Tetrahedron Letters,Vol.25,No.34,pp 3731-3734,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

FIVE NEW STEROIDAL GLYCOSIDES, PREGNEDIOSIDE-A, -B, AND THEIR THREE MONOACETATES, FROM AN OKINAWAN SOFT CORAL OF ALCYONIUM SP.

Motomasa Kobayashi,[a)] Yutaka Kiyota,[a)] Satomi Orito,[a)]
Yoshimasa Kyogoku,[b)] and Isao Kitagawa[a)]*

a) Faculty of Pharmaceutical Sciences, Osaka University, 1-6, Yamada-oka, Suita, Osaka 565, Japan
b) Institute for Protein Research, Osaka University, 3-2, Yamada-oka, Suita, Osaka 565, Japan

Summary: Five new pregnene-type steroidal glycosides, named pregnedioside -a (1), 4'-O-acetyl-pregnedioside-a (2), 3'-O-acetyl-pregnedioside-a (3), pregnedioside-b (5), and 4'-O-acetyl-pregnedioside-b (6), were isolated from an Okinawan soft coral of *Alcyonium* sp. and their structures were elucidated. These are rare examples of steroidal glycosides from soft coral.

In search of new bioactive compounds from marine organisms, we have been engaged in chemical studies of metabolites of Okinawan coral-reef organisms. As s continuing study on the soft coral constituents,[1)] we have isolated two new pregnene-type steroidal glycosides together with their three monoacetates from a soft coral of *Alcyonium* sp.[2)] These seem to be unprecedented examples of steroidal glycosides isolated from marine organisms except echinoderm saponins.[3,4)]

An acetone extract of fresh soft coral, which was collected in July at Taketomijima, Okinawa Prefecture, was partitioned into an AcOEt-water mixture and the AcOEt-soluble portion was subjected to silica gel column chromatography to afford Fr.1 and Fr.2 containing glycosides. Fr.2, containing free glycosides, was further purified *via* acetylation, silica gel column chromatography (benzene-AcOEt), and deacetylation to furnish pregnedioside-a (1) and pregnedioside-b (5) in 0.1% and 0.2% yields respectively from the AcOEt-soluble portion. Fr.1, containing monoacetylated glycosides, was purified by Lobar column chromatography (LiChroprep SiO_2 60, $CHCl_3$-MeOH) and HPLC (Cosmosil 5C_{18}, MeOH-H_2O) to furnish 4'-O-acetyl-pregnedioside-a (2), 3'-O-acetyl-pregnedioside -a (3), 4'-O-acetyl-pregnedioside-b (6), in 0.1%, 0.07%, and 0.04% yields from the AcOEt-soluble portion.

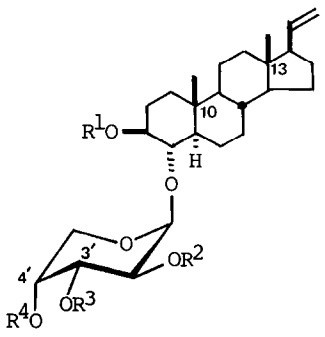
Pregnedioside-a (1), colorless needles, mp 279°C, $C_{26}H_{42}O_6$,[5)] $[\alpha]_D$-92° (pyridine), showed IR absorption bands characteristic of a glycosidic structure : 3350 (br), 1068 cm^{-1}. Acidic hydrolysis of 1 liberated the aglycone (8),

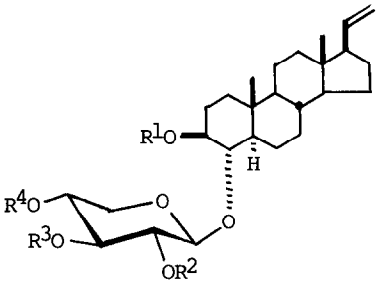
mp 205°C, $C_{21}H_{34}O_2$, and arabinose. The ^1H NMR spectrum[6] of 8 showed signals assignable to 13-CH_3 (δ 0.60, 3H s), 10-CH_3 (δ 0.87, 3H s), 17-vinyl (δ 5.82, 1H ddd, J=15.5, 12.0, 9.0 Hz, 20-H; δ 5.08, 1H d, J=12.0, 21-H; δ 5.07, 1H d, J=15.5, 21-H), and two protons geminal each to a hydroxyl function (δ 3.81, 1H ddd, J=13.5, 9.0, 4.0, 3α-H; δ 3.68, 1H dd, J=9.0, 9.0, 4β-H). Acetylation of 8 gave a diacetate (9), $C_{25}H_{36}O_4$, whereas PCC oxidation of 8 yielded two monoketones: 10, $C_{21}H_{32}O_2$, δ 4.23 (1H d, J=11.0, 4β-H), $[\theta]_{282}^{MeOH}$ +2800 (pos. max.) and 11, $C_{21}H_{32}O_2$, δ 4.41 (1H dd, J=11.5, 8.0, 3α-H), $[\theta]_{288}^{MeOH}$ -3500 (neg. max.). Based on these findings together with ^{13}C NMR data (Table), a pregnene-type structure 8 was presumed for the aglycone of 1.

