and extracted with ether. Working-up of the ether extract in the usual manner furnished the product (490 mg). Preparative TLC (developing with $CHCl_3$) gave genin-1a (**1a**, 60 mg). Genin-1a (**1a**), colorless glassy, $[\alpha]_D^{13}$ +59° ($c=3.2$, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1743, 1712. PMR ($CDCl_3$) δ: 0.55 (3H, s, 13-CH_3), 1.02 (3H, s, 10-CH_3), 2.01 (6H, s, OAc×2), 2.11 (3H, s, 20-CH_3), 4.50—5.00 (2H, m, 3α-H, 6β-H), 5.40 (1H, m, 11-H). CD ($c=6.65\times10^{-2}$, MeOH): $[\theta]_{327}$ 0, $[\theta]_{287}$ +6400 (pos. max.), $[\theta]_{254}$ 0, $[\theta]_{220}$ −1300 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{212}$ +2300!. ORD ($c=6.65\times10^{-2}$): $[\Phi]_{450}$ +500°!, $[\Phi]_{304}$ +4700° (peak), $[\Phi]_{282}$ 0°, $[\Phi]_{263}$ −3600° (trough), $[\Phi]_{234}$ 0°, $[\Phi]_{220}$ +4200°!. MS *m/e* (%): 416 (M^+, 6), 296 (M^+−2AcOH, 60), 281 (296−CH_3, 63).

Alkaline Hydrolysis of Genin-1a (1a) giving Genin-1 (1)——A solution of genin-1a (**1a**, 30 mg) in 5% KOH–MeOH (5 ml) was refluxed for 2 hr. While adding water gradually, MeOH was removed under reduced pressure from the reaction mixture. The aqueous mixture thus obtained was extracted with AcOEt. The AcOEt extract was neutralized, washed with water, and evaporated under reduced pressure to give genin-1

35) The following instruments were used for obtaining the physical data: mp (Yanagimoto Micro-melting-point Apparatus, recorded uncorrected); Specific rotations (Rex Photoelectric Polarimeter NEP-2, 1= one dm); UV spectra (Shimadzu MPS-50L Spectrophotometer); IR spectra (Hitachi IR Spectrometer EPI-G3 or EPI-S2); CD spectra (JASCO UV/ORD-5 or Model J-20 Automatic Recording Spectropolarimeter, c=g/100 ml); MS (Hitachi RMU-6E Mass Spectrometer, at 70 eV unless specified otherwise, direct inlet); High resolution MS (JEOL JMS-01SG Mass Spectrometer); PMR spectra (Hitachi R-22 (90 MHz) NMR Spectrometer, tetramethylsilane as an internal standard). Silica gel (Merck, 60—230 mesh) was used for column chromatography, and silica gel (Camag D-5) and Kieselgel 60 F_{254} (Merck, DC-Fertigplatten) were used for TLC. Detection was made by spraying 1% $Ce(SO_4)_2$–10% H_2SO_4 with heating. For preparative TLC, detection was made by spraying water or by I_2 vapor.

(**1**, 20 mg), mp 158—160° (MeOH–H_2O). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3420, 1708. PMR ($CDCl_3$) δ: 0.53 (3H, s, 13-CH_3), 0.93 (3H, s, 10-CH_3), 2.09 (3H, s, 20-CH_3), 3.30—3.60 (2H, m, 3α-H, 6β-H), 5.35 (1H, m, 11-H). CD ($c=1.66\times10^{-1}$, MeOH): $[\theta]_{318}$ 0, $[\theta]_{285}$ +6300 (pos. max.), $[\theta]_{240}$ 0. ORD ($c=1.66\times10^{-1}$, MeOH): $[\Phi]_{350}$ +1660°!, $[\Phi]_{302}$ +4980° (peak), $[\Phi]_{280}$ 0°, $[\Phi]_{267}$ −4580° (trough), $[\Phi]_{224}$ 0°, $[\Phi]_{212}$ +5000°!. MS *m/e* (%): 332 (M^+, 34), 296 (M^+-2H_2O, 34), 281 (296−CH_3, 59). **1** in lit.[16a]: mp 162—163°, $[\theta]_{287}$ +7380 (pos. max.); $[\Phi]_{305}$ +5199° (peak), $[\Phi]_{265}$ −3812° (trough); δ ($CDCl_3$): 0.57, 0.95, 2.11 (3H each, all s), 3.09 (1H, t, J=9.5 Hz), 3.57 (2H, m), 5.37 (1H, distorted t, J=5.5 Hz). Lit.[15b]: mp 193—196° (MeOH–H_2O), $[\alpha]_D^{23}$ +98.4° (MeOH): ν: 3400, 1700 cm^{-1}; MS *m/e*: 332 (M^+), 314, 299, 296, 281, 230, 229, 211, 43; δ ($CDCl_3$): 0.53, 0.95, 2.11 (3H each, all s), 3.6 (2H), 5.37 (1H, unresolved t).

