Tetrahedron Letters,Vol.28,No.20,pp 2291-2294,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

STARFISH SAPONINS PART 32. STRUCTURE OF A NOVEL STEROIDAL 5-O-METHYL GALACTOFURANOSIDE FROM THE STARFISH ASTROPECTEN INDICUS.

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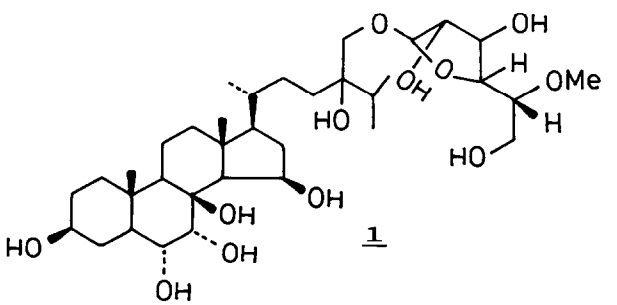
Summary: Extended spectroscopic analysis has led to the derivation of structure for a novel steroidal glycoside isolated from the starfish Astropecten indicus; this compound, named indicoside A, contains a 5-O-methyl-β-galactofuranosyl moiety linked to C-28 of 24-methyl-5α-cholestane-3β,6α,7α,8,15β,24-,28-heptol aglycone (1).

Steroidal glycosides, composed of polyhydroxysteroidal aglycone and a carbohydrate portion made up from only one or two monosaccharide units, are a growing subgroup of active compounds among the glycosides isolated from starfishes[1,2]. The more common monosaccharides are xylopyranose, often 2-O-methylated, and arabinofuranose.

In our continuing research on active metabolites from starfish we have isolated from Astropecten indicus a novel steroidal galactofuranoside, assigned as indicoside A, and now describe the structure elucidation.

The compound was purified from the aqueous extracts of the fresh animals, collected from the Clifton coast in Karachi, by the following successive chromatographic steps: recovery of the polar material on a column of Amberlite XAD-2, chromatography of methanol eluates on column of Sephadex LH-60 to separate the penta- and hexa-glycosides "asterosaponins" from the monoglycosides, DCCC of the monoglycosides fractions ($CHCl_3$/MeOH/H_2O, 7:13:8, ascending mode) followed by HPLC (C_{18} μ-Bondapak, 65% aq. MeOH). The compound thus isolated (12 mg from 2.2 kg fresh animals), glassy material, $[\alpha]_D$ -69.4° (c = 1, MeOH), shows in FAB-MS (negative mode) an intense ion at m/z 673 $(M-H)^-$. Next to the quasi-molecular ion the spectrum displayed a fragment with m/z 497, which corresponds to the loss of a methylated hexose unit. The ^{13}C nmr spectrum, measured in CD_3OD at 62.9 MHz, shows the presence of 35 carbon atoms, chemical shifts and multiplicities for the aglycone and sugar moieties are listed in Tables 1 and 2, respectively.



1

Table 1. The ^{13}C nmr data[a] for aglycone moiety.

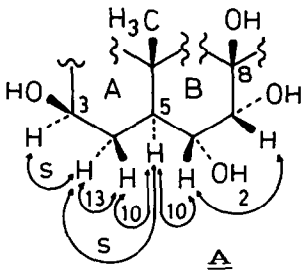
Carbon No.	1	Carbon No.	1
1	39.4 CH_2	15	71.2 CH
2	31.5 CH_2	16	42.7 CH_2
3	72.3 CH	17	58.0 CH
4	32.1 CH_2	18	16.5 CH_3
5	44.4 CH	19	13.9 CH_3
6	69.5 CH	20	36.8 CH
7	76.5 CH	21	19.0 CH_3
8	79.5 C	22	30.0 CH_2
9	50.2 CH	23	31.9 CH_2
10	37.5 C	24	76.5 C
11	19.5 CH_2	25	34.5 CH
12	43.1 CH_2	26	17.2 CH_3
13	44.3 C	27	17.4 CH_3
14	56.6 CH	28	72.5 CH_2

a. δ Values relative to CD_3OD = 49.00 (central peak).
Multiplicities determined by DEPT experiments using polarization transfer pulses of 90°, CH only, and 135°, positive signals for CH and CH_3 and negative ones for CH_2.

