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Saponin and Sapogenol. XXVI.[1] Steroidal Saponins from the Starfish *Acanthaster planci* L. (Crown of Thorns). (2). Structure of the Major Saponin Thornasteroside A

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The major saponin named thornasteroside A (**5**) has been isolated from the whole body of starfish *Acanthaster planci* L. (crown of thorns). In order to facilitate the structural study, thornasteroside A (**5**) has been converted to a pentaglycoside (designated as dsp-1), which corresponds to a desulfated pregnane-type derivative of **5**. On the bases of chemical reactions and physicochemical evidence, the structure of dsp-1 has been elucidated as **10**. Based on the accumulated evidence in this and the preceding papers, the structure of thornasteroside A has been established as 20-hydroxy-6α-O-{β-D-fucopyranosyl(1→2)-β-D-galactopyranosyl(1→4)-[β-D-quinovopyranosyl(1→2)]-β-D-xylopyranosyl(1→3)-β-D-quinovopyranosyl}-5α-cholest-9(11)-en-23-on-3β-yl sodium sulfate (**5**).

Keywords——starfish; crown of thorns; *Acanthaster pianci*; thronasteroside A; crude naringinase; methylation of pregenolone; MS; CD

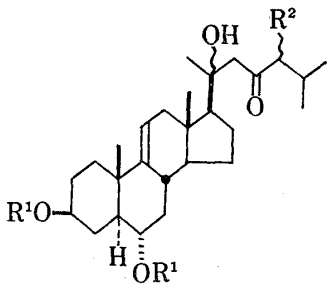
In the preceding paper,[1] we have reported the structure elucidation of two genuine sapogenols named thornasterol A (**1**) and thornasterol B (**2**), which are obtained by enzymatic hydrolysis followed by solvolysis of the saponin mixture isolated from the starfish *Acanthaster planci* L. (crown of thorns). In addition, the sulfate group contained in the parent saponins of thornasterol A (**1**) and thornasterol B (**2**) has been shown to connect at 3β–OH of the respective sapogenols. In a continuing study, we have been able to isolate the major saponin (named thornasteroside A) of *A. planci* and have elucidated the total structure. This paper provides the full account on the structure elucidation of thornasteroside A (**5**), which is a pentaglycoside of thornasterol A sulfated at 3β–OH.

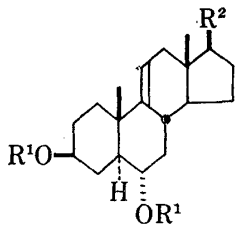
Silica gel column chromatography of the saponin mixture, which was isolated from *A. planci* collected in Okinawa prefecture in May,[1] furnished the major saponin thornasteroside A (**5**), mp 203—204°. The infrared (IR) spectrum of thornasteroside A (**5**) shows the strong absorption bands at 3400 (br) and 1065 (br) cm^{-1} due to the glycosidic structure. The ultraviolet (UV) spectrum of **5** shows no significant absorption maximum above 210 nm, while the circular dichroism (CD) spectrum shows a negative maximum at 285 nm ([θ] −3600) and a positive maximum at 198 nm ([θ] +40000), both of which are respectively ascribable to the chiral chromophores contained in the sapogenol part of **5**.[1]

The elemental analysis including atomic absorption spectrometry has shown that thornasteroside A (**5**) contains a sodium sulfate group in its molecule. On enzymatic hydrolysis using a glycosidase mixture of *Charonia lampas*[1] followed by solvolysis using a dioxane-pyridine mixture[1] and acetylation, thornasteroside A (**5**) afforded diacetyl-thornasterol A (**1a**)[1] as a sole sapogenol derivative. Detailed examinations by thin-layer chromatography (TLC) of the total acetylated hydrolysate have disclosed the absence of other acetylated sapogenols such as diacetyl-thornasterol B (**2a**),[1] genin-1a (**3a**),[1] and genin-2a (**4a**).[1]

As described in our preliminary communication,[3] the side chains in the steroidal aglycones of saponins of *A. planci* (including thornasteroside A (**5**)) are readily changeable. For

1) Part XXV: I. Kitagawa, M. Kobayashi, and T. Sugawara, *Chem. Pharm. Bull.* (Tokyo), **26**, 1852 (1978).
2) Location: *133–1, Yamada-kami, Suita, Osaka 565, Japan.*
3) I. Kitagawa and M. Kobayashi, *Tetrahedron Lett.*, **1977**, 859.





1 : $R^1=R^2=H$ thornasterol A

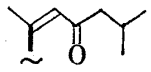
1a : $R^1=Ac$, $R^2=H$

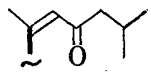
2 : $R^1=H$, $R^2=CH_3$ thornasterol B

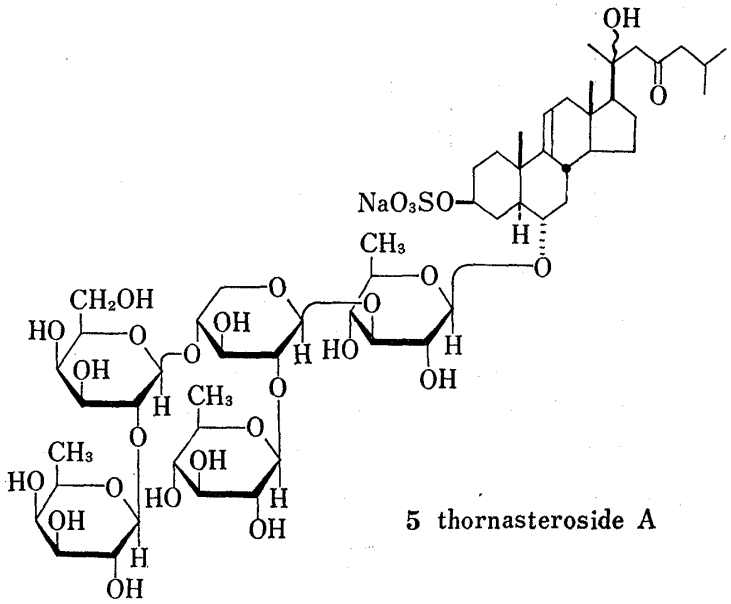
2a : $R^1=Ac$, $R^2=CH_3$

3 : $R^1=H$, $R^2=$  (genin-1)

3a : $R^1=Ac$, $R^2=$  (genin-1a)

4 : $R^1=H$, $R^2=$  (genin-2)

4a : $R^1=Ac$, $R^2=$  (genin-2a)

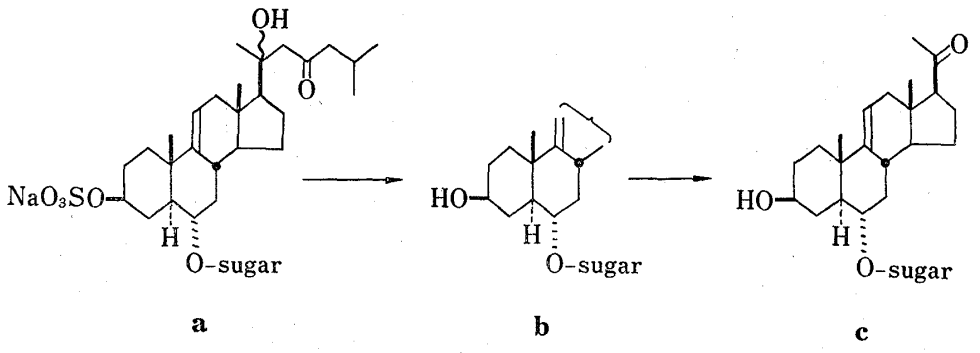


5 thornasteroside A

Chart 1

example, the saponins, which are contained in the total methanolic extractive, gradually decompose partly even while preserved at room temperature, thus resulting in the formation of an inseparable mixture of saponins. Therefore, treatment of the fresh methanolic extractive seems to be desirable for isolation of the saponins. Although thornasteroside A (**5**) could be isolated in a pure form by rapid chromatographic separation (using a medium pressure column) from a complex mixture of saponins, some devices have been needed for enough supply of the saponin for the structure study.