Enzymatic Hydrolysis of Saponin Mixture with Glycosidase Mixture of *Charonia lampas*——To a solution of saponin mixture (1 g)[36] in McIlvain buffer solution (pH 4.0, 200 ml), was added the glycosidase mixture of *Charonia lampas* (Seikagaku Kogyo, Tokyo, 1 g). The total mixture was kept gentle stirring at 40° for 3 days. After addition of a small amount (*ca.* 5 ml) of EtOH, the mixture was warmed for a while and filtered. The aqueous filtrate was then extracted with *n*-BuOH and evaporation of the *n*-BuOH extract under reduced pressure yielded the residue (0.84 g), which was purified by silica gel (40 g) column chromatography developing with AcOEt–MeOH (10: 1) to furnish the sapogenol sulfate mixture (94 mg) and other hydrolysates. Sapogenol sulfate mixture: positive (yellow) for potassium rhodizonate reagent. IR ν_{max}^{KBr} cm^{-1}: 3450, 1230. UV λ_{max}^{EtOH} nm: 246.5 ($E_{1cm}^{1\%}$ *ca.* 16).

Enzymatic Hydrolysis of Sapogenol Sulfate Mixture with Sulfatase——To a solution of sapogenol sulfate mixture (3 mg) in AcONa–AcOH buffer solution (pH 5.6, 2 ml), was added sulfatase (from edible snail *Helix pomatia*, EC3.1.6.1, Mannheim Boehringer, 0.2 ml), and the total mixture was kept stirring at 25° for 6 days. After addition of EtOH (0.5 ml) followed by warming for a while, the mixture was extracted with AcOEt. TLC examinations (benzene–acetone=1: 1) of the AcOEt extractive revealed the hydrolysis of sapogenol sulfate linkage resulting in the formation of aglycone mixture (Rf=0.60).

