Tetrahedron Letters No. 11, pp 967 - 970, 1975. Pergamon Press. Printed in Great Britain.

THORNASTEROL A AND B, TWO GENUINE SAPOGENOLS FROM THE STARFISH ACANTHASTER PLANCI

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(Received in Japan 8 January 1975; received in UK for publication 11 February 1975)

For these years, several steroidal sapogenols and a prosapogenol have been isolated by acid hydrolysis of the saponin mixture of the starfish Acanthaster planci, and their structures have been demonstrated respectively to be 5α-pregn-9(11)-ene-3β,6α-diol-20-one (1),[1,2] 5α-cholesta-9(11),20(22)-diene-3β,6α-diol-23-one (2),[1] 5α-cholesta-9(11),17(20),24-triene-3β,6α-diol (3),[3] 24ξ-methyl-5α-cholesta-9(11),20(22)-diene-3β,6α-diol (4),[3] and 6-O-quinovopyranoside of 2 (5).[4] However, the genuineness of these sapogenols in regard to the constituents of the parent saponins has not yet been assured.

As a continuation of our recent study on the sea-cucumber saponin (holotoxin A from Stichopus japonicus SELENKA[5]), we have been working on the saponin constituents of the starfish Acanthaster planci and have isolated two genuine sapogenols named thornasterol A (6) and B (7) as their diacetates (6a, 7a).[6] This paper provides with the evidence being consistent with the structures 6 and 7 for thornasterol A and B, respectively.

Silica gel column and droplet countercurrent chromatography[7] of the MeOH extractive obtained from the defatted whole body of the starfish (collected at Kushimoto in Wakayama prefecture) furnished the saponin mixture, which on enzymatic hydrolysis using the commercial enzyme of Charonia lampas yielded the sapogenol sulfate [potassium rhodizonate test[8]: positive; IR[9]: 1230 cm^{-1}; PMR (d_5-pyridine)[10]: δ4.6-4.9 (1H, m, >C<u>H</u>-OSO_3^-)]. Treatment of the sulfate with pyridine (making pyridinium salt) and then with dioxane/K_2CO_3 under reflux (cf. solvolysis with dioxane only[11]) afforded the desulfated product, which, after acetylation with Ac_2O/pyridine,

was purified by preparative TLC to furnish two new acetylated sapogenols: diacetyl-thornasterol A (6a) and B (7a), in addition to 1a (minor) and 2a (less than 6a) which are presumed to be formed secondarily during the above mentioned solvolysis.[12]

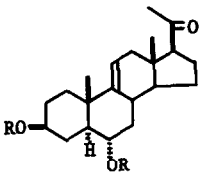
Diacetyl-thornasterol A (6a), $C_{31}H_{48}O_6$, mp 158.5-159.5° (n-hexane), $[\alpha]_D^{15}$ +23° (MeOH); UV (EtOH): transparent above 210 nm; CD (MeOH): $[\theta]_{292}$ -2500, possesses hydroxyl (3500 cm^{-1}), acetoxyl (1742 cm^{-1}) and ketone (1705 cm^{-1}) as revealed by its IR spectrum (CCl_4). The PMR spectrum (90 MHz, $CDCl_3$, δ) of 6a shows the proton signals at 0.74 (3H, s, $C_{(13)}$-CH_3), 0.99 (3H, s, $C_{(10)}$-CH_3), 0.87 (6H, d, J= 6 Hz, $C_{(25)}$-$(CH_3)_2$), 1.30 (3H, s, $C_{(20)}$(OH)-CH_3), 2.00 (6H, s, AcO×2), 2.51 (2H, br.s, -$C_{(22)}H_2$-CO-), 4.55-4.90 (2H, m, $C_{(3)}$H-OAc, $C_{(6)}$H-OAc), and at 5.30 (1H, m, =$C_{(11)}$H-), among which the signals ascribable to a methyl on the carbon bearing a hydroxyl (δ1.30) and to a methylene adjacent to the ketone (δ2.51) are characteristic as compared with those reported for 2a.[1]