For this purpose, we have found that modification of the saponin to the more stable form is favorable for effecting the facile isolation and also for the structure study of the carbohydrate moiety. Thus, as schemed in Chart 2, the saponin (**a**, *e.g.* thornasteroside A (**5**)) has been first subjected to solvolysis to remove the sulfate group (giving **b**) and then treated with alkali to convert its changeable steroidal side chain to a methyl carbonyl *via* a retro aldol



a b c

Chart 2

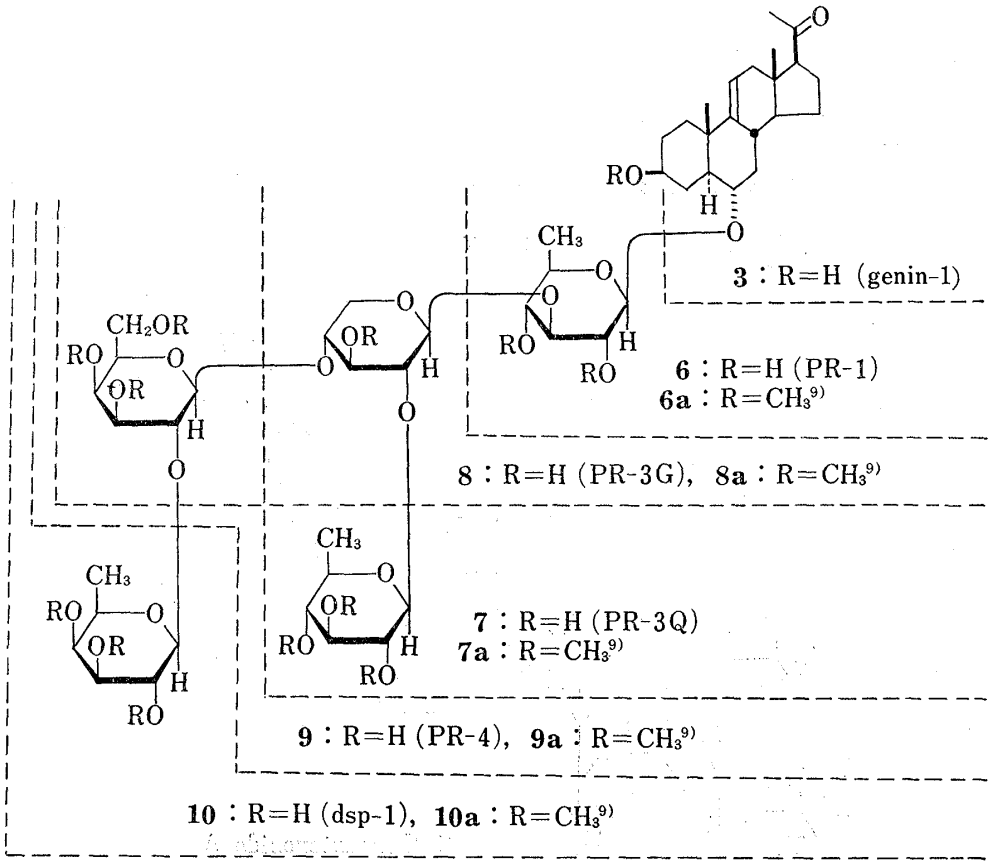


Chart 3

reaction (giving **c**, *e,g.* dsp-1 (**10**), *vide infra*), and chromatographed. The carbohydrate portion of the oligoglycoside thus obtained from thornasteroside A (**5**) (now designated as dsp-1 (**10**)) has been investigated. In practice, the oligoglycoside dsp-1 (**10**), which corresponds to a desulfated pregnane-type derivative of thornasteroside A (**5**), has been obtained in *ca.* 50% yield from the total saponin mixture *via* direct solvolysis followed by alkaline treatment and chromatographic separation.

The IR spectrum of dsp-1 (**10**), mp 264—265°, shows the carbonyl absorption band at 1703 cm^{-1} together with the strong absorption bands at 3380 (br) and 1065 (br) cm^{-1} as observed in the IR spectrum of thornasteroside A (**5**). The CD spectrum of **10** shows a positive maximum ($[\theta]_{287}$ +6300) which is attributable to a methyl carbonyl moiety in the pregnane-type aglycone.[1] On enzymatic hydrolysis using crude naringinase,[4] dsp-1 (**10**)

4) Kindly provided by Prof. O. Tanaka of Hiroshima University, to whom the authors deepest thanks are due.

afforded genin-1 (**3**) as a sole sapogenol. As for the carbohydrate constituents of **10**, D-quinovose, D-xylose, D-fucose,[5] and D-galactose have been identified by gas-liquid chromatography (GLC) of the methanolysis product of **10**.

On mild acid hydrolysis, dsp-1 (**10**) furnished genin-1 (**3**) and four prosapogenols: PR-1 (**6**), PR-3Q (**7**), PR-3G (**8**), and PR-4 (**9**) (in the order of increasing polarity). Methanolysis of these prosapogenols has revealed the respective carbohydrate ingredients as follows: quinovose in **6**, quinovose and xylose in **7**, quinovose, xylose, and galactose in **8** and **9**.

PR-1 (**6**), mp 168—169°, exhibits hydroxyl (3390 (br) cm^{-1}) and carbonyl (1701 cm^{-1}) absorption bands in its IR spectrum. The presence of a methyl carbonyl moiety in **6** has been suggested by its positive CD maximum ($[\theta]_{285}$ +8800).[1] Methylation of **6** with methyl iodide–dimethyl sulfoxide(DMSO)–sodium hydride[7] gave a tetra-O-methyl derivative (**6a**). As described below, the methyl carbonyl moiety in **6** has been simultaneously affected during the methylation, so that **6a** is a mixture of methylated derivatives in regard to its steroidal side chain.

The proton magnetic resonance (PMR) spectrum of **6a** shows a one-proton doublet at δ 4.22 (J=7 Hz) which is ascribable to the anomeric proton of β-quinovopyranoside linkage ($^4C_1$ form). Methanolysis of **6a** yielded methyl 2,3,4-tri-O-methyl-quinovopyranoside, thus the structure of PR-1 being established as 6-O-β-D-quinovopyranoside of genin-1 (**6**).

