

A CHEMICAL INVESTIGATION OF THE TREPANG (*Stylochopus Japonicus* Selenka):  
THE STRUCTURE OF TRITERPENOID AGLYCONES OBTAINED FROM TREPANG GLYCOSIDES.

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We have previously (1) demonstrated that the methanol extract of the Far-Eastern sea cucumber — the trepang — contains 2 glycosides called stichoposides A and C.

The present work cites data on the structure of two substances, namely stichopogenins  $A_2$  and  $A_4$ , obtained at hydrolyzing stichoposide A with a 12% hydrochloric acid aqueous solution. Stichopogenins  $A_2$  and  $A_4$  are the main hydrolysis products, the chromatographically uniform stichopogenin  $A_4$  being capable, under these conditions, of partially converting into stichopogenin  $A_2$ .

Stichopogenins  $A_2$  and  $A_4$  were separated by partition chromatography on a silica gel in the benzene — benzene : ethyl acetate (2:1) gradient elution system.

Stichopogenin  $A_2$ , Ia,  $C_{30}H_{44}O_4$ , m.p. 238-240° (from MeOH),  $[\alpha]_D^{25}$  - 48° (C 2.5,  $CHCl_3$ ), IR spectra: 1755-1760  $cm^{-1}$  ( $\gamma$ -lactone). Found: C 76.71 H 9.57; mol.wt. 468 (mass spectrometry). Calcd. for  $C_{30}H_{44}O_4$ : C 76.88 H 9.46; mol.wt. 468.

Stichopogenin  $A_2$  monoacetate, Ic,  $C_{32}H_{46}O_5$ , m.p. 216-219° (from abs. MeOH),  $[\alpha]_D^{25}$  - 36.3 (C 3.3,  $CHCl_3$ ), IR spectra in  $CHCl_3$ : 3600  $cm^{-1}$  (OH group), 1255  $cm^{-1}$ , 1720  $cm^{-1}$  ( $\overset{O}{\parallel}C-O$ ). Found: C 75.11 H 9.38, mol.wt. 510 (mass spectrometry). Calcd. for  $C_{32}H_{46}O_5$ : C 75.26 H 9.08, mol.wt. 510.

Stichopogenin  $A_4$ , IIa,  $C_{30}H_{46}O_5$ , m.p. 238-240° (from aqueous  $Me_2O$ ), IR spectra in  $CHCl_3$ : 1755  $cm^{-1}$  ( $\gamma$ -lactone). Found: C 72.65 H 9.49, ( $M^+$ -18) — 468 (mass spectrometry). Calcd. for  $C_{30}H_{46}O_5 \cdot 1/2H_2O$ : C 72.72 H 9.49, mol.wt. 486.

Stichopogenin A<sub>4</sub> monoacetate, IIb, C<sub>32</sub>H<sub>48</sub>O<sub>6</sub>, m.p. 221-223° (abs. EtOH), IR spectra in CHCl<sub>3</sub>: 1755 cm<sup>-1</sup> (γ-lactone), 1265 cm<sup>-1</sup>, 1720 cm<sup>-1</sup> (-C(=O)-). Found: C 72.76 H 9.10, mol.wt. 528 (mass spectrometry). Calcd. for C<sub>32</sub>H<sub>48</sub>O<sub>6</sub>: C 72.72 H 9.09, mol.wt. 528.

Stichopogenin A<sub>4</sub> diacetate, IIc, C<sub>34</sub>H<sub>50</sub>O<sub>7</sub>, m.p. 212-216° (from MeOH), IR spectra in CHCl<sub>3</sub>: 1755 cm<sup>-1</sup> (γ-lactone), 1265 cm<sup>-1</sup>, 1725 cm<sup>-1</sup> (-C(=O)-). Found: C 71.92 H 9.13, mol.wt. 570 (mass spectrometry). Calcd. for C<sub>34</sub>H<sub>50</sub>O<sub>7</sub>: C 71.57 H 8.84, mol.wt. 570.

The analytical data for stichopogenins A<sub>2</sub> and A<sub>4</sub> and their acetates, as well as the fact that the NMR spectra of the said compounds contain only 7 methyl signals, four of which are bonded to quaternary carbon atoms (Table 1), are indicative of the tetracyclic triterpenoid nature of the stichopogenins.

The eighth methyl group, indispensable for this class of compounds, is oxidized in the stichopogenins into lactone; this is, likewise, observed in the aglycones of sea cucumbers *Actinopyga agassizi* (2) and *Halodeima Grisea* (3). The presence and position of the γ-lactone is corroborated by NMR spectra analysis in stichopogenins A<sub>2</sub> and A<sub>4</sub> and their acetates: the proton signal for methyl group 21 is shifted to the weak field (δ = 1.38-1.45 p.p.m.) which may be accounted for only by the neighbouring ester group (2,3).