The mass spectrum of 6a discloses that the above mentioned two functions are assembled in the 20-hydroxy-23-ketone moiety in the side chain of 6a. Thus, the prominent ion peaks[13] observed at m/e 356 (i), 296 (ii), and 281 (iii, base peak) are formally explained as derived through the McLafferty-type rearrangement of the molecular ion.[14] In addition, the two rich ion peaks of m/e 211 (iv)[15] and m/e 85 (viia) accompanied by a weak ion peak at m/e 441 (vi) respectively suggest that 6a possesses the same steroidal skeleton as 2a and also the isobutyl ketone moiety in the side chain.

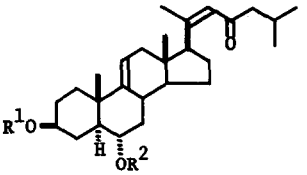
The accumulated evidence has led us to propose 3β,6α-diacetoxy-5α-cholest-9(11)-en-20-ol-23-one (6a) for diacetyl-thornasterol A, and the proposal has been further substantiated by conversion of 6a to 1a and 2a (identified by comparison of the physical data with the reported values[1]) on treatment with 2N HCl/benzene under reflux for 27 hr.

Diacetyl-thornasterol B (7a), $C_{32}H_{50}O_6$, mp 147-148° (n-hexane), $[\alpha]_D^{15}$ +18° ($CHCl_3$); UV (EtOH): transparent above 210 nm; CD (MeOH): $[\theta]_{305}$ +1000; IR (CCl_4, cm^{-1}): 3500 (OH), 1743 (OAc), 1700 (CO); PMR (90 MHz, $CDCl_3$, δ): 0.76 (3H, s, $C_{(13)}$-CH_3), 1.00 (3H, s, $C_{(10)}$-CH_3), 0.79 -0.94 (9H, >CH-CH_3×3), 1.32 (3H, s, $C_{(20)}$(OH)-CH_3), 2.00 (6H, s, AcO×2), 2.56 (2H, br.s, -$C_{(22)}H_2$-CO-), 4.50-4.85 (2H, m, $C_{(3)}$H-OAc, $C_{(6)}$H-OAc), 5.28 (1H, m, =$C_{(11)}$H-), possesses one more secondary methyl than 6a.

In the mass spectrum of 7a, were observed the same prominent ion peaks as in the case of 6a, i.e.,at m/e 356 (i), 296 (ii), 281 (iii, base peak), and 211 (iv), which indicate that 7a holds the same steroidal nucleus as 6a, and the ion peaks at m/e 441 (vi) and 99 (viib) suggest

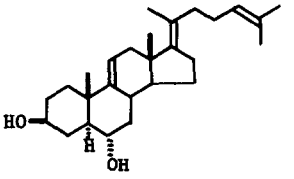


1 R= H; 1a R= Ac

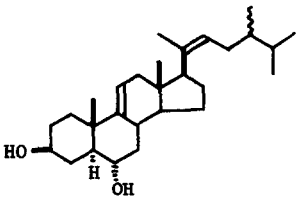


2 R^1= R^2= H; 2a R^1= R^2= Ac

5 R^1= H, R^2= β-D-quinovopyranosyl



3



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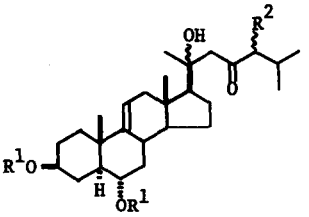
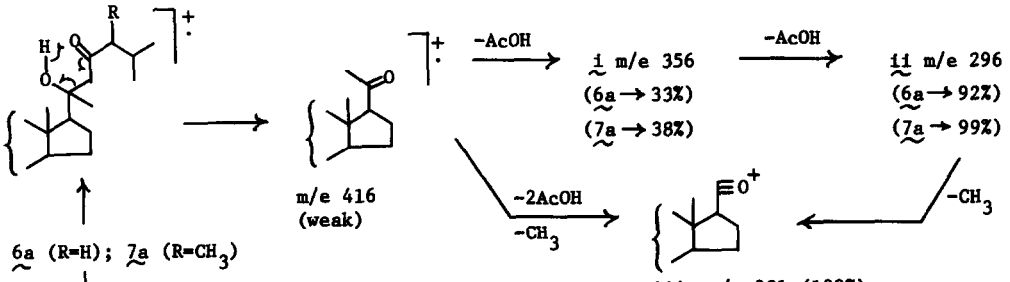