As for the modification of the methyl carbonyl moiety in **6** during the methylation procedure, the CD spectrum and mass spectrometric (MS) analysis of **6a** have been informative. Thus, the positive CD maximum at 285 nm (in methanol) of **6** shifts to 291 nm (in methanol) or to 294 nm (in ether) in the case of **6a**, which suggests occurrence of some modification on the methyl carbonyl moiety in **6**. The MS of **6a** (M^+=i) gives three fragment ion peaks at *m/e* 371 (ii, 12%), *m/e* 357 (iii, 34%), and *m/e* 343 (iv, 13%), which are derived from the steroidal portion of **6a**. These three ions are further accompanied by ion peaks at *m/e* 339 (v, 10%), *m/e* 325 (vi, 24%), and *m/e* 311 (vii, 13%), which are presumably formed through elimination of methanol.[8] It has been assumed therefore that the methyl carbonyl moiety in **6** is methylated during the methylation procedure converting to a tertiary butyl carbonyl, an isopropyl carbonyl, and an ethyl carbonyl moieties.[9] In order to make sure of these observations, pregnenolone has been subjected to the methylation. The structures of four products (**11** (2.5% yield), **12** (65%), **13** (7%), and **14** (5%)) thus obtained have been assigned on the basis of the elemental analyses together with their physicochemical properties (IR, CD, PMR, and MS).

PR-3Q (**7**), mp 263—265°, shows a positive maximum at 286 nm ($[\theta]$ +9100) in its CD spectrum. On methylation, was obtained an octa-O-methyl derivative (**7a**),[9] whose PMR spectrum shows three anomeric proton signals at δ 4.23 (1H, d, J=8 Hz), δ 4.54 (1H, d, J=7.5 Hz), and δ 4.84 (1H, d, J=6 Hz). Methanolysis of **7a** furnished methyl 2,3,4-tri-O-methyl-quinovopyranoside, methyl 3,4-di-O-methyl-xylopyranoside, and methyl 2,4-di-O-methyl-quinovopyranoside, thus the structure of PR-3Q being expressed as **7** including its three anomeric configurations.

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5) Since L-fucose has been known to occur as a constituent of fucoidan isolated from brown seaweed,[6] D-fucose obtained here was determined by its specific rotation.

6) T. Fujikawa and K. Nakashima, *Nippon Nogeikagaku Kaishi*, **49**, 455 (1975).

7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

8) The MS and GLC analyses of the aglycone of **6a** have also shown that the methyl carbonyl moiety of **6** is methylated to furnish a mixture of *tert.* butyl carbonyl, isopropyl carbonyl, and ethyl carbonyl derivatives with the ratio of 7 : 13 : 1 (see Experimental).

9) The similar type of methylations have been observed upon methylation of other prosapogenols (PR-3Q (**7**), PR-3G (**8**), PR-4 (**9**)) and dsp-1 (**10**). The modification of the methyl carbonyl moiety has been suggested by the shift of the CD maximum due to the methyl carbonyl in respective methylated derivatives (**7a**, **8a**, **9a**, and **10a**) (regardless of the solvent used) as compared with those of parent prosapogenols (**7**—**10**) (see Experimental).

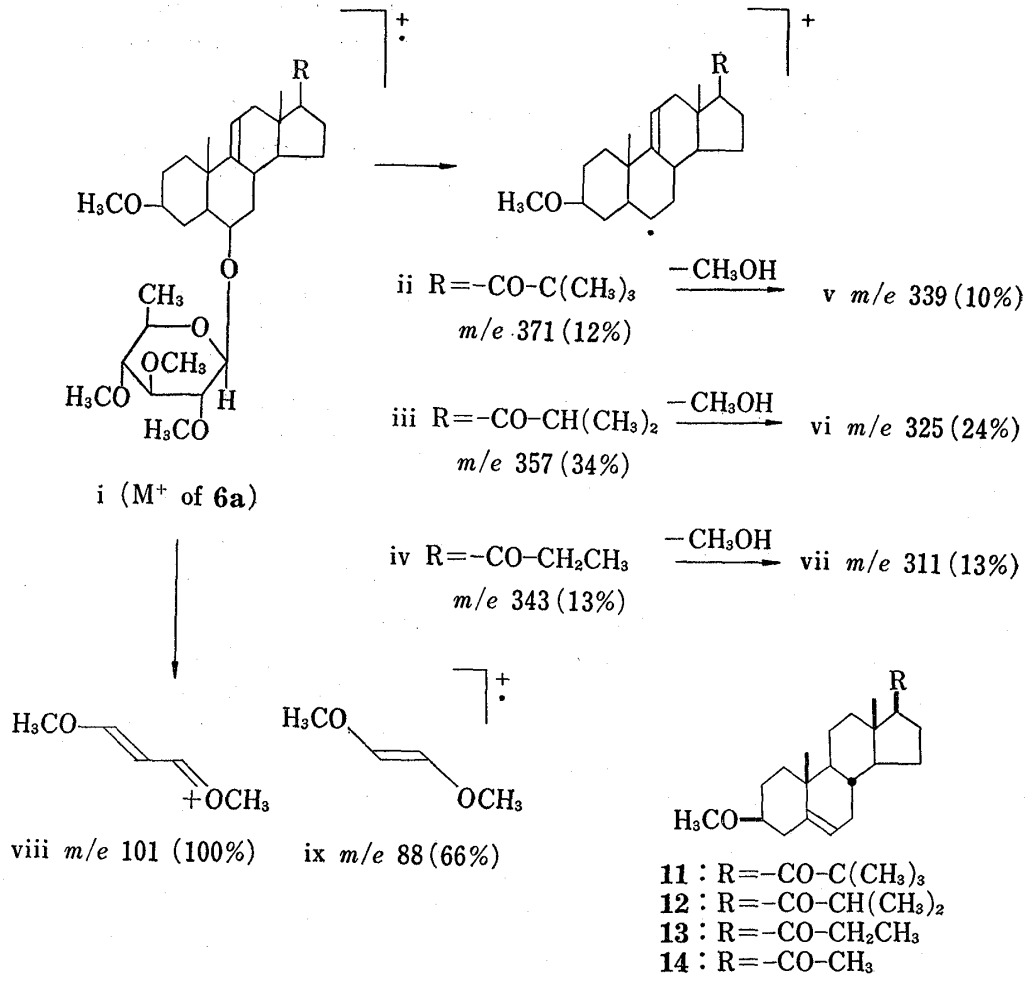


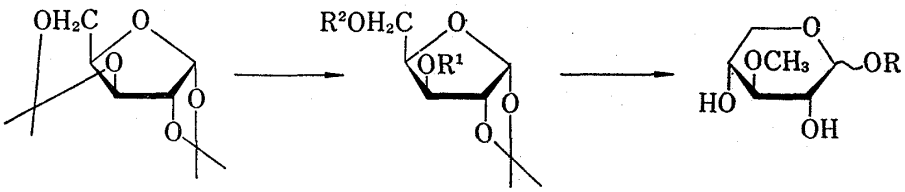
Chart 4

PR-3G (**8**), mp 169—171°, $[\theta]_{288}$ +5500 (positive maximum), gave a nona-O-methyl derivative (**8a**) by methylation as above.[9] In the PMR spectrum of **8a**, are observed three one-proton doublets at δ 4.24 (J=8 Hz), δ 4.26 (J=6 Hz), and δ 4.70 (J=8 Hz) due to anomeric protons. On methanolysis, **8a** liberated methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, methyl 2,3-di-O-methyl-xylopyranoside, and methyl 2,4-di-O-methyl-quinovopyranoside, thus the structure of PR-3G being substantiated as **8**.

Methylation of the fourth prosapogenol PR-4 (**9**), mp 220—221°, $[\theta]_{285}$ +7300 (positive maximum), yielded an undeca-O-methyl derivative (**9a**),[9] which shows four anomeric proton signals at δ 4.24 (d, J=8 Hz), δ 4.26 (d, J=7 Hz), δ 4.55 (d, J=7 Hz), and δ 4.86 (d, J=6 Hz) in its PMR spectrum. Methanolysis of **9a** liberated methyl 2,3,4-tri-O-methyl-quinovopyranoside, methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, methyl 3-O-methyl-xylopyranoside (*vide infra*), and methyl 2,4-di-O-methyl-quinovopyranoside. Consequently, the structure of PR-4 has been clarified as **9**.

