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JEREISTEROL A AND B : TWO 3β-METHOXY-SECOSTEROIDS FROM THE PACIFIC SPONGE *JEREICOPSIS GRAPHIDIOPHORA*.

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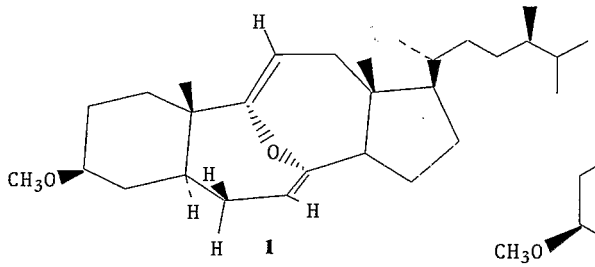
Summary: Two 3 β-methoxy secosteroids, named jereisterol A and B were isolated from the pacific sponge *Jereicopsis graphidiophora* Lévi & Lévi. Their structures, which combine rare 3 β-methoxy and seco features, were determined as (24 R) 24-methyl-3β-methoxy-8α,9α-oxido-8,9-secocholesta-7,9(11)-diene (1) and (24R) 24-methyl-3β-methoxy-8,14-secocholesta-8,14-dione (2).

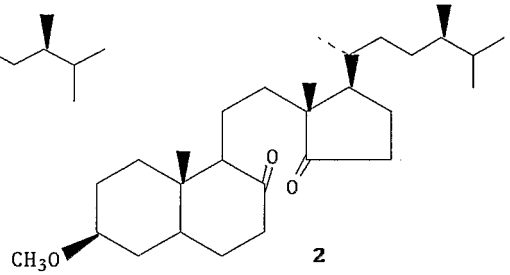
Although a large number of new sterol structures have been discovered from marine organisms during the last twenty years[1], very few secosteroids have been reported. After the discovery in 1972 of the unique 3β,11-dihydroxy-9,11-secogorgost-5-en-9-one from a gorgonian[2], three new 9,11-secosterols were isolated from the soft coral *Sinularia sp.*[3], a polhydroxylated 9,11-secosterol from the sponge *Disidea herbacea*[4] and a trihydroxylated 5,6-secosterol has been described from the sponge *Hippospongia communis*[5]. Very recently an unique group of norsterols, the incisterols, derived from biodegradation of the sterol nucleus with loss of the ring A including 19-methyl group, has been isolated from the sponge *Dictyonella incisa*[6], and the Authors have proposed these degraded sterols to originate biogenetically from a 5,6-secosterol through the further cleavage of the 9,10 bond.

During our on going program to isolate novel bioactive molecules from New Caledonian marine invertebrates, the opportunity occurred recently to examine the lipidic extracts of the sponge *Jereicopsis graphidiophora*, Lévi & Lévi[7]. In a related paper[8] we have reported the occurrence in *J. graphidiophora* of the first 3β-*O*-methylsterols, while the conventional 3β-hydroxysteroids were totally absent. Herein we describe the isolation and structure determination of two more 3β-methoxysteroids, jereisterol A (1) and B (2), which combine the 3β-methoxyl group with a rare seco-structure.

The sponge was ground, freeze-dried (0.7 K.) and extracted with *n*-hexane. Silica gel chromatography of the extract using increasing amounts of ethyl acetate in *n*-hexane gave a major fraction containing 3β-methoxysteroids together



1



2

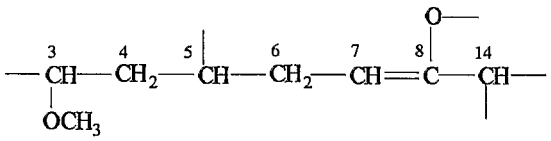
with some more polar fractions containing the oxygenated 3β-methoxysteroids.

Reverse-phase HPLC (Whatman Partisil ODS-2) of the fraction eluted with *n*-hexane/EtOAc 98:2 yielded, with MeOH/CH_2Cl_2 9:1, jereisterol A (**1**, 0.8 mg), while HPLC with MeOH/H_2O 95:5 of the fraction eluted with *n*-hexane/EtoAc 9:1 yielded jereisterol B(**2**, 5 mg).

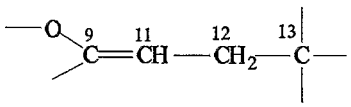
Jereisterol A (**1**) showed a molecular ion (EI) at *m/z* 428.3650 ($C_{29}H_{48}O_2$) and a major fragment at *m/z* 301.2167 ($C_{20}H_{29}O_2$) due to the loss of a C_9H_{19} fragment and suggesting a conventional saturated steroidal C_9-side chain. The ^{1}H-NMR spectrum also indicated a steroidal structure. The methyl signals at δ 0.53 (3H, s), 0.79 (3H, d, J=7.0), 0.81 (3H, d, J=7.0), 0.86 (3H, d, J=7.0), 0.98 (3H, d, J=6.5) and 1.09 (3H, s) could be assigned to carbons 18, 26, 27, 28, 21 and 19 respectively of a "ergostane" sketeton. The 1H multiplet at δ 3.22 ppm and the 3H singlet at δ 3.36 are assigned to a 3β-methoxyl group. Remaining relevant ^{1}H NMR data, are summarized in Figure 1.