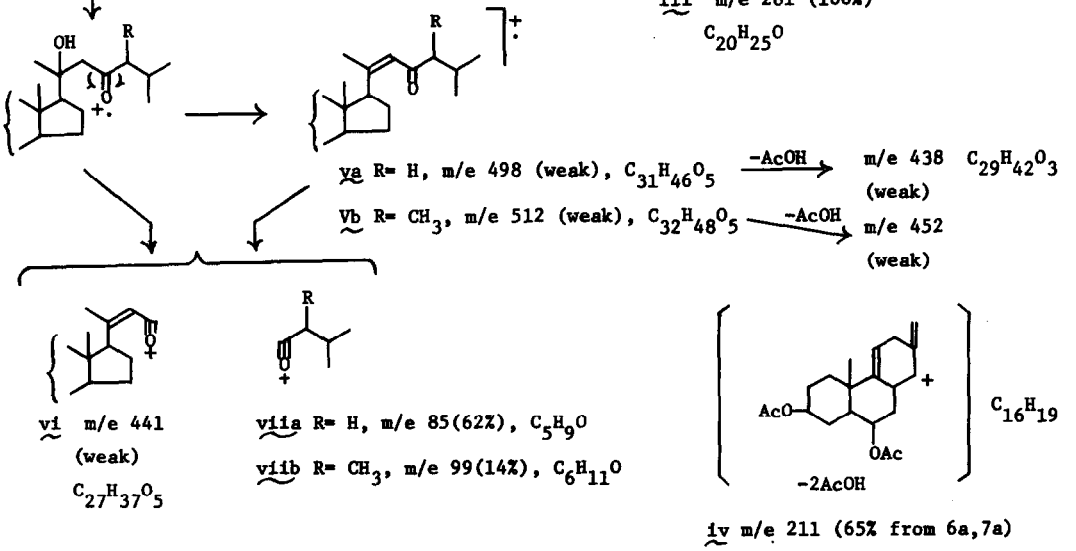
6 R^1= R^2= H thornasterol A

6a R^1= Ac, R^2= H

7 R^1= H, R^2= CH_3 thornasterol B

7a R^1= Ac, R^2= CH_3





the presence of the 23-ketone function in 7a and the location of the additional secondary methyl at C-24. Consequently, diacetyl-thornasterol B is now formulated as 24ξ-methyl-3β,6α-diacetoxy-cholest-9(11)-en-20-ol-23-one (7a). The configurations at C-20 of 6a and 7a and at C-24 of 7a are not yet defined.

Thornasterol A (6) and B (7) carrying the 20-hydroxy-23-ketone moiety are considered to be the two genuine sapogenols of the starfish Acanthaster planci, since i) the acid treatment of 6a furnished 1a and 2a and ii) the CD spectra of 6a and 7a resemble the CD spectrum of the parent saponin. In the recent paper,[16] it has been suggested that 5α-cholest-9(11)-ene-3β, 6α,20-triol-23-one (6) might be a biogenetic precursor of 1 and 2, however, the present results demonstrate that 6 may not be the biogenetic precursor of 1, but is one of the genuine sapogenols and also that 1 could be an artefact sapogenol.[12]

The authors are grateful to Mr. A. Tatsuki and Mr. K. Hayashi of Sabiura Res. Lab., Kushimoto, Wakayama, for collecting the starfish and to Prof. I. Ninomiya of Kobe Women's College of Pharmacy for measuring the high resolution mass spectra.

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