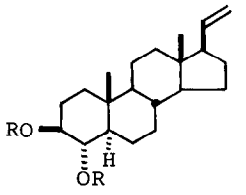
The presumption was corroborated by a following partial synthesis. $NaBH_4$ reduction of progesterone (12) in MeOH in the presence of $CeCl_3 \cdot 7H_2O$ afforded a diol mixture which, *via* hydroboration-oxidation, was converted to a 3β,4α,20-triol mixture (13), $C_{21}H_{36}O_3$. After protection of the 3β,4α-diol moiety by acetonide formation, the triol mixture was oxidized with PCC to furnish 14, $C_{24}H_{38}O_3$, δ (d_6-benzene): 0.54, 0.64 (both 3H s), 1.52 (6H s), 1.81 (3H s), 3.2-3.5 (2H m). The methylketone (14) was then subjected to successive reactions (tosylhydrazone formation, acetonidation, n-BuLi treatment, and deacetonidation) to furnish 3β,4α-dihydroxy-5α-pregn-20-ene (8) (12% overall yield from 12) which was shown to be identical in all respects with the aglycone of pregnedioside-a (1).

In the ^1H NMR spectrum of 1, signals due to the arabinoside moiety were observed at δ 5.74 (1H d, J=4.5, 1'-H), 4.78 (1H dd, J=10.0, 4.5, 2'-H), 4.58 (1H dd, J=10.0, 1.5, 3'-H), 4.45 (1H dd, J=1.5, 1.5, 4'-H), 4.18 (1H dd, J=11.5, 1.5, 5'β-H), and 4.43 (1H d, J=11.5, 5'α-H), thus the $^1C_4$ conformation with β-linkage being elucidated. Furthermore, the 3α-H signal of the tetraacetate (4), $C_{34}H_{50}O_{10}$, was observed at δ 4.73 (1H ddd, J=11.5, 9.0, 5.5), so that the arabinoside linkage of 1 was shown to be attached to 4α-OH of 8. The molecular rotation difference of 4 and 9 ($\Delta[M]_D$=-534°; *cf.*[7] $[M]_D$ of methyl 2,3,4-tri-O-acetyl-β-D-arabinopyranoside=-534°), evidenced the D-arabinoside moiety. Thus, the structure of pregnedioside-a has been determined as 4α-O-β-D-arabinopyranosyloxy-3β-hydroxy-5α-pregn-20-ene (1).

Another glycoside, pregnedioside-b (5), mp 260°C, $C_{26}H_{42}O_6$, $[\alpha]_D$ +2.6° (pyridine), was shown to be a xyloside of 8 by acidic hydrolysis. In the ^1H NMR spectrum of 5, signals suggesting presence of a β-xylopyranoside moiety of $^4C_1$ form were observed at δ 5.17 (1H d, J=8.0, 1'-H), 4.12 (1H dd, J=8.5, 8.0, 2'-H), 4.16 (1H dd, J=8.5, 8.5, 3'-H), 4.24 (1H ddd, J=11.0, 8.5, 5.0, 4'-H), 4.38 (1H dd, J=11.0, 5.0, 5'β-H), 3.71 (1H dd, J=11.0, 11.0, 5'α-H). The ^1H NMR spectrum of the tetraacetate (7) showed that the xyloside moiety of 5 was attached to 4α-OH of the aglycone (8) by the 3α-H signal observed at δ 4.98 (1H ddd, J=11.5, 9.0, 5.5). Furthermore, the molecular rotation difference ($\Delta[M]_D$=-235°; *cf.*[8] $[M]_D$ of methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside=-173°) suggested the xylose to be D, thus the structure of pregnedioside-b has been determined as 4α-O-β-D-xylopyranosyloxy-3β-hydroxy-5α-pregn-20-ene (5).



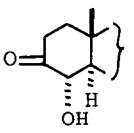




8 : R= H , 9 : R= Ac

1 : R^1= R^2= R^3= R^4= H (pregnedioside-a)
2 : R^1= R^2= R^3= H, R^4= Ac
3 : R^1= R^2= R^4= H, R^3= Ac
4 : R^1= R^2= R^3= R^4= Ac

5 : R^1= R^2= R^3= R^4= H (pregnedioside-b)
6 : R^1= R^2= R^3= H, R^4= Ac
7 : R^1= R^2= R^3= R^4= Ac



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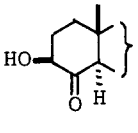