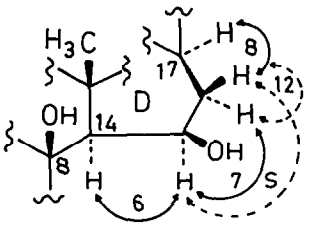
Table 2. The ^{13}C nmr data for sugar moiety.

Carbon No.	1[a]	Ref. Compound[b]
1'	109.8 CH	109.0
2'	82.9 CH	81.8
3'	79.0 CH	77.8
4'	84.7 CH	83.4
5'	83.4 CH	81.8
6'	62.4 CH_2	60.9
OCH_3	59.5 CH_3	

a. δ-Values relative to CD_3OD = 49.00 (central peak). b. Methyl 5-O-methyl-ß-D- galacto-furanoside from D_2O solution[3]. Differences in the two spectra can be due to the different solvents used. We have always observed downfield shifts of ca. 1.0 - 1.5 ppm of the sugar signals on passing from D_2O to CD_3OD.



A



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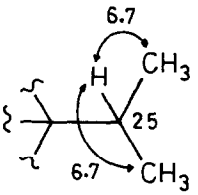

C

H-3: 3.55 m
H-4α: 2.12 dt
H-4β: 1.18 m
H-5: 1.58 m
H-6: 3.85 dd
H-7: 3.90 d

H-14: 1.43 d
H-15: 4.56 dt
H-16β: 2.43 sextet
H-17: 1.08 m

H-25. 1.88 heptet
CH_3-26,27: 0.95 d

Fig. 1. Partial structures of 1. Full arrows indicate the results of double resonance experiments at 500 MHz in both ways (J-values given in Hz). (s) = small (less than 4 Hz). Arrows with broken lines are used where the indicated connectives and J-values have not corroborated by direct irradiation experiments.

H-1': 5.05 br s
H-2': 5.10 br d (2)
H-3': 5.19 dd (6,2)
H-4': 4.11 dd (6,3)
H-5': 3.70 dt (3, 5.5)
H_2-6': 4.28 d (5.5)
OCH_3: 3.58 s

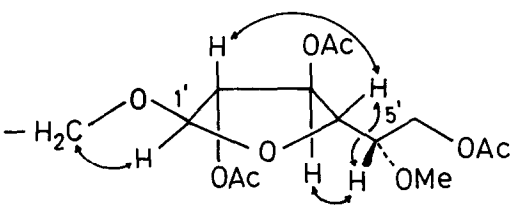

Fig. 2. ^{1}H nmr daca of 1 3,6,2',3',6'-pentaacetate. Assignments based on sequential decoupling (J-values in Hz). Arrows indicate the results of NOEDS experiments.

The ^{1}H nmr spectra were recorded in CD_3OD at 250 and 500 MHz and the relevant data, including the out-come of double resonance and NOEDS experiments, are summarized in Figures 1 and 2.

The ^{13}C nmr spectrum of the aglycone moiety reveals the presence of seven (four CH, two C and one CH_2) oxygen carrying carbon atoms; thus the molecular formula $C_{28}H_{50}O_7$, which corresponds to a C_{28} saturated steroid with seven hydroxyl groups, is established for the aglycone moiety. In agreement with a steroid structure the ^{1}H nmr shows the presence of two tertiary, δ 1.31 (CH_3-18) and 1.01 (CH_3-19), and three secondary methyls, δ 0.95 (6H, J= 6.7 Hz; CH_3-26,27) and 0.98 (3H, J= 5.5, CH_3-21) and also the structural moieties A and B (Fig. 1), which seem obviously connected through a quaternary carbon, very probably C-8 bearing a tert-OH. NOEDS on 1 induced intense enhancement of the proton at C-7 upon irradiation of the C-15 signal and enabled us to connect the moieties A and B. The presence of a tertiary OH at C-8 is corroborated by the expected high field shielding of C-11 (19.5 ppm; in 8β-H steroids around 21 ppm[4]) and the low field shielding of CH_3-18 in both ^{13}C nmr (16.5 ppm) and ^{1}H nmr (1.31 ppm) spectra. Thus the pentahydroxy tetracyclic moiety of the steroid aglycone is clarified, leaving