The fact that under normal acetylating conditions stichopogenin A<sub>2</sub> forms monoacetate Ic, and on oxidizing in pyridine with chromic anhydride — mono-ketone Ib, m.p. 174-178°, mol.wt. 466 (mass spectrometry), shows the presence in Ia of one secondary hydroxyl group, situated (by analogy with all known triterpenoids) at the third carbon atom; the proton signals at C-3 in the NMR spectra of Ia (δ = 3.40 p.p.m.) and Ic (δ = 4.30 p.p.m.) are likewise indicative of this. The spin-spin interaction (I<sub>aa</sub> + I<sub>ae</sub> = 10 c/s + 5 c/s = 15 c/s) constant magnitudes signify the equatorial configuration of this hydroxyl group. The presence of a small absorption band at 3600 cm<sup>-1</sup> in the IR spectra of Ic indicates the existence in Ia of a non-acetylated tertiary hydroxyl group. On comparing the NMR spectra of 22-, 25-oxydoholothurinogenin and its 17-desoxy analog, it is apparent (2) that the presence of an OH group at C-17 shifts the methyl group proton signal at C-14 to a weaker

field (from 1.00 p.p.m. in the 17-desoxy analog spectrum to 1.22 p.p.m. in the 22-, 25-oxydoholothurinogenin spectrum). Table 1 shows that the chemical shift value for  $\text{CH}_3$  groups at C-14 in the Ia and Ic NMR spectra coincides with the data for 22-, 25-oxydoholothurinogenin; hence, the hydroxyl group is located at C-17. The above reasoning is likewise valid for IIa.

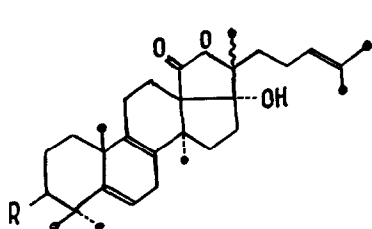
Table 1: NMR Spectra ( $\delta$  values)

| Ord. Nos. | Compound                             | 4 Me | 4 Me | 10 Me | 14 Me | 21 Me | 26 Me | 27 Me | Ac Me        | 3 H  | 7 H  | 11 H | 22 H | 24 H |
|-----------|--------------------------------------|------|------|-------|-------|-------|-------|-------|--------------|------|------|------|------|------|
| 1         | A <sub>2</sub>                       | 0.87 | 0.93 | 1.02  | 1.24  | 1.45  | 1.62  | 1.74  | -            | 3.40 | -    | 5.34 | -    | 4.70 |
| 2         | A <sub>2</sub> acetate               | 0.90 | 0.90 | 0.90  | 1.22  | 1.38  | 1.58  | 1.63  | 2.00         | 4.30 | -    | 5.15 | -    | 4.50 |
| 3         | A <sub>4</sub>                       | 0.87 | 0.93 | 1.02  | 1.25  | 1.45  | 1.25  | 1.25  | -            | 3.35 | -    | 5.35 | -    | -    |
| 4         | A <sub>4</sub> acetate               | 0.90 | 0.90 | 0.90  | 1.20  | 1.38  | 1.20  | 1.20  | 2.00         | 4.35 | -    | 5.15 | -    | -    |
| 5         | A <sub>4</sub> diacetate             | 0.90 | 0.90 | 0.90  | 1.20  | 1.38  | 1.40  | 1.40  | 1.90<br>2.00 | 4.35 | -    | 5.15 | -    | -    |
| 6         | 22-,25-oxydoholothurinogenin acetate | 0.91 | 0.89 | 1.15  | 1.22  | 1.38  | 1.26  | 1.28  | 2.08         | 4.55 | 5.57 | 5.25 | 4.23 | -    |
| 7         | 17-desoxyholothurinogenin acetate    | 0.90 | 0.96 | 1.13  | 1.00  | 1.35  | 1.22  | 1.26  | 2.08         | 4.56 | 5.59 | 5.27 | 4.08 | -    |

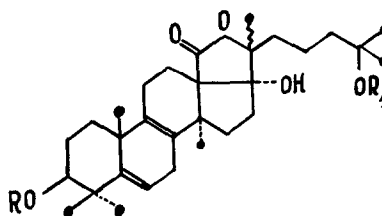
The chemical shift magnitude of the 26 and 27 methyl group protons in the NMR spectra for Ia and Ic is indicative of the presence of a  $\Delta$  24.25 double bond therein (4). In the NMR spectra for IIa, IIb and IIc there are no signals in the 1.6 p.p.m. range, where 26 and 27 methyl groups are absorbed with a  $\Delta$  24.25 double bond, and in the 4.5 p.p.m. range, where 24 H is absorbed with the said double bond. The signals of the above-mentioned methyl groups appear at 1.25 p.p.m. in the IIa spectrum, and at 1.20 p.p.m. in the IIb spectrum (see Table 1). This signifies the presence of a tertiary hydroxyl group at C-25 (5). The acetoxy group at C-25 shifts the signals of the 26 and 27 methyl group protons to the weaker field still more (6). The relatively low peak intensity of the side chain ( $m/e$  109), ascribed to the fragment  $\left[ \begin{array}{c} \text{---} \\ \text{---} \\ \text{---} \end{array} \right]$  in the mass spectra of stichopogenins A<sub>2</sub> and A<sub>4</sub> and their acetates is explicable by the presence of the C 18  $\rightarrow$  20 lactone

bridge which impedes the forming of a similar ion.

According to the analysis and mass spectra data for stichopogenins  $A_2$  and  $A_4$  and their acetates, each said substance skeleton contains 2 double bonds, whose sites are to be determined. The NMR spectra for stichopogenins  $A_2$  and  $A_4$  and their acetates, as well as the UV spectra for Ia and Ic, indicate the absence of a heteroannular diene (2) in these compounds; only one of their vinyl proton signals is observed in the NMR spectra (see Table 1). In this case, the 2 double bonds have only two sites:  $\Delta$  8.9 and  $\Delta$  5.6. All the above-cited data are in accordance with structures I and II for stichopogenins  $A_2$  and  $A_4$  and their respective derivatives:



- I    a    R = OH  
       b    R = O  
       c    R = OAc



- II    a    R = R<sub>1</sub> = H  
       b    R = Ac    R<sub>1</sub> = H  
       c    R = Ac = R<sub>1</sub>

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