Since the authentic sample of methyl 3-O-methyl-xylopyranoside (identified above) was unavailable, it was synthesized as schemed in Chart 5. Acid treatment of 1,2: 3,5-di-O-isopropylidene-α-D-xylofuranose (**15**)[10] gave 1,2-O-isopropylidene-α-D-xylofuranose (**16**)[11] which, on tritylation, was converted to 1,2-O-isopropylidene-5-O-trityl-α-D-xylofuranose (**17**). Methylation of **17** with methyl iodide and silver oxide furnished 1,2-O-isopropylidene-3-O-methyl-5-O-trityl-α-D-xylofuranose (**18**), whose PMR spectrum shows the signals due to a methoxyl

10) P.A. Levene and A.L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933).
11) R.S. Tipson, H.S. Isbell, and J.E. Stewart, *J. Research Natl. Bur. Standards*, **62**, 257 (1959).



15

16 : $R^1=R^2=H$
17 : $R^1=H$, R^2=trityl
18 : $R^1=CH_3$, R^2=trityl

19 : R=H
20 : $R=CH_3$

Chart 5

at δ 3.20 (3H, s) and an anomeric proton at δ 5.69 (d, J=4 Hz). Detritylation of **18** with acid gave 3-O-methyl-D-xylose (**19**),[12] which, on methanolysis, was converted to authentic methyl 3-O-methyl-D-xylopyranoside (**20**).

Finally, the structure of dsp-1 (**10**) has been clarified on the basis of the above-mentioned and additional following evidence. Thus, methylation of **10** gave a trideca-O-methyl derivative (**10a**),[9] whose PMR spectrum shows five anomeric proton signals at δ 4.25 (d, J=8 Hz), δ 4.36 (d, J=7 Hz), δ 4.56 (d, J=6 Hz), δ 4.59 (d, J=7.5 Hz), and δ 4.81 (d, J=7 Hz). On methanolysis, **10a** liberated methyl 2,3,4-tri-O-methyl-quinovopyranoside, methyl 2,3,4-tri-O-methyl-fucopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside, methyl 2,4-di-O-methyl-quinovopyranoside, and methyl 3-O-methyl-xylopyranoside. Therefore, the structure of dsp-1 has been corroborated as **10**.[3]

On the basis of the accumulated evidence mentioned above and the direct correlation of dsp-1 (**10**) with thornasteroside A (*vide supra*), the structure of thornasteroside A, the major saponin of *A. planci*, has now been established as 20-hydroxy-6α-O-{β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl(1→4)-[β-D-quinovopyranosyl(1→2)]-β-D-xylopyranosyl(1→3)-β-D-quinovopyranosyl}-5α-cholest-9(11)-en-23-on-3β-yl sodium sulfate (**5**). Thornasteroside A (**5**) seems to be the first example of saponin isolated from the *Asteroidea* whose total structure is elucidated including its genuine sapogenol part.[13]

Experimental[14]

Isolation of Thornasteroside A (5)——Saponin mixture (500 mg),[1] which was isolated from the starfish *A. planci* collected in Okinawa in May, was subjected to medium pressure column chromatography (Kieselgel H 80 g; pressure for preparing column: 5 kg/cm^2; pressure for elution: 3 kg/cm^2; column: 20 mm×70 cm; flow rate: 1 ml/min) developing successively with $CHCl_3$–MeOH–H_2O (7:3:1, lower layer→65:30:10, lower layer→65:32:10, lower layer) to furnish thornasteroside A (**5**, 50 mg). Thornasteroside A (**5**), mp 203—204 (*n*-BuOH–H_2O), $[\alpha]_D^{25}$ −7.0° (c=0.5, H_2O). *Anal.* Calcd. for $C_{56}H_{92}O_{28}SNa\cdot H_2O$: C, 52.28; H, 7.37; S, 2.50. Found: C, 52.18; H, 7.59; S, 2.95. Na was detected by atomic absorption spectrometry by using a Na lamp: 70% absorbance for the calculated value. IR ν_{max}^{Nujol} cm^{-1}: 3400 (br), 1695, 1065 (br). UV $\lambda_{max}^{H_2O}$ nm: transparent above 210 nm. CD ($c=5.17\times10^{-1}$, H_2O): $[\theta]_{325}$ 0, $[\theta]_{285}$ −3600 (neg. max.), $[\theta]_{226}$ 0; ($c=6.3\times10^{-2}$, MeOH): $[\theta]_{198}$ +40000 (pos. max.), $[\theta]_{195}$ +30000!.

Enzymatic Hydrolysis of Thornasteroside A (5) with Glycosidase Mixture of *Charonia lampas* followed by Solvolysis and Acetylation giving 1a——A solution of thornasteroside A (**5**, 60 mg) in AcONa–AcOH buffer solution (pH 5.0, 8 ml) was treated with glycosidase mixture of *Charonia lampas* (Seikagaku-Kogyo, Tokyo,

12) R.J. Ferrier, D. Prasad, A. Rudowski, and I. Sangster, *J. Chem. Soc.*, **1964**, 3330.
13) Profs. T. Kawasaki and T. Komori of Kyushu University recently reported the structure elucidation of a saponin which was isolated from *A. planci* and is a pentaglycoside of thornasterol B (**2**) sulfated at 3β-OH. T. Komori, J. Matsuo, Y. Sanechika, and T. Kawasaki, The 97th Annual Meeting of Pharmacetuical Society of Japan, Tokyo, April 1977, Abstract Paper II-217.
14) The instruments used in the experimental section and the experimental conditions for chromatography were same as in the preceding paper,[1] except for the followings: Atomic absorption spectrometry (Hitachi Perkin Elmer Type 303); GLC (Hitachi Gas Chromatograph Model 063 or 163 with FID); Specific rotations (JASCO DIP-181 Digital Polarimeter, 1=0.5 and 1 dm); Column chromatography (silica gel, Merck, 60—230 mesh or 230—400 mesh; Kieselgel H nach Stahl, Type 60, Merck).

300 mg). The total mixture was kept stirring at 36° for 3 days, treated with *n*-BuOH, and warmed for a while. After filtration with the aid of Celite, the filtrate was extracted with *n*-BuOH. The *n*-BuOH extract was washed with water and evaporated under reduced pressure to give the residue (195 mg). Purification of the residue with silica gel column chromatography (Merck, 230—400 mesh, 2 g, $CHCl_3$–MeOH=10: 1) furnished sapogenol sulfate (14 mg). The solution of sapogenol sulfate (14 mg) in pyridine (0.5 ml) was heated under reflux for 10 min, treated with dioxane (2 ml), and refluxed further for 25 min. After evaporation of the solvent under reduced pressure, the residue was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) at 25° for 20 hr. The reaction mixture was poured into ice-water, extracted with $CHCl_3$, and the $CHCl_3$ extract was worked up in the usual manner. The acetate (8 mg) thus obtained was purified by silica gel column chromatography (230—400 mesh, 400 mg, $CHCl_3$) to furnish the pure sample of diacetyl-thornasterol A (**1a**, 5 mg), which was identified with the authentic sample[1] by mixed mp, IR, TLC, and CD. Detailed TLC examinations of total acetate (Kieselgel 60 F_{254}, benzene–ether=8: 1) showed the presence of only diacetyl-thornasterol A (**1a**, *Rf*=0.50), but the absence of diacetyl-thornasterol B (**2a**, *Rf*=0.55), genin-1a (**3a**, *Rf*=0.52), and genin-2a (**4a**, *Rf*=0.60).

