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STRUCTURE OF STICHOPOGENIN A_4, THE GENUINE AGLYCONE OF HOLOTOXIN A ISOLATED FROM STICHOPUS JAPONICUS SELENKA

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Recently, we reported[1] the elucidation of the carbohydrate portion of holotoxin A, a major antifungal glycoside of Stichopus japonicus SELENKA, and also described the identity of its triterpenoid aglycone with stichopogenin A_4, to which the structure 1 was previously proposed by Elyakov, et al.[2] The structure 1 was characteristic by the possession of a rare example of an unconjugated $\Delta^{5,8}$-diene moiety and only limited numbers of sterols having the similar diene system have so far been isolated in the free states.[3] Since survival of the $\Delta^{5,8}$-diene system during acid hydrolysis of the parent glycoside appeared significant and the diene system was expected to show a red shift in its CD spectrum due to the interaction of the two $\pi \rightarrow \pi^*$ transitions[4] and in order to elucidate the genuine aglycone of holotoxin A, we have examined the CD spectra of holotoxin A and its aglycone (= stichopogenin A_4). However, the spectra have revealed that both stichopogenin A_4 and holotoxin A possess unexpectedly the ketone chromophore along with the olefin and γ-lactone chromophores in their molecules (stichopogenin A_4: $[\theta]_{200}$ +41000, $[\theta]_{233}$ -21000, $[\theta]_{305}$ -16800; holotoxin A: $[\theta]_{205}$ +22000, $[\theta]_{233}$ -13700, $[\theta]_{305}$ -9900). On the basis of the following evidence, we have reached the conclusion that stichopogenin A_4 is expressed as 2 rather than 1 and consequently that the total structure of holotoxin A is formulated as 3 (the configuration at C-20 being undefined yet).

Stichopogenin A_4 (2), $C_{30}H_{46}O_5 \cdot 1/2H_2O$, mp 240-243°, $[\alpha]_D^{23}$ -77° ($CHCl_3$), Mass (m/e): 486 (M^+), 468 (M^+-H_2O), shows no UV absorption above 210 nm but a broad IR absorption band at 1750

cm^{-1}(KBr). On acetylation with Ac_2O/pyridine, 2 gave a monoacetate (2a), $C_{32}H_{48}O_6$, M^+: m/e 528, mp 220-222° (250-252°[5]), $[\alpha]_D^{23}$ -53° ($CHCl_3$), IR ($CHCl_3$): 1732 (br), 1716 (sh) cm^{-1}. These physical data are in accord with those reported for stichopogenin A_4 and its monoacetate by Elyakov, et al.[2]

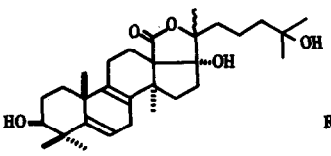
Since the CD spectrum of 2 shows the presence of the ketone, which does not locate in the 6-membered ring as revealed by the IR spectrum of 2, 2 is considered to carry one double bond and one 5-membered ring ketone in place of two double bonds and one hydroxyl as seen in 1. The ketone is assigned at C-16 in the lanostane skeleton as based on the negative CD curve ($[\theta]_{305}$ -16800: cf. 6 - 10[6c]). The assignment is further supported by a fact that the addition of alkali alters the CD curve of 2 to a curve ($[\theta]_{270}$ -22400, $[\theta]_{330}$ -5300 (sh)) ascribable to an enone chromophore (4).[7] Therefore, the IR absorption band due to the C-16 ketone in 2 seems to be overlapped by the band due to the γ-lactone (vide infra).

In the PMR spectrum of stichopogenin A_4 (2), a one-proton signal (br, $W_{h/2}$= 6 Hz) is observed at δ5.27 and hence the double bond could be assigned as Δ^5, Δ^7, or $\Delta^{9(11)}$. Among them, $\Delta^{9(11)}$ is preferred, since the observed strong positive CD maximum ($[\theta]_{200}$ +41000) is in good accord with the reported values for the $\Delta^{9(11)}$-lanostene triterpenoids (cf. 11 - 15[8]). In addition, the olefinic proton chemical shift is quite alike to those reported for the $\Delta^{9(11)}$-lanostene derivatives.[9]

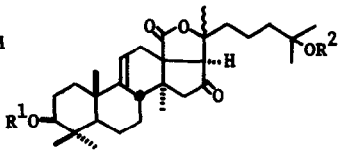
As for the γ-lactone moiety of stichopogenin A_4 (2), the $n\rightarrow\pi^*$ transition is observed with the strong negative CD maximum ($[\theta]_{233}$ -21000). The sign is well explained by the Beecham's rule,[10] and the red shift and the enhanced magnitude could be attributable to the spacial interaction of the $\Delta^{9(11)}$ double bond located near the γ-lactone moiety.[11] The explanation is also supported by comparing the CD data of 2 with those of the 9α,11α-epoxy derivative (5) ($[\theta]_{228}$ -5400, $[\theta]_{305}$ -12000), which was prepared from 2 by m-chloroperbenzoic acid oxidation.