Solvolysis of Sapogenol Sulfate Mixture followed by Acetylation giving Diacetyl-thornasterol A (8a) and Diacetyl-thornasterol B (9a) together with 1a and 5a——A solution of sapogenol sulfate mixture (135 mg) in pyridine (3 ml) was left standing at 40° for 2 hr. After removing pyridine under reduced pressure, the residue was treated with dioxane (20 ml) and K_2CO_3 (7 mg), and heated under reflux for 20 min. The reaction mixture was diluted with AcOEt, filtered, and evaporated under reduced pressure to give sapogenol mixture (118 mg). Sapogenol mixture (335 mg), obtained by repeated experiments as above, was acetylated with Ac_2O (4 ml) and pyridine (4 ml) at 43° for 20 hr. The reaction mixture was treated with ice-water, extracted with $CHCl_3$, and the $CHCl_3$ extract was worked up in the usual manner. The acetate (370 mg) thus obtained was purified by preparative TLC (benzene–ether=20: 1, developing three times) to furnish genin-1a (**1a**, 20 mg), genin-2a (**5a**, 90 mg), diacetyl-thornasterol A (**8a**, 96 mg), and diacetyl-thornasterol B (**9a**, 31 mg). Genin-1a (**1a**) was identified with the sample (obtained above by acid hydrolysis of saponin-containing fraction) by TLC, IR, PMR, CD, and MS. Genin-2a (**5a**), colorless glassy, UV λ_{max}^{EtOH} nm: 248 (ε=11000). CD ($c=4.70\times10^{-2}$, MeOH): $[\theta]_{280}$ 0, $[\theta]_{246}$ −10600 (neg. max.), $[\theta]_{215}$ 0, $[\theta]_{210}$ +9200!. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1743, 1689, 1607. PMR ($CDCl_3$) δ: 0.51 (3H, s, 13-CH_3), 1.02 (3H, s, 10-CH_3), 0.92 (6H, d, J=6 Hz, 25-(CH_3)$_2$), 2.02 (6H, s, OAc×2), 2.14 (3H, br.s, 20-CH_3), 4.50—5.00 (2H, m, 3α-H, 6β-H), 5.35 (1H, m, 11-H), 6.04 (1H, br.s, 22-H). MS *m/e* (%): 498 (M^+, 32), 378 (M^+−2AcOH, 100), 441 ($M^+-C_4H_9$, 56). **5a** in lit.[16a]: λ_{max}^{MeOH} nm: 246.5 (ε=13000); ν ($CHCl_3$): 1735, 1685 cm^{-1}; CD (MeOH): $[\theta]_{247.5}$ −12000 (neg. max.); MS *m/e*: 438, 378, 441, 311, 211; δ ($CDCl_3$): AcO×2 and olefinic CH_3×1 (around 2.0), 4.80 (2H, m), 5.30 (1H, dt), 6.06 (1H, s). Diacetyl-thornasterol A (**8a**), mp 158.5—159.5° (hexane), $[\alpha]_D^{15}$ +23° (c=0.1, MeOH). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.04; H, 9.35. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 1742, 1705. UV λ_{max}^{EtOH} nm: transparent above 210 nm. CD ($c=1.04\times10^{-1}$, MeOH): $[\theta]_{328}$ 0, $[\theta]_{292}$ −2500 (neg. max.), $[\theta]_{246}$ 0, $[\theta]_{219}$ −1300 (neg. max.), $[\theta]_{214}$ 0; ($c=3.0\times10^{-2}$, MeOH): $[\theta]_{194}$ +40000!, $[\theta]_{197}$ +45000 (pos. max.). PMR ($CDCl_3$) δ: as given in the text. MS (at 30 eV) *m/e* (%): as shown in Chart 3. High resolution MS: Found 498.334, 438.313, 281.190, 211.148, 85.066. Calcd. for $C_{31}H_{46}O_5$ (va)=498.334, $C_{29}H_{42}O_3$ (va–AcOH)=438.313, $C_{20}H_{25}O$ (iii)=281.190, $C_{16}H_{19}$ (iv)=211.148, C_5H_9O (viia)=85.065. Diacetyl-thornasterol B (**9a**), mp 147—148° (hexane), $[\alpha]_D^{15}$ +18° (c=0.3, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.38; H, 9.62. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 1743, 1700. UV λ_{max}^{EtOH} nm: transparent above 210 nm. CD ($c=1.06\times10^{-1}$, MeOH): $[\theta]_{334}$ 0, $[\theta]_{305}$ +1000 (pos. max.), $[\theta]_{280}$ 0, $[\theta]_{220}$ −1600 (neg. max.), $[\theta]_{216}$ 0; ($c=3.5\times10^{-2}$, MeOH): $[\theta]_{194}$ +34000!, $[\theta]_{197}$ +38000 (pos. max.). PMR ($CDCl_3$) δ: as given in the text. MS (at 30 eV) *m/e* (%): as shown in Chart 3. High resolution MS: Found 512.350, 441.264, 281.190, 211.148, 99.081. Calcd. for $C_{32}H_{48}O_5$ (vb)=512.350, $C_{27}H_{37}O_5$ (vi)=441.264, $C_{20}H_{25}O$ (iii)=281.190, $C_{16}H_{19}$ (iv)=211.148, $C_6H_{11}O$ (viib)=99.080.

Acid Treatment of Diacetyl-thornasterol A (8a) giving 1a and 5a——To a solution of **8a** (30 mg) in benzene (1.5 ml), was added aqueous 2 N HCl (1 ml), and the total mixture was heated under reflux for 27 hr. After dilution with water, the reaction mixture was extracted with benzene. The benzene extract was washed with water, dried over $MgSO_4$, and evaporated under reduced pressure to give the product (27 mg). Pre-

36) Saponin mixture, used in the Experimental section hereafter, was obtained from the starfish collected in Okinawa prefecture in May and June.

parative TLC purification (benzene–ether=20: 1, developing twice) of the product furnished genin-1a (**1a**, 4 mg), genin-2a (**5a**, 14 mg), and recovered **8a** (3 mg). Genin-1a (**1a**) obtained here was identified with the sample obtained above by TLC, IR, CD, and MS. Genin-2a (**5a**) was identified with the sample, which was obtained above by enzymatic hydrolysis followed by solvolysis of saponin mixture, by TLC, IR, UV, CD, PMR, and MS.