the primary and one tertiary hydroxyl group for the side chain. The $-CH_2-O$ group that appears in ^1H nmr as an isolated AB system at δ 3.69 d (J= 12 Hz) and 3.36 (J= 12 Hz) must be located on a quaternary carbon; further, in a double resonance experiment the 1H heptet at δ 1.88, assigned to 25-H, was simplified to a singlet upon irradiation of the CH_3-26,27 doublet at δ 0.95. Thus both the $-CH_2-O$ and tert-OH are located at C-24. Treatment with excess acetic anhydride in pyridine at room temperature produced a pentaacetate[5] showing in the ^1H nmr the $-CH_2-O-$ proton signals virtually unshifted relative to 1 at δ 3.63 and 3.34 ppm. This clarified that the sugar is attached at C-28. The stereochemistry of the steroid is based on J values (Fig. 1), chemical shifts of the tertiary methyl protons, NOEDS (NOE positive CH3-19/H-6 and H-7/H-15), ^{13}C nmr and comparison with similar polyhydroxylated steroids[2,6]. The stereochemistry at C-24 await to be disclosed.

The ^{13}C (Table 2)- and the ^1H-resonances for the sugar residue were strongly suggestive for a methylated hexofuranose structure. The ^{13}C-shifts match those of methyl-5-O-methyl-ß-galactofuranoside (Table 2). In the ^1H nmr spectrum of 1 [δ 4.85 (1H, d, 1.5, 1'-H), 4.04-3.95 (3H, m, 2'-, 3'- and 4'-H), 3.74 (2H, d, 6, 6'-H_2), 3.57 (3H, s, OCH_3) and 3.44 (1H, q, 6, 5'-H)] three sugar protons overlap, but, when we measured the spectrum of the derived pentaacetate, the sugar protons gave rise to six resolved bands (Fig. 2), that were correlated by sequential decoupling, thus also establishing the location of the methoxyl group at C-5 (H-5' virtually unshifted relative to 1). The relative stereochemistry of the substituents in the furanose ring was then confirmed by NOEDS experiments (Fig. 2). Thus the structure for indicoside A is suggested as 1.

Acknowledgement. We thank the Italian Ministry of Education (Ministero della Pubblica Istruzione) for financial support. We are most grateful to Professor D.Arigoni, ETH Zurich, for spectra of galactofuranoside reference compounds.

References and Footnotes

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5) characterized as 3,6,2',3',6'-pentaacetate from ^1H nmr data: δ in $CDCl_3$ 5.36 (1H, dd, J= 12.5, 2.5 Hz, 6-H), 4.68 (1H, m, 3-H), 4.54 (1H, br m, 15-H), 4.00 (1H, t, J= 1.5 Hz, 7-H), 3.63 and 3.34 (each 1H, d, J= 10 Hz, 28-H_2), 3.73 and 1.90 (both 1H, s, tert-OH), 2.45 (1H, d, J= 2.5 Hz, 15-OH), 1.95 (1H, d, J= 1.5 Hz, 7-OH), 2.13, 2.12, 2.11, 2.10, 2.04 (each 3H, s, $OCOCH_3$), 1.28 (3H, s, CH_3-18), 1.10 (3H, s, CH_3-19), 0.93 (9H, d, J= 6.5 Hz, CH_3-21,-26 and -27), the sugar proton signals in Fig. 2.

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(Received in UK 17 March 1987)