In the structural study by Elyakov, et al.[2] who proposed the structure 1 for stichopogenin A_4, 17α-OH in 1 was based on the angular CH_3 chemical shifts which were assigned in comparison with those of 22,25-oxido-holothurinogenin (16). However, since 10-CH_3's in the $\Delta^{9(11)}$-lanostene triterpenoids are also observed at the similar positions[7a,12], the above assignment could not be the definite criterion. Furthermore, the following evidence excludes the presence of 17α-OH in stichopogenin A_4 (2).

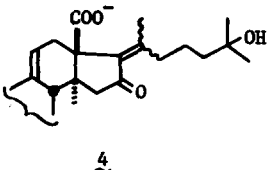
As described previously,[1] the mild acid hydrolysis (aq. 2% H_2SO_4/MeOH/benzene) of holotoxin A (3) furnished 2 and another aglycone (2b), $C_{31}H_{48}O_5$, mp 240-243°, $[\alpha]_D^{24}$ -96° (MeOH); Mass(m/e)



1 (Elyakov, et al.)[2)]



2 R^1= R^2= H
stichopogenin A_4
2a R^1= Ac, R^2= H
2b R^1= H, R^2= CH_3
2c R^1= Ac, R^2= CH_3



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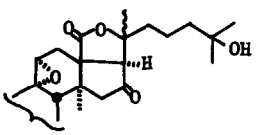

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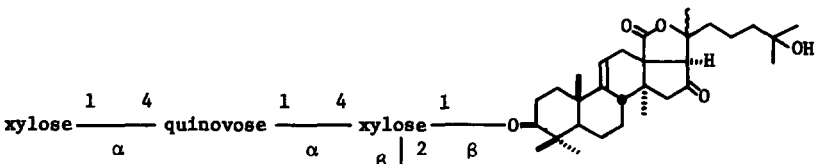
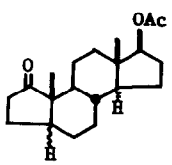
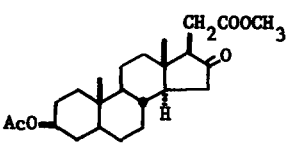


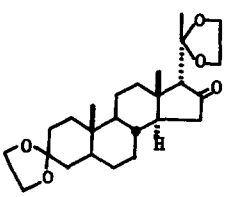
3 holotoxin A



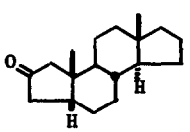
6 5β-H $[\theta]_{294}$ -4870[6c)]
7 5α-H $[\theta]_{302}$ -5620[6c)]



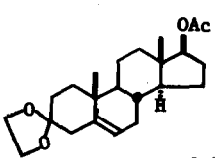
8 $[\theta]_{294}$ -17450[6c)]



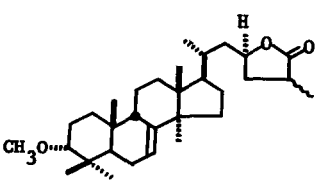
9 $[\theta]_{305}$ -9760[6c)]



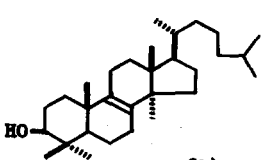
10 $[\theta]_{295}$ -9520[6c)]



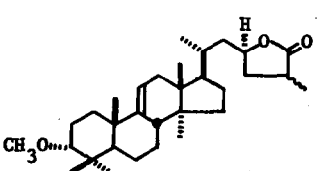
11 $[\theta]_{199}$ +8600[8b)]
12 4,4-dimethyl deriv. negative[8b)]



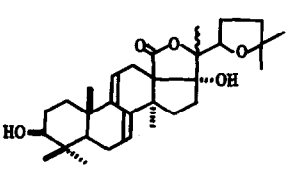
13 $[\theta]_{205}$ -30000[8a)]



14 $[\theta]_{205}$ +10600[8b)]



15 $[\theta]_{205}$ +45000[8a)]



16

: 500 (M^+), 468 (M^+-CH_3OH), 73 ($(CH_3)_2C=\overset{+}{O}CH_3$)[13]; PMR ($CDCl_3$, δ): 0.84, 0.89, 0.99, 1.19 (3H each, all s), 1.12 (6H, s), 1.41 (3H, s)(CH_3 x 7), 3.17 (3H, s, CH_3O), 3.00-3.33 (2H, m, 3α-H, 8β-H), 5.29 (1H, m, 11-H). The CD spectrum of 2b shows the presence of the same chromophore as in 2 ($[\theta]_{200}$ +33000, $[\theta]_{233}$ -16400, $[\theta]_{303.5}$ -15500). Acetylation of 2b with Ac_2O/pyridine gave a monoacetate (2c), mp 238-241°, $[\alpha]_D^{23}$ -11° ($CHCl_3$), the IR spectrum (CCl_4) of which shows no OH absorption band, but the absorption bands due to γ-lactone, 5-membered ring ketone, and acetoxyl (1768 (sh), 1751, 1742 (sh) cm^{-1}).

Stichopogenin A_4 (2) is considered to be a genuine aglycone of holotoxin A (3), since i) 2 is obtained by the mild acid hydrolysis, and ii) the CD spectra of both 2 and 3 show the presence of the same chromophores in both compounds, although the maxima intensities in the CD spectrum of 3 are slightly lower than in 2, the reason of which will be a subject of the further investigation.

References and Footnotes

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