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STRUCTURE OF HOLOTOXIN A, A MAJOR ANTIFUNGAL GLYCOSIDE OF STICHOPUS JAPONICUS SELENKA

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In 1968, Elyakov, et al. reported the isolation of two triterpene glycosides (stichoposide A and C) from the MeOH extractive of Far Eastern sea cucumber (Stichopus japonicus SELENKA)[1] and proposed the structures of two aglycones, stichopogenin A_2 (1) and A_4 (2), which were obtained by acid hydrolysis of stichoposide A.[2] Later on, they described the carbohydrate composition of both glycosides as xylose, 3-O-methyl-glucose, quinovose, and glucose.[3] On the other hand, in 1969 Shimada isolated an antifungal glycoside named holotoxin from the same species of sea cucumber.[4] Although the direct comparison of these glycosides has not been realized, Scheuer suggested that Shimada's holotoxin is probably identical with or closely related to one of the stichoposides reported by Elyakov, et al.[5] However, the structures of these glycosides have not been elucidated.

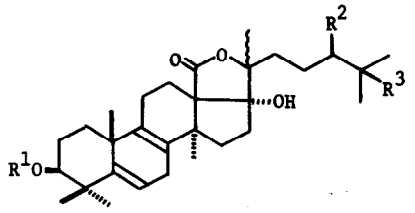
We have isolated the antifungal glycoside, which is identical with Shimada's holotoxin, from the MeOH extractive of Stichopus japonicus and have separated it by droplet countercurrent chromatography[6] to three glycosides now named holotoxin A (major), B, and C. This communication presents the evidence which is consistent with the structure of oligosaccharide portion of holotoxin A (4).

Holotoxin A (4), mp 248-250°, $[\alpha]_D$ -53° (Py.), shows no UV absorption maximum above 210 nm but shows the hydroxyl (3400 cm^{-1}) and γ-lactone (1754 cm^{-1}) absorption bands in its IR spectrum (KBr). On aq. 2N HCl hydrolysis, it furnished xylose, quinovose, 3-O-methyl-glucose, glucose, and two aglycones. Based on the comparison of the physicochemical properties (UV, IR, PMR, mass) of two aglycones and their derivatives with those of stichopogenin A_2 (1),

A_4 (2), and their derivatives,[2] it has been disclosed that one aglycone is identical with 2 while the other being a mixture of 1 and its unidentified isomer which were derived secondarily during the acid hydrolysis. On mild acid treatment (aq. 2% H_2SO_4-MeOH(1:1)/benzene), holotoxin A furnished 2 along with a minor methoxy derivative 3 (the location of the methoxyl function at C-25 being based on the comparison of its physicochemical properties with those of 1 and 2), but 1 was not formed.

On enzymatic hydrolysis using a partially purified takadiastase A preparation, holotoxin A afforded two prosapogenols, 7 and 9, while on treatment with cellulase III it gave 7 and xylose, thus showing that holotoxin A is a xyloside of 7. 7, mp 259-262°, IR: 3400, 1750 cm^{-1}, gave, on acid hydrolysis, xylose, quinovose, 3-O-methyl-glucose, glucose, and two aglycones as in the case of holotoxin A. Methylation of 7 with CH_3I/DMSO/NaH gave 8, whose free hydroxyl in the aglycone part was not readily acetylated. Therefore, the oligosaccharide moiety in 7 attaches to 3-OH of the aglycone. The PMR spectrum of 8 (C_6D_6) shows the signals due to an olefinic proton (δ 5.51, m) and four anomeric protons: δ 4.96 (d, J= 7), 4.93 (d, J= 7), 4.81 (br.s), and 4.60 (d, J= 7). Since all the monosaccharide constituents (D series) of holotoxin A are considered to take the C1 conformation, the coupling patterns of the anomeric protons indicate the presence of three β- and one α-glycosidic linkages. Methanolysis of 8 yielded Me 3-O-methyl-xylopyranoside, Me 2,3,4-tri-O-methyl-quinovopyranoside, Me 2,4,6-tri-O-methyl-glucopyranoside, and Me 2,3,4,6-tetra-O-methyl-glucopyranoside. Another prosapogenol 9, mp 274.5-277°, IR: 3350, 1750 cm^{-1}, gave, on acid hydrolysis, xylose, quinovose, and two aglycones as above. Methanolysis of 10, a methylated product of 9, furnished Me 2,3,4-tri-O-methyl-quinovopyranoside and Me 2,3-di-O-methyl-xylopyranoside. Consequently, two prosapogenols obtained by the enzymatic hydrolysis have been elucidated to be 7 and 9 (anomeric configurations undefined yet), respectively.