Oxidation of Sapogenol Sulfate Mixture followed by Solvolysis and Acetylation giving 10, 11, and 12——To a solution of sapogenol sulfate mixture (295 mg) in pyridine (3 ml), was added a suspension (2 ml) of CrO_3–pyridine (300 mg–3 ml), and the total mixture was kept stirring at 38° for 5 hr. The reaction mixture was poured into ice-water and extracted with *n*-BuOH. The *n*-BuOH extract was washed with water and evaporated under reduced pressure to furnish the residue (225 mg). The residue was dissolved in dioxane (10 ml)–pyridine (1 ml) and heated under reflux for 15 min. After dilution with $CHCl_3$ (10 ml), the total mixture was filtered. Evaporation of the filtrate under reduced pressure gave the residue (185 mg). The residue was then acetylated with Ac_2O (1.5 ml) and pyridine (3 ml) at room temperature for 20 hr and poured into ice-water. The total mixture was extracted with $CHCl_3$. Usual work-up of the $CHCl_3$ extract yielded the product (160 mg) which was purified by preparative TLC (benzene–ether=10: 1, developing twice) affording KA-1 (**10**, 22 mg), KA-2 (**11**, 28 mg), and KA-3 (**12**, 96 mg). KA-1 (**10**), mp 169—170° (hexane–$CHCl_3$), $[\alpha]_D^{18}$ −40° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 76.35; H, 9.29. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1741, 1722, 1687, 1606. UV λ_{max}^{MeOH} nm: 248 (ε=12200). CD ($c=3.42\times10^{-2}$, MeOH): $[\theta]_{314}$ 0, $[\theta]_{292}$ −6100 (neg. max.), $[\theta]_{266}$ −4200 (neg. min.), $[\theta]_{244}$ −12000 (neg. max.), $[\theta]_{222}$ 0, $[\theta]_{210}$ +9400!. PMR ($CDCl_3$) δ: 0.54 (3H, s, 13-CH_3), 0.92 (3H, s, 10-CH_3), 0.93 (6H, d, J=6 Hz, 25-$(CH_3)_2$), 2.03 (3H, s, OAc), 2.16 (3H, br.s, 20-CH_3), 4.50—4.75 (1H, m, 3α-H), 5.51 (1H, m, 11-H), 6.06 (1H, br.s, 22-H). MS *m/e* (%): 454 (M^+, 8), 394 (M^+−AcOH, 8), 397 ($M^+-C_4H_9$, 16), 85 (viia, 100). KA-2 (**11**), mp 184—185° (hexane–$CHCl_3$), $[\alpha]_D^{18}$ −24° (c=0.2, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found: C, 73.90; H, 9.80. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3490, 1740, 1720, 1703 (sh.). CD ($c=1.53\times10^{-1}$, MeOH): $[\theta]_{330}$ 0, $[\theta]_{293}$ −6600 (neg. max.), $[\theta]_{245}$ 0. ORD ($c=1.53\times10^{-1}$, MeOH): $[\Phi]_{400}$ −1000°!, $[\Phi]_{308}$ −5260° (trough), $[\Phi]_{290}$ 0°, $[\Phi]_{272}$ +4900° (peak), $[\Phi]_{240}$ +3600° (trough). PMR ($CDCl_3$) δ: 0.80 (3H, s, 13-CH_3), 0.91 (3H, s, 10-CH_3), 0.80—1.03 (9H, 24-CH_3, 25-$(CH_3)_2$), 1.34 (3H, s, 20-CH_3), 2.02 (3H, s, OAc), 2.58 (2H, br.s, 22-H_2), 4.53—4.77 (1H, m, 3α-H), 5.48 (1H, m, 11-H). MS *m/e* (%): 468 (M^+-H_2O, 11), 408 (M^+-H_2O–AcOH, 4), 372 (derived through McLafferty-type rearrangement of M^+, 100), 99 (viib, 9). KA-3 (**12**), mp 178—179° (hexane–$CHCl_3$), $[\alpha]_D^{18}$ −16° (c=0.3, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.65; H, 9.13. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 1742, 1720, 1706 (sh.). CD ($c=1.76\times10^{-1}$, MeOH): $[\theta]_{326}$ 0, $[\theta]_{293}$ −7420 (neg. max.), $[\theta]_{244}$ 0. ORD ($c=1.76\times10^{-1}$, MeOH): $[\Phi]_{450}$ −750°!, $[\Phi]_{308}$ −5890° (trough), $[\Phi]_{292}$ 0°, $[\Phi]_{272}$ +5570° (peak), $[\Phi]_{240}$ +3900° (trough). PMR ($CDCl_3$) δ: 0.81 (3H, s, 13-CH_3), 0.91 (3H, s, 10-CH_3), 0.92 (6H, d, J=6 Hz, 25-$(CH_3)_2$), 1.34 (3H, s, 20-CH_3), 2.02 (3H, s, OAc), 2.54 (2H, br.s, 22-H_2), 4.50—4.75 (1H, m, 3α-H), 5.49 (1H, m, 11-H). MS *m/e* (%): 454 (M^+-H_2O, 1), 372 (derived through McLafferty-type rearrangement of M^+, 5), 85 (viia, 20), 43 (100).

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