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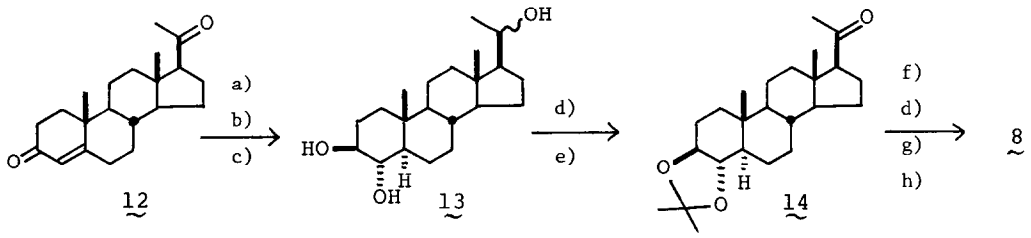

a) $NaBH_4$, $CeCl_3$-MeOH , b) $BH_3 \cdot Et_3N$, DMSO , c) H_2O_2, OH^- , d) 2,2-dimethoxypropane, PPTS , e) PCC, CH_2Cl_2-pyridine , f) $TsNHNH_2$, HCl-EtOH , g) n-BuLi, THF , h) aq.HCl-MeOH

Table ^{13}C NMR Data for 1, 2, 3, 5, 6, and 8[a)]

carbon	1	2	3	5	6	8
1	36.5	36.5	36.5	36.6	36.6	37.0
2	29.1	29.1	29.3	29.0	29.0	29.7
3	76.4	76.3	76.2	76.0[b]	76.0[b]	76.7
4	88.5	88.7	88.6	88.0	87.7	75.4
5	50.4	50.3	50.3	50.3	50.1	51.8
6	23.6	23.6	23.6	23.5	23.5	23.5
7	32.2	32.1	32.1	32.1	32.1	32.3
8	35.4	35.4	35.4	35.5	35.5	35.5
9	55.0[c]	55.0[c]	55.0[c]	55.0[c]	55.0[c]	55.2[c]
10	38.0	38.0	38.0	37.9	37.9	37.5
11	20.9	20.9	21.0	20.9	20.9	20.9
12	37.8	37.8	37.8	37.7	37.7	37.8
13	43.7	43.8	43.8	43.7	43.7	43.8
14	55.7[c]	55.8[c]	55.8[c]	55.6[c]	55.6[c]	55.8[c]
15	24.9	24.9	25.0	24.9	24.9	25.0
16	27.5	27.5	27.5	27.4	27.5	27.5
17	55.6[c]	55.6[c]	55.6[c]	55.6[c]	55.6[c]	55.6[c]
18	13.1	13.1	13.1	13.1	13.1	13.1
19	13.8	13.6	13.6	13.8	13.7	13.9
20	140.1	140.1	140.1	140.1	140.1	140.1
21	114.7	114.8	114.8	114.7	114.7	114.7

carbon	1	2	3	5	6
1'	104.0	103.5	103.9	107.2	106.8
2'	70.5[b]	71.1	68.0	76.5[b]	75.2
3'	70.7[b]	68.5	74.7	78.7	76.5[b]
4'	71.2	73.3	68.0	71.0	72.8
5'	64.9	62.2	65.0	67.5	63.4

a) All compounds were measured in d_5-pyridine (at 22°C) at 22.5 MHz and the assignments were made by off-resonance experiments and INEPT methods and by comparisons with data reported for 3β,4α-dihydroxy-5α-cholestane,[14] methyl β-D-arabinopyranoside,[15] and methyl β-D-xylopyranoside.[15]

b,c) The assignments for these signals within the same vertical column may be interchanged.

The structures of three monoacetylated glycosides: 4'-O-acetyl-pregnedioside-a (2), mp 199°C, $C_{28}H_{44}O_7$, $[\alpha]_D$ -96° ($CHCl_3$), 3'-O-acetyl-pregnedioside-a (3), mp 126°C, $C_{28}H_{44}O_7$, $[\alpha]_D$ -148° ($CHCl_3$), and 4'-O-acetyl-pregnedioside-b (6), mp 193°C, $C_{28}H_{44}O_7$, $[\alpha]_D$ -2.2° ($CHCl_3$), were determined on the bases of alkaline hydrolysis (giving respective free glycoside quantitatively) and IR, ^1H NMR, and ^{13}C NMR analyses (*e.g.* refering to esterification shift[9]).

Some pregn-20-ene-type steroids have been reported from several soft corals such as *Gersemia rubiformis*,[10] *Telesto riisei*,[11] and an unidentified sp. (collected at Canton Island),[12] and a gorgonian *Eunicella carolini*.[13] However, those steroids have never been isolated as their glyco-conjugates. The physiological function of the present steroidal glycosides may be of interest.

Acknowledgement - One of the authors (M.K.) is grateful to the Foundation for the Promotion of Research on Medicinal Resources for financial support.

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2) The species is most likely *Alcyonium okinawanum* Utinomi, which is yet under study. The authors are grateful to Mr. T. Yoshino, Ryukyu University, for the identification.
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(Received in Japan 7 May 1984)