Sequential decoupling experiments identified the structural moieties **A** (C-3 to C-14) and **B** (C-9 to C-13). Evidence to connect the structural part from C-3 to C-7 and C-14, through a quaternary carbon, was provided by a clearly detected homoallylic coupling between the H-6α signal at δ 2.12 ppm and the broad double doublet downfield shifted to δ 2.38 ppm (H-14).

The ^{13}C NMR spectrum[9], even if quite poor, contained signals for rings A and B and for the side chain, and revealed



A



B

-OCH_3	:	3.36 s		H-11	:	4.92 dd (J=8, 6 Hz)
H-3	:	3.22 m		H-12	:	2.31 dd (J=14, 8 Hz)
H-4α	:	1.78 brd	(J=12.5 Hz)			2.21 dd (J=14, 6 Hz)
H-4β	:	1.95 m[a]				
H-5	:	2.79 tt	(J=13.2, 3.7 Hz)			
H-6α	:	2.12 brdt	(J=18.4, 3.7 Hz)			
H-6β	:	1.95 m[a]				
H-7	:	4.53 t	(J=3.7 Hz)			
H-14	:	2.38 brdd	(J=11.8, 5.9 Hz)			

Fig.1 ^{1}H NMR data ($CDCl_3$, 500 MHz) of jereisterol A (**1**)
[a] overlapping signals

two sp^2 CH signals at relatively high field (δ 106.8 and 107.2 ppm), which, along with the ^1H NMR high field shifted olefinic signals at δ 4.92 and 4.53 ppm, indicated the presence of enol ether structure.

Thus, the remaining oxygen atom indicated by MS was fixed between the olefin carbons C-8 and C-9 giving rise to the unique 24-methyl-3β-methoxy-8α,9α-oxido-8,9-secocholesta-7,9(11)-diene structure. The 8α,9α-oxido stereochemistry is suggested by the chemical shift of the proton of C-5 downfield shifted to δ 2.79 ppm implying the oxygen function and H-5 to be located on same face of the molecule. The 24R stereochemistry is assigned by comparison of ^1H and ^{13}C NMR spectra with known compounds[10].

Jereisterol B (2). The molecular formula $C_{29}H_{50}O_3$ was established by MS, *m/z* 446.3745 (M^+). The MS spectrum also displayed, aside the molecular ion peak, an intense ion at *m/z* 319.2268 ($C_{20}H_{31}O_3$, due to the loss of a C_9H_{19} side chain), thereby locating all three oxygen functions in the nucleus.

The ^1H NMR and ^{13}C NMR spectra[11] indicated a "3β-methoxy-24-methylcholestane" skeleton. Two IR carbonyl bands at 1727 and 1700 cm^{-1} and the ^{13}C NMR signals at 224.0 and 214.0 ppm indicated the presence of two ketone groups in **2**, which must be incorporated into a steroidal tricyclic nucleus to account for the molecular formula $C_{29}H_{50}O_3$, requiring five unsaturations. All the data can readily be explained if the two ketone groups are at C-8 and C-14 of a 8,14-secosteroid. The structural deduction was confirmed by synthesis of the model 3β-acetoxy-8,14-seco-ergostane-8,14-dione. The synthesis was accomplished by oxidation with ruthenium tetroxide of 3β-acetoxy-ergost-8(14)-ene[12] by using the procedure described by Sharpless *et al.*[13] to yield the desired 8,14 seco-compound[14] along with major amounts of the product of allylic oxidation, 3β-acetoxy-ergost-8(14)-en-15-one, identified by comparison with published spectral data for the 3β-hydroxycholesta-8(14)-en-15-one[15].

Comparison of the ^1H NMR data of **2**[11] with those of the synthetic 3β-acetoxy-8,14-secoergostane-8,14-dione[14] showed identical chemical shift values for C-18, C-19 and C-21 methyl resonances; the small differences observed in the pattern of the C-26, C-27 and C-28 methyl resonances gives strong support to the assignement of the 24R-configuration to **2** (the synthetic model has the 24S-configuration). Comparison of the ^{13}C NMR data of **2** with those of the model equally supports the 8,14-secosteroid-8,14-dione structure and the 24R-configuration for the natural compound.