Solvolysis followed by Alkali Treatment of Thornasteroside A (5) giving dsp-1 (10)——A solution of **5** (10 mg) in dioxane (0.5 ml)–pyridine (2 ml) was heated under reflux for 2 hr. The residue, which was obtained by evaporation of the solvent under reduced pressure to dryness, was treated with 1 N NaOMe–MeOH (1.5 ml) and heated under reflux for 2 hr. After dilution with MeOH (5 ml), the total solution was neutralized with Dowex 50W-X8, filtered, and evaporated under reduced pressure. The residue was then partitioned into *n*-BuOH–H_2O. The *n*-BuOH layer was taken and evaporated under reduced pressure to give the residue (10 mg). Column chromatography of the residue with Amberlite XAD-2 (1 g) eluting with MeOH furnished dsp-1 (**10**, 4 mg), being identical with the sample obtained below by IR, TLC, CD, and TLC analysis of the mild acid hydrolysates.

Isolation of dsp-1 (10) from Saponin Mixture——A solution of saponin mixture (5 g) in 1 N NaOMe–MeOH (75 ml) was heated under reflux for 4 hr. After dilution with aq. 50% MeOH (100 ml), the total mixture was neutralized with Dowex 50W-X8 (50 ml) and filtered. Evaporation of the filtrate under reduced pressure yielded the residue (4.5 g) which was dissolved in pyridine (60 ml)–dioxane (15 ml) and heated under reflux for 1.5 hr. The total reaction mixture was diluted with MeOH (100 ml), filtered, and evaporated under reduced pressure to furnish an oligoglycoside mixture (3.44 g). Purification of the oligoglycoside mixture (280 mg) was effected by medium pressure column chromatography (Kieselgel H 80 g; the other conditions were same as for isolation of **5**) eluting with $CHCl_3$–MeOH–H_2O (10: 3: 1, lower layer→9: 3: 1, lower layer→7: 3: 1, lower layer) to afford dsp-1 (**10**, 107 mg) and dsp-1 containing fraction (29 mg). dsp-1 (**10**), mp 264—265° (MeOH–H_2O), $[\alpha]_D^{16}$ +22° (*c*=0.6, MeOH–H_2O). *Anal.* Calcd. for $C_{50}H_{80}O_{24}$: C, 56.38; H, 7.57. Found: C, 56.49; H, 7.37. IR ν_{max}^{Nujol} cm^{-1}: 3380 (br), 1703. CD ($c=4.35\times10^{-1}$, MeOH): $[\theta]_{326}$ 0, $[\theta]_{287}$ +6300 (pos. max.), $[\theta]_{240}$ 0; ($c=5.0\times10^{-2}$, MeOH): $[\theta]_{199}$ +32000 (pos. max.), $[\theta]_{195}$ +26000!. Potassium rhodizonate test: negative.

Enzymatic Hydrolysis of dsp-1 (10) giving Genin-1 (3)——A solution of dsp-1 (**10**, 30 mg) in AcONa–AcOH buffer solution (pH 4.0, 30 ml) was treated with crude naringinase (7000 U/g, pH 2.8, Lot No. 1—22, 40 mg)[4] and the total mixture was kept stirring at 42° for 3 days. After addition of EtOH (1 ml), the reaction mixture was warmed for a while, filtered, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried, and evaporated under reduced pressure to afford genin-1 (**3**, 11 mg), which was identified with the authentic sample[1] by TLC, IR, CD, PMR, and MS.

Mild Acid Hydrolysis of dsp-1 (10) giving 3, 6, 7, 8, and 9——A solution of dsp-1 (**10**, 580 mg) in aq. 1 N HCl (10 ml)–AcOH (10 ml)–EtOH (10 ml) was heated under reflux for 45 min. After neutralization with aq. NaOH, the total mixture was diluted with water (100 ml) and extracted with *n*-BuOH. Evaporation of the *n*-BuOH extract under reduced pressure gave the extractive (657 mg), which was dissolved in dry MeOH (100 ml) and treated with 1 N NaOMe–MeOH (40 ml). The total solution was kept stirring at 26° for 30 min, neutralized with Dowex 50W-X8 (15 ml), and filtered. Evaporation of the filtrate under reduced pressure gave the residue (800 mg), which was subjected to medium pressure column chromatography (Kieselgel H 80 g; $CHCl_3$–MeOH=20: 1→$CHCl_3$–MeOH–H_2O=10: 3: 1, lower layer; the other conditions were same as for isolation of **5**) to furnish genin-1 (**3**, 28 mg), PR-1 (**6**, 53 mg), PR-3Q (**7**, 36 mg), PR-3G (**8**, 66 mg), and PR-4 (**9**, 153 mg). PR-1 (**6**), mp 168—169° ($CHCl_3$–MeOH), $[\alpha]_D^{23}$ +52° (*c*=0.4, MeOH). *Anal.* Calcd. for $C_{27}H_{42}O_7\cdot1/2H_2O$: C, 66.50; H, 8.89. Found: C, 66.70; H, 9.14. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390 (br), 1701. CD ($c=3.9\times10^{-1}$, MeOH): $[\theta]_{330}$ 0, $[\theta]_{285}$ +8800 (pos. max.), $[\theta]_{230}$ 0. PR-3Q (**7**), mp 263—265° (MeOH), $[\alpha]_D^{16}$ +39° (*c*=0.3, MeOH). *Anal.* Calcd. for $C_{38}H_{60}O_{15}\cdot1/2H_2O$: C, 59.59; H, 8.02. Found: C, 59.37; H, 8.08. IR ν_{max}^{Nujol} cm^{-1}: 3400 (br), 1697. CD ($c=2.9\times10^{-1}$, MeOH): $[\theta]_{326}$ 0, $[\theta]_{286}$ +9100 (pos. max.), $[\theta]_{225}$ 0. PR-3G (**8**), mp 169—171° (isoPrOH–ether), $[\alpha]_D^{20}$ +13° (*c*=0.4, EtOH). *Anal.* Calcd. for $C_{38}H_{60}O_{16}\cdot2H_2O$: C, 56.41; H, 7.98. Found: C, 56.71; H, 8.01. IR ν_{max}^{Nujol} cm^{-1}: 3350 (br), 1704. CD ($c=3.4\times10^{-1}$, EtOH): $[\theta]_{325}$ 0, $[\theta]_{288}$ +5500 (pos. max.), $[\theta]_{236}$ 0. PR-4 (**9**), mp 220—221° (MeOH–isoPrOH), $[\alpha]_D^{16}$ +24° (*c*=0.4, MeOH). *Anal.* Calcd. for $C_{44}H_{70}O_{20}\cdot H_2O$: C, 56.63; H, 7.75. Found: C, 56.48; H, 7.67. IR ν_{max}^{Nujol} cm^{-1}: 3380 (br), 1696. CD ($c=2.4\times10^{-1}$, MeOH): $[\theta]_{326}$ 0, $[\theta]_{285}$ +7300 (pos. max.), $[\theta]_{240}$ 0.