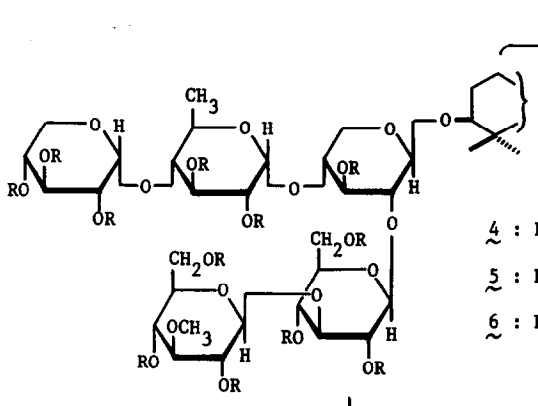
Since 5, a methylated derivative of 4, shows an additional anomeric proton (as compared with 8) at δ 4.30 (d, J= 4)(C_6D_6) due to a xyloside, the terminal xylose in 4 is linked with α-orientation (supported also by application of the Klyne's rule[7]: $[M]_D$ of 4 - $[M]_D$ of 7 = +238°; $[M]_D$(Me α-D-xylopyranoside) = +249°; $[M]_D$(Me β-D-xylopyranoside) = -107°[8]). Methanolysis of 5 gave Me 2,3,4,6-tetra-O-methyl-glucopyranoside, Me 2,3,4-tri-O-methyl-xylopyranoside, Me 2,3-di-O-methyl-quinovopyranoside, Me 2,4,6-tri-O-methyl-glucopyranoside, and Me 3-O-methyl-xylopyranoside, thus leading to the formulation of the oligosaccharide portion of holotoxin A as shown in 4 (anomeric configurations partly undefined yet).



1 R^1= H, R^2, R^3= Δ^{24} stichopogenin A_2

2 R^1= R^2= H, R^3= OH stichopogenin A_4

3 R^1= R^2= H, R^3= OCH_3



stichopogenin A_4

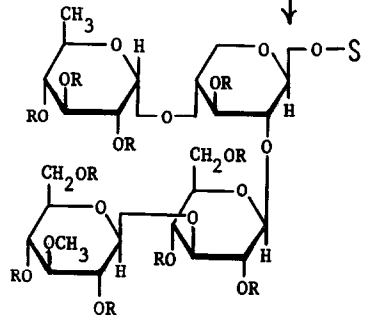
4 : R= H holotoxin A

5 : R= CH_3

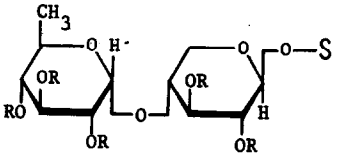
6 : R= Ac

enzyme

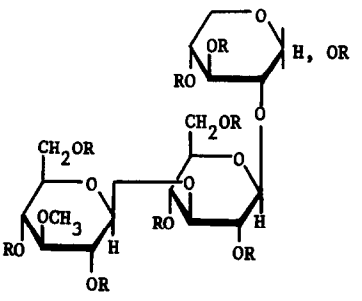
$Ac_2O/ZnCl_2$



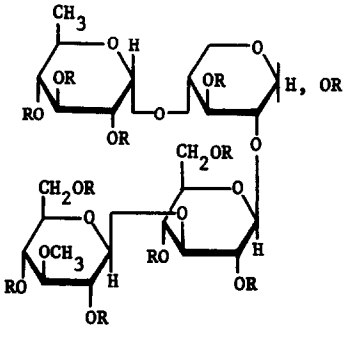
7: R= H, 8: R= CH_3



9: R= H, 10: R= CH_3



11: R= Ac, 12: R= CH_3



13: R= Ac, 14: R= CH_3

Finally, the total structure of the oligosaccharide portion in 4 has been elucidated on the basis of the following evidence. Thus, treatment of holotoxin A with $Ac_2O/ZnCl_2$ [9] furnished two acetyl-oligosaccharides, 11, amorphous, IR: no OH, 1750 cm^{-1}, PMR: δ 3.37 (OCH_3), and 13, amorphous, IR: no OH, 1750 cm^{-1}, PMR: δ 3.37 (OCH_3), 1.27 (>CH-CH_3). The PMR analysis and methanolysis of their permethylates: 12, three anomeric protons at δ4.27 (2H, d, J= 8) and δ4.57 (1H, d, J= 7), and 14, an additional anomeric proton at δ4.22 (1H, br.s), have disclosed the structures of the acetyl-oligosaccharides to be 11 and 13 including the anomeric configurations. The coupling pattern of the quinovoside anomeric proton (δ4.22, br.s) in 14 indicates that the terminal quinovose in 13 is connected with α-linkage (vide supra).

The established structure of the oligosaccharide moiety of holotoxin A (4) is characteristic by the possession of two α-linkages of xylose and quinovose. Since no aglycone has yet been obtained through the enzymatic hydrolysis of holotoxin A, a further examination seems to be needed for the definite proof of the genuineness of stichopogenin A_4 moiety in holotoxin A. The high activity of holotoxin A and B against the pathogenic fungi, such as Trichophyton mentagrophytes, Microsporum gypseum, Candida albicans, and C. utilis, will be reported elsewhere.

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