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ON THE STRUCTURE OF THE MAJOR SAPONIN FROM THE STARFISH ACANTHASTER PLANCI

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Recently we reported the structures of two genuine sapogenols named thornasterol A (1) and B (2) which were obtained from a saponin mixture isolated from the whole body of starfish Acanthaster planci.[1] This communication provides evidence which has led us to presume the structure of the major saponin contained in the starfish.

A saponin mixture isolated from the starfish (collected in Okinawa prefecture in June) was hydrolyzed with a commercial glycosidase mixture of Charonia lampas as reported previously[1] and the resulting sapogenol sulfate mixture was successively subjected to CrO_3-pyridine oxidation (converting free hydroxyl to carbonyl), solvolysis using dioxane-pyridine (for splitting sulfate bond[1]), acetylation, and TLC separation to afford three products: KA-1 (3, 14%), KA-2 (4, 18%), and KA-3 (5, 60%).[2]

The major one, KA-3 (5), $C_{29}H_{44}O_5$,[3] mp 178-179°, $[\alpha]_D^{18}$ -16° ($CHCl_3$), IR (CCl_4, cm^{-1}): 3500 (OH), 1742 (AcO), 1720 (C_6-CO), 1706 (C_{23}-CO), shows a resembled PMR spectrum to that of thornasterol A diacetate (1a)[1] except that only one acetoxyl signal (δ2.02, 3H, s) is observed in the former while two in the latter. The CD spectrum (MeOH) of KA-3 exhibits a negative maximum ($[\theta]_{293}$ -7420)[4] which suggests the presence of a carbonyl function at C_6,[5] thus the structure 5 being substantiated for KA-3. Structures of two minor products are respectively supported by their spectral properties: KA-2 (4), $C_{30}H_{46}O_5$, mp 184-185°, $[\alpha]_D^{18}$ -24° ($CHCl_3$), CD (MeOH): $[\theta]_{293}$ -6600 (neg. max.),[6] which is resulting from a minor sapogenol thornasterol B(2), and KA-1 (3), $C_{29}H_{42}O_4$, mp 169-170°, $[\alpha]_D^{18}$ -40° ($CHCl_3$), UV (MeOH): 248 nm (max, ε= 12200), CD (MeOH): $[\theta]_{292}$ -6100 (neg. max.), $[\theta]_{244}$ -12000 (neg. max.), which corresponds to a dehydration

product of KA-3 (5). It follows therefore that a sulfate group in saponin connects to C_3 hydroxyl of its aglycone.

Next, the structure of oligosaccharide portion in saponin has been investigated. In the course of the studies, we have noticed that the side chains (as seen in 1 and 2) in the steroidal aglycones of saponins are readily changeable and this may cause formation of a complex mixture. Therefore, our effort has been focused to isolate the major saponin by removing its sulfate group first and then by converting its aglycone part to a pregnane-type steroid.

The above mentioned saponin mixture was treated with 1N NaOMe-MeOH under reflux for 4 hr (the side chains as in 1 and 2 being converted to a methyl carbonyl moiety via a retro aldol reaction) and subjected to solvolysis with dioxane-pyridine (1:4, under reflux for 1.5 hr, to remove the sulfate group) and to column chromatographic separation (Kiesel gel H).

The major oligoglycoside dsp-1 (6), $C_{50}H_{80}O_{24}$, mp 264-265° (MeOH-H_2O), $[\alpha]_D^{16}$ +22° (MeOH-H_2O), CD (MeOH): $[\theta]_{287}$ +6300 (pos. max.), IR (Nuj.): 3380 (br, OH), 1703 (CO), gave 5α-pregn-9(11)-ene-3β,6α-diol-20-one (7)[1] on hydrolysis using crude naringinase.[7] On the other hand, methanolysis of 6 liberated quinovose, xylose, galactose, and fucose[8] (GLC as TMS deriv.). Methylation of 6 with CH_3I-NaH-DMSO[9] gave a trideca-O-methyl derivative (6a),[10] IR (CCl_4): no OH, 1710 (CO), the PMR spectrum ($CDCl_3$) of which exhibits five anomeric proton signals at 4.25 (d, J= 8 Hz), 4.36 (d, J= 7), 4.56 (d, J= 6), 4.59 (d, J= 7.5), and 4.81 (d, J= 7), thus indicating all anomeric configurations in 6a to be β (Cl form). Upon methanolysis, 6a furnished methyl 2,3,4-tri-O-methyl-quinovopyranoside, methyl 2,3,4-tri-O-methyl-fucopyranoside, methyl 2,4-di-O-methyl-quinovopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside, and methyl 3-O-methyl-xylopyranoside (GLC, TLC), thus showing fucose and one of two quinovoses in 6 being located terminally.

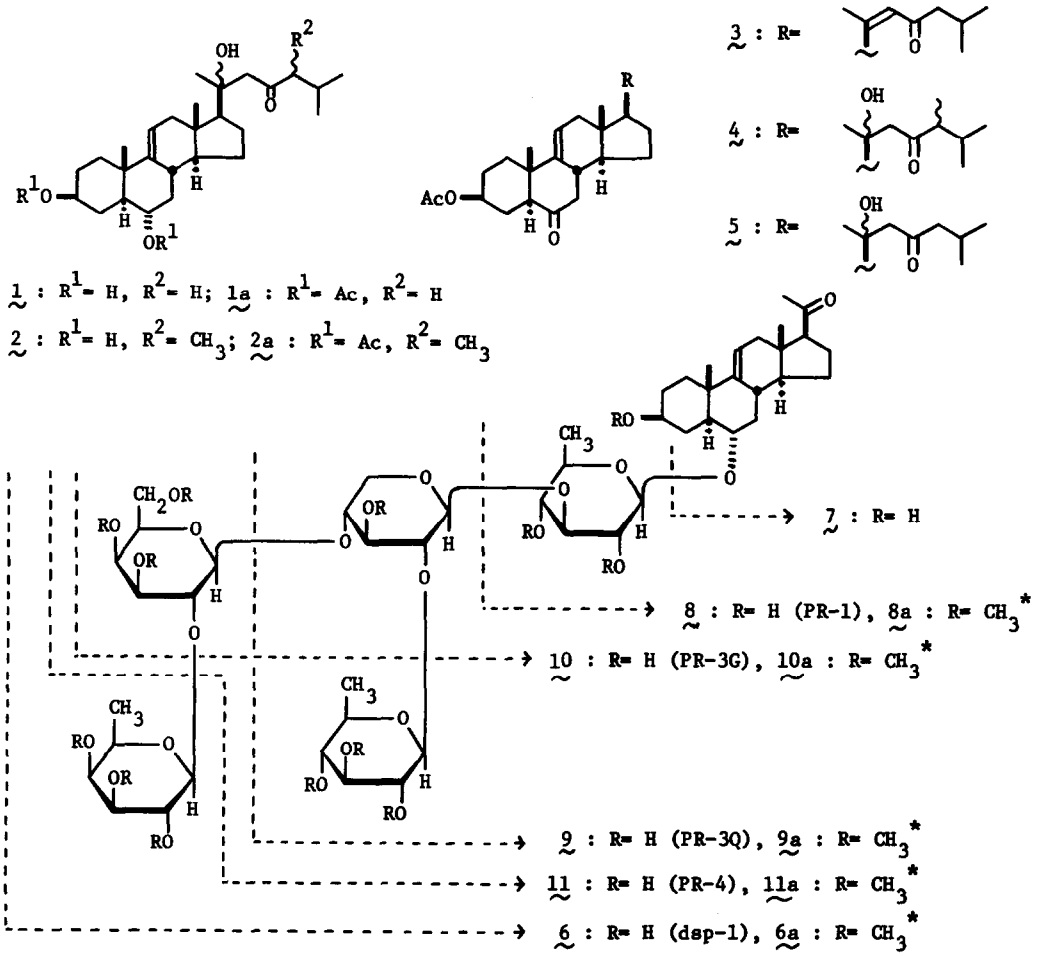
On mild acid hydrolysis, 6 gave 7 and four prosapogenols: PR-1 (8), PR-3Q (9), PR-3G (10), and PR-4 (11), which, on respective methanolysis, liberated quinovose (from 8), quinovose and xylose (from 9), quinovose, xylose, and galactose (from 10 and 11).

PR-1 (8), $C_{27}H_{42}O_7 \cdot H_2O$, mp 168-169° ($CHCl_3$-MeOH), $[\alpha]_D^{23}$ +52° (MeOH), CD (MeOH): $[\theta]_{285}$ +8800 (pos. max.), IR ($CHCl_3$): 3390 (br, OH), 1701 (CO), was converted by methylation to a tetra-O-methyl derivative (8a),[10] IR (CCl_4): no OH, 1702 (CO), PMR ($CDCl_3$): 4.22 (1H, d, J= 7, anomeric proton), which, on methanolysis, gave methyl 2,3,4-tri-O-methyl-quinovopyranoside.