Carbohydrate Ingredients of dsp-1 (10) and Other Prosapogenols (6, 7, 8, 9)——A solution of dsp-1 (**10**, 3 mg) in anhydrous 2.5 N HCl–MeOH (0.5 ml) was heated under reflux for 40 min. After cooling, the reaction

mixture was neutralized with Ag_2CO_3, filtered, and the filtrate was evaporated to dryness. The residue was then dissolved in pyridine (0.1 ml), treated with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (0.2 ml), left standing at room temperature for 5 min, and subjected to GLC analysis (2% SE-52 on chromosorb WAWDMCS 80—100 mesh; column 3 mm×2 m; column temp. 119°; N_2 flow rate 45 ml/min). The other prosapogenols (**6**, 2 mg; **7**, 2 mg; **8**, 3 mg; **9**, 3 mg) were also treated in the similar manner. As for the carbohydrate standards, quinovose (10 mg), fucose (10 mg), xylose (9 mg), and galactose (11 mg) were respectively subjected to methanolysis as above, dissolved in pyridine (0.2 ml), silylated with hexamethyldisilazane (0.2 ml) and trimethylsilyl chloride (0.2 ml). The following carbohydrates were identified: PR-1 (**6**)—quinovose (t_R 11′30″); PR-3Q (**7**)—xylose (9′09″) and quinovose; PR-3G (**8**)—xylose, quinovose, and galactose (20′45″, 23′45″); PR-4 (**9**)—xylose, quinovose, and galactose; dsp-1 (**10**)—xylose, quinovose, galactose, and fucose (7′17″, 8′00″).

Isolation of D-Fucose from the Hydrolysate of dsp-1 (10)——A solution of dsp-1 (**10**, 200 mg) in aq. 0.06 N HCl (16 ml)–MeOH (6 ml) was heated under reflux for 1 hr and 15 min. The reaction mixture was neutralized with Amberlite IR-45, filtered, and evaporated under reduced pressure to remove MeOH. The residue was then partitioned into *n*-BuOH–H_2O. Evaporation of the aqueous layer under reduced pressure gave the residue (46 mg) which was subjected to preparative PPC (Toyo Filter Paper No. 50; ascending with *n*-BuOH–benzene–pyridine–H_2O=5: 1: 3: 3, upper layer; detection with aniline hydrogen phthalate) and preparative TLC (silica gel Camag D-5, $CHCl_3$–MeOH–H_2O=7: 3: 1, lower layer, developing twice), successively to furnish fucose (3 mg), $[\alpha]_D^{16}$ +59° (*c*=0.3, MeOH) (taken after 3 days). In lit. D-fucose $[\alpha]_D^{19}$ +76° (final),[15] L-fucose $[\alpha]_D^{20}$ −75.6° (final).[16]

Methylation of PR-1 (6) followed by Methanolysis——a) To a solution of **6** (27 mg) in DMSO (3 ml) was added dimsyl carbanion solution (2 ml) [prepared from NaH (2 g, washed with dry hexane beforehand) and DMSO (35 ml) with stirring at 70° for 1 hr under N_2 atmosphere]. The total mixture was kept stirring under N_2 atmosphere at 25° for 1 hr, treated with CH_3I (1 ml), and kept stirring for further 1.5 hr in the dark. After pouring into ice-water, the total mixture was extracted with AcOEt. The AcOEt extract was washed with water, dried over $MgSO_4$, and evaporated to dryness under reduced pressure. The residue was then purified by preparative TLC ($CHCl_3$–AcOEt=5: 1) to furnish tetra-O-methyl derivative (**6a**, 15 mg). **6a**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1702. PMR ($CDCl_3$) δ: 3.32, 3.51, 3.55, 3.58 (3H each, all s, MeO×4), 4.22 (1H, d, J=7 Hz, anomeric H), 5.27 (1H, m, 11-H). MS *m/e* (%): as given in Chart 4 (for viii and ix, *cf.* lit.[17]). CD (MeOH): $[\theta]_{330}$ 0, $[\theta]_{291}$ (pos. max.), $[\theta]_{245}$ 0.

b) A solution of **6a** (6 mg) in anhydrous 2.5 N HCl–MeOH (2.5 ml) was heated under reflux for 4 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3, filtered, and evaporated. The product was subjected to GLC (15% NPGS on chromosorb WAW 80—100 mesh; column 3 mm×2 m; column temp. 171°; N_2 flow rate 35 ml/min) and TLC (benzene–acetone=3: 1) analyses to identify with methyl 2,3,4-tri-O-methyl-quinovopyranoside [I] (t_R=1′41″, 2′05″; *Rf*=0.60, 0.65). The aglycone part was obtained by preparative TLC (benzene–acetone=3: 1) of the methanolysis product and subjected to MS and GLC analyses. MS *m/e* (%): 388 (M^+ from *tert.* butyl carbonyl deriv. (a), 0.5), 374 (M^+ from isopropyl carbonyl deriv. (b), 7), and 360 (M^+ from ethyl carbonyl deriv. (c), 4). GLC (10% SE-30 on chromosorb W 80—100 mesh, 3 mm×1 m; column temp. 222°; N_2 flow rate 30 ml/min): (c) t_R=26′40″, (b) 29′00″, and (a) 36′00″; the ratio of peak intensities of (c): (b): (a)=7: 13: 1.[18]

Methylation of Pregnenolone giving 11, 12, 13, and 14——A solution of pregnenolone (180 mg) in DMSO (3 ml) and benzene (5 ml) was treated with dimsyl carbanion solution (3 ml, prepared as above). The total mixture was kept stirring under N_2 atmosphere at room temperature for 1 hr, treated with CH_3I (2 ml), and kept stirring for further 2 hr in the dark. The reaction mixture was then poured into ice-water, and extracted with AcOEt. Working up of the AcOEt extract as above gave the product (200 mg). Preparative TLC (hexane–AcOEt=30: 1, developing twice) of the product (160 mg) furnished **11** (4 mg), **12** (104 mg), **13** (11 mg), and **14** (8 mg). **11**, mp 146.5—147° (petr. ether), $[\alpha]_D^{13}$ −17° (*c*=1.2, ether). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1695. CD (*c*=1.2, ether): $[\theta]_{340}$ 0, $[\theta]_{302}$ +2200 (pos. max.), $[\theta]_{250}$ 0. PMR ($CDCl_3$) δ: 0.82, 1.00 (3H each, both s, 13-CH_3, 10-CH_3), 1.10 (9H, s, 21-$(CH_3)_3$), 3.35 (3H, s, MeO), 5.32 (1H, m, 6-H). MS *m/e* (%): 372 (M^+, 5), 315 ($M^+-C_4H_9$, 73), 255 ($M^+-C_5H_9O-CH_3OH$, 100). High resolution MS: Found 372.303. Calcd. for $C_{25}H_{40}O_2$ (M^+)=372.303. **12**, mp 143.5—144° (hexane), $[\alpha]_D^{26}$ +34° (*c*=0.2, ether). *Anal.* Calcd. for $C_{24}H_{38}O_2$: C, 80.39; H, 10.68. Found: C, 80.25; H, 10.67. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1711. CD ($c=1.8\times10^{-1}$, ether): $[\theta]_{335}$ 0, $[\theta]_{294}$ +12500 (pos. max.), $[\theta]_{230}$ 0. PMR ($CDCl_3$) δ: 0.62, 1.00 (3H each, both s, 13-CH_3, 10-CH_3), 1.02, 1.04 (3H each, both d, J=7 Hz, 21-$(CH_3)_2$), 3.33 (3H, s, MeO), 5.31 (1H, m, 6-H). MS *m/e* (%): 358 (M^+, 17), 315 ($M^+-C_3H_7$, 45), 255 ($M^+-C_4H_7O-CH_3OH$, 100). **13**, mp 118—119° (petr. ether), $[\alpha]_D^{18}$ +17° (*c*=0.4, ether). *Anal.* Calcd. for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53. Found: C, 79.99; H, 10.48. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1703.

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18) The retention time of (a), (b), or (c) was determined analogously as in the case of methylation products (**11**—**14**) of pregnenolone.

CD ($c=1.8\times10^{-1}$, ether): $[\theta]_{335}$ 0, $[\theta]_{291}$ +10200 (pos. max.), $[\theta]_{225}$ 0. PMR ($CDCl_3$) δ: 0.63, 1.01 (3H each, both s, 13-CH_3, 10-CH_3), 1.04 (3H, t, $J=7$ Hz, 21-CH_3), 2.38 (2H, q, $J=7$ Hz, 21-H_2), 3.36 (3H, s, MeO), 5.34 (1H, m, 6-H). MS *m/e* (%): 344 (M^+, 19), 312 (M^+-CH_3OH, 52), 57 (C_3H_5O, 100). **14**, mp 128—129° (petr. ether), $[\alpha]_D^{18}$ +14° ($c=0.6$, ether). *Anal.* Calcd. for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.79; H, 10.38. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1703. CD ($c=5.9\times10^{-1}$, ether): $[\theta]_{340}$ 0, $[\theta]_{288}$ +11700 (shoulder), $[\theta]_{293}$ +11900 (pos. max.), $[\theta]_{250}$ 0. PMR ($CDCl_3$) δ: 0.63, 1.00 (3H each, both s, 13-CH_3, 10-CH_3), 2.11 (3H, s, 20-CH_3), 3.35 (3H, s, MeO), 5.35 (1H, m, 6-H). MS *m/e* (%): 330 (M^+, 45), 298 (M^+-CH_3OH, 77), 43 (CH_3CO^+, 100). Pregnenolone, CD ($c=3.33\times10^{-1}$, MeOH): $[\theta]_{330}$ 0, $[\theta]_{285}$ +11800 (pos. max.), $[\theta]_{225}$ 0.

Methylation of PR-3Q (7) followed by Methanolysis——a) A solution of **7** (20 mg) in DMSO (2 ml) was treated with dimsyl carbanion solution (2.5 ml), kept stirring for 1.5 hr, treated with CH_3I (1.25 ml), and kept stirring for further 1.5 hr as above. Working up of the reaction mixture as for **6** followed by preparative TLC (benzene–acetone=5: 1) gave octa-O-methyl derivative (**7a**, 9 mg). **7a**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1701. PMR ($CDCl_3$) δ: 3.35—3.61 (MeO×8), 4.23 (1H, d, $J=8$ Hz), 4.54 (1H, d, $J=7.5$ Hz), 4.84 (1H, d, $J=6$ Hz) (anomeric H×3), 5.32 (1H, m, 11-H). CD (ether): $[\theta]_{330}$ 0, $[\theta]_{296}$ (pos. max.), $[\theta]_{266}$ 0.

b) A solution of **7a** (3 mg) in anhydrous 2.5 N HCl–MeOH (1.5 ml) was heated under reflux for 1 hr, and worked up as for **6a**. GLC (15% NPGS on chromosorb WAW 80—100 mesh; column 3 mm×2 m; column temp. 181°; N_2 flow rate 30 ml/min) and TLC (benzene-acetone=2: 1) of the product identified [I] ($t_R=1'44''$, $2'07''$; $Rf=0.70$, 0.75), methyl 2,4-di-O-methyl-quinovopyranoside [II] ($t_R=3'39''$, $4'52''$; $Rf=0.30$), and methyl 3,4-di-O-methyl-xylopyranoside [III] ($t_R=4'31''$, $5'09''$ (w); $Rf=0.40$).

Methylation of PR-3G (8) followed by Methanolysis——a) A solution of **8** (40 mg) in DMSO (2 ml) was treated with dimsyl carbanion solution (5 ml) and methylated with CH_3I (4 ml) as for **6**. Preparative TLC (benzene–acetone=3: 1) of the reaction product gave nona-O-methyl derivative (**8a**, 35 mg). **8a**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1706. PMR ($CDCl_3$) δ: 3.34—3.60 (MeO×9), 4.24 (1H, d, $J=8$ Hz), 4.26 (1H, d, $J=6$ Hz), 4.70 (1H, d, $J=8$ Hz) (anomeric H×3), 5.31 (1H, m, 11-H). CD (ether): $[\theta]_{340}$ 0, $[\theta]_{295}$ (pos. max.), $[\theta]_{250}$ 0.

b) A solution of **8a** (9 mg) in anhydrous 2.5 N HCl–MeOH (2 ml) was heated under reflux for 2 hr and worked up as for **6a**. GLC (15% PEGS on chromosorb WAW 80—100 mesh; column 3 mm×2 m; column temp. 181°; N_2 flow rate 30 ml/min) and TLC (benzene–acetone=2: 1) of the product identified [II] ($t_R=3'27''$, $4'51''$; $Rf=0.25$), methyl 2,3-di-O-methyl-xylopyranoside [IV] ($t_R=5'07''$ (w), $6'01''$, $Rf=0.25$, 0.35), and methyl 2,3,4,6-tetra-O-methyl-galactopyranoside [V] ($t_R=5'14''$, $5'26''$; $Rf=0.55$, 0.60).

Methylation of PR-4 (9) followed by Methanolysis——a) A solution of **9** (50 mg) in DMSO (2 ml) was treated with dimsyl carbanion solution (6 ml) and methylated with CH_3I (4 ml) as for **6**. Silica gel column chromatography (5 g, benzene-acetone=15: 1) of the product gave undeca-O-methyl derivative (**9a**, 39 mg). **9a**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1710. PMR ($CDCl_3$) δ: 3.36, 3.37 (3H each, both s, MeO×2), 3.51—3.60 (MeO×9), 4.24 (1H, d, $J=8$ Hz), 4.26 (1H, d, $J=7$ Hz), 4.55 (1H, d, $J=7$ Hz), 4.86 (1H, d, $J=6$ Hz) (anomeric H×4), 5.33 (1H, m, 11-H). CD (ether): $[\theta]_{350}$ 0, $[\theta]_{294}$ (pos. max.), $[\theta]_{230}$ 0.

b) A solution of **9a** (5 mg) in anhydrous 2.5 N HCl–MeOH (1 ml) was heated under reflux for 1.5 hr and worked up as for **6a**. GLC (15% PEGS on chromosorb WAW 80—100 mesh, column 3 mm×1 m, column temp. 162°; N_2 flow rate 40 ml/min) and TLC (benzene–acetone=2: 1) of the product identified [I] ($t_R=1'17''$, $1'47''$; $Rf=0.70$, 0.75), [III] ($t_R=4'26''$, $6'34''$; $Rf=0.30$), [V] ($t_R=7'29''$, $7'59''$; $Rf=0.40$, 0.50, and methyl 3-O-methyl-xylopyranoside [VI] ($Rf=0.15$). [VI] was further identified by GLC with column temp. 189° (with the other conditions as above) ($t_R=6'34''$, $10'06''$).

1,2: 3,5-Di-O-isopropylidene-α-D-xylofuranose (15)——To a solution of D-xylose (1.75 g) in dry acetone (30 ml), was added anhydrous $CuSO_4$ (3 g) and conc. H_2SO_4 (0.1 ml). The total mixture was kept stirring at room temperature for 7.5 hr and filtered. The filtrate was added with $Ca(OH)_2$ powder (1 g), kept stirring for 1 hr, and filtered. In order to neutralize completely, the reaction mixture was treated with Amberlite IR-45 (1 ml), filtered, and evaporated under reduced pressure to dryness. Silica gel (100 g) column chromatography (benzene–acetone=3: 1) of the product (2.2 g) furnished 1,2: 3,5-di-O-isopropylidene-α-D-xylofuranose (**15**, 1.5 g) and 1,2-O-isopropylidene-α-D-xylofuranose (**16**, 570 mg). **15**, $[\alpha]_D^{19}$ +5.5° ($c=3.9$, $CHCl_3$) (lit.[10] $[\alpha]_D$ +6.0° ($CHCl_3$)), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1078. PMR ($CDCl_3$) δ: 1.33, 1.38, 1.45, 1.49 (3H each, all s, isopropylidene $CH_3\times4$), 4.08 (3H, m, 5-H_2, 4-H), 4.29 (1H, d, $J=2$ Hz, 3-H), 4.51 (1H, d, $J=4$ Hz, 2-H), 5.99 (1H, d, $J=4$ Hz, 1-H). **16**, $[\alpha]_D^{16}$ −18° ($c=1.1$, acetone) (lit.[11] $[\alpha]_D$ −17.5° (H_2O)), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1068. PMR ($CDCl_3$) δ: 1.31, 1.48 (3H each, both s, isopropylidene $CH_3\times2$), 3.98 (2H, m), 4.19 (1H, m), 4.29 (1H, m) (3,4,5-H_4), 4.52 (1H, d, $J=4$ Hz, 2-H), 5.96 (1H, d, $J=4$ Hz, 1-H).

Acid Treatment of 15 giving 16——A solution of **15** (1.3 g) in aq. 0.04 N H_2SO_4 (6 ml)–MeOH (18 ml) was left standing at 25° for 14 hr, neutralized with $BaCO_3$ powder, and warmed on a hot water-bath for a while. After cooling, the total mixture was filtered and evaporated under reduced pressure to afford **16** (1.09 g).

Tritylation of 16 giving 17——To a solution of **16** (460 mg) in pyridine (3 ml), was added trityl chloride (1.0 g). The total mixture was left standing at room temperature (19°) for 15 hr, poured into ice water, and extracted with ether. The ether extract was washed successively with dil. AcOH, and aq. $NaHCO_3$, and water, and dried over $MgSO_4$. The residue, obtained by evaporation of the solvent, was purified by silica gel (15 g) column chromatography (hexane–acetone=10: 1) to furnish **17** (980 mg). **17**, $[\alpha]_D^{16}$ +17° ($c=0.71$, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 74.67; H, 6.68. IR $\nu_{max}^{CCl_4}$ cm^{-1}:

3480, 3050, 1597 (w), 1488. PMR ($CDCl_3$) δ: 1.23, 1.39 (3H each, both s, isopropylidene $CH_3\times2$), 3.42 (2H, m), 4.11 (2H, m) (3,4,5-H_4), 4.34 (1H, d, J=4 Hz, 2-H), 5.80 (1H, d, J=4 Hz, 1-H), 7.13—7.45 (15H, trityl H_{15}).

Methylation of 17 giving 18——A solution of **17** (140 mg) in CH_3I (2 ml) was treated with Ag_2O (300 mg) and kept stirring at room temperature (18°) for 14 hr. After dilution with ether (10 ml), the reaction mixture was filtered. Evaporation of the filtrate gave the product, which was extracted again with ether to furnish **18** (150 mg). **18**, $[\alpha]_D^{18}$ −52° (c=1.3, $CHCl_3$). High resolution MS: Found 446.209. Calcd. for $C_{28}H_{30}O_5$ (M^+)=446.209. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3050, 1595 (w), 1485. PMR ($CDCl_3$) δ: 1.22, 1.42 (3H each, both s, isopropylidene $CH_3\times2$), 3.20 (3H, s, MeO), 3.25—3.50 (2H, m), 4.21 (1H, m) (4,5-H_3), 3.64 (1H, d, J=3 Hz, 3-H), 4.38 (1H, d, J=4 Hz, 2-H), 5.69 (1H, d, J=4 Hz, 1-H), 7.10—7.47 (15H, trityl H_{15}).

Methyl 3-O-Methyl-D-xylopyranoside (20)——A solution of **18** (120 mg) in MeOH (2 ml) was treated with aq. 0.08 N H_2SO_4 (1.5 ml) and heated under reflux for 30 min. After neutralization with $Ba(OH_2)$, the total mixture was centrifuged. Evaporation of the supernatant under reduced pressure gave the reaction product, which was purified by silica gel (5 g) column chromatography ($CHCl_3$–MeOH–H_2O=7:3:1, lower layer) to furnish 3-O-methyl-D-xylose (**19**, 30 mg). **19**, $[\alpha]_D^{18}$ +14° (c=0.5, H_2O, taken after 90 min) (lit.[12] $[\alpha]_D$ +14.7° (H_2O, 90 min). A solution of **19** (10 mg) in anhydrous 2 N HCl–MeOH (1.5 ml) was heated under reflux for 2 hr and the product, which was obtained after the usual work-up, has been used as the authentic sample for above mentioned GLC and TLC identifications.

Methylation of dsp-1 (10) followed by Methanolysis——a) A solution of **10** (55 mg) in DMSO (1 ml) was treated with dimsyl carbanion solution (7 ml) and methylated with CH_3I (4 ml) as for **6**. Preparative TLC (AcOEt–MeOH=15:1) of the reaction product gave trideca-O-methyl derivative (**10a**, 55 mg). **10a**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1710. PMR ($CDCl_3$) δ: 3.36—3.62 (MeO×13), 4.25 (1H, d, J=8 Hz), 4.36 (1H, d, J=7 Hz), 4.56 (1H, d, J=6 Hz), 4.59 (1H, d, J=7.5 Hz), 4.81 (1H, d, J=7 Hz) (anomeric H×5), 5.33 (1H, m, 11-H). CD (MeOH): $[\theta]_{330}$ 0, $[\theta]_{293}$ (pos. max.), $[\theta]_{240}$ 0.

b) A solution of **10a** (13 mg) in anhydrous 2.5 N HCl–MeOH (3 ml) was heated under reflux for 1.5 hr and worked up as for **6a**. GLC (15% NPGS on chromosorb WAW 80—100 mesh; column 3 mm×2 m; column temp. 181°; N_2 flow rate 25 ml/min) and TLC (benzene–acetone=2:1) of the product identified [I] (t_R=2′17″, 2′58″; Rf=0.70, 0.75), [II] (t_R=5′35″, 7′44″; Rf=0.30), methyl 2,3,4-tri-O-methyl-fucopyranoside [VII] (t_R=4′17″; Rf=0.65), [VI] (Rf=0.15) and methyl 3,4,6-tri-O-methyl-galactopyranoside [VIII] (Rf=0.20). Identifications of [VI] and [VIII] by GLC were carried out with column temp. 200° and N_2 flow rate 45 ml/min: [VI] (t_R=7′00″, 9′47″) and [VIII] (t_R=8′22″, 12′08″).

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