PR-3Q (9), $C_{38}H_{60}O_{15} \cdot 1/2H_2O$, mp 263-265° (MeOH), $[\alpha]_D^{16}$ +39° (MeOH), CD (MeOH): $[\theta]_{286}$ +9100 (pos. max.), IR (Nuj.): 3400 (br, OH), 1697 (CO), gave an octa-O-methyl derivative (9a),[10]

IR (CCl_4): no OH, 1701 (CO), PMR ($CDCl_3$): 4.23 (1H, d, J= 8), 4.54 (d, J= 7.5), 4.84 (d, J= 6) (three anomeric protons). Methanolysis of 9a liberated methyl 2,3,4-tri-O-methyl-quinovopyranoside, methyl 3,4-di-O-methyl-xylopyranoside, and methyl 2,4-di-O-methyl-quinovopyranoside.

PR-3G (10), $C_{38}H_{60}O_{16}\cdot 3H_2O$, mp 169-171° (PrOH-ether), $[\alpha]_D^{20}$ +13° (EtOH), CD (EtOH): $[\theta]_{288}$ +5500 (pos. max.), IR (Nuj.): 3350 (br, OH), 1704 (CO), gave, on methylation, a nona-O-methyl-derivative (10a),[10] IR (CCl_4): no OH, 1706 (CO). The PMR spectrum of 10a ($CDCl_3$) shows three anomeric proton doublets at 4.24 (J= 8), 4.26 (J= 6), and 4.70 (J= 8), thus suggesting that quinovose, galactose, and xylose in 10 are connected with β orientation. Methanolysis of 10a 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.



* The methyl carbonyl at C_{17} in 6a, 8a, 9a, 10a, or 11a is converted to a mixture of an ethyl carbonyl and an isopropyl carbonyl.[10]

PR-4 (11), mp 220-221° (MeOH-PrOH), $[\alpha]_D^{16}$ +24° (MeOH), CD (MeOH): $[\theta]_{285}$ +7300 (pos. max.), IR (Nuj.): 3380 (br, OH), 1696 (CO), gave an undeca-O-methyl derivative (11a),[10] IR (CCl_4): no OH, 1710 (CO), on methylation. In the PMR spectrum ($CDCl_3$) of 11a, four anomeric proton signals are observed as doublets at 4.24 (J= 8), 4.26 (J= 7), 4.55 (J= 7), and 4.86 (J= 6) (each showing β orientation). Methanolysis of 11a liberated methyl 2,3,4-tri-O-methyl-quinovopyranoside, methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, methyl 2,4-di-O-methyl-quinovopyranoside, and methyl 3-O-methyl-xylopyranoside.

Based on the accumulated evidence given above, the structure of dsp-1 is now formulated as 3β-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-pregn-9(11)-ene (6), and consequently the major saponin of Acanthaster planci is presumed to be the one in which the aglycone thornasterol A (1) is linked with a sulfate group at its C_3 hydroxyl and with a same oligosaccharide moiety as in dsp-1 (6) at its C_6 hydroxyl.

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References and Footnotes

1) I. Kitagawa, M. Kobayashi, T. Sugawara, and I. Yosioka, Tetrahedron Letters, 1975, 967.

2) The yields are based on the final acetylation product.

3) All compounds given with the chemical formulae gave satisfactory analytical values.

4) The observed molar ellipticity is larger than that of the ordinary C_6-keto steroid (cf. 3β-acetoxy-5α-cholestan-6-one: $[\phi]_{306}$ -3580°, $[\phi]_{270}$ +4050°[5]) due to enhancement caused by a negative maximum due to C_{23} carbonyl.[1]

5) C. Djerassi and W. Klyne, J. Chem. Soc., 1963, 2390.

6) The positive maximum due to C_{23} carbonyl of thornasterol B diacetate (2a)[1] is overcome by a large negative maximum due to C_6 carbonyl.

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

8) Determined to be D-fucose by measuring the $[\alpha]_D$ value in MeOH.

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

10) Methylation of pregnenolone with CH_3I-NaH-DMSO revealed that the methyl carbonyl moiety at C_{17} was also methylated simultaneously to give a mixture of an ethyl carbonyl and an isopropyl carbonyl derivatives. Therefore, it is presumed that the methyl carbonyl moieties in the aglycones of 6, 8, 9, 10, and 11 are also similarly affected on methylation as deduced from the sgift of the CD maxima: e.g. 293 nm in 6a, 287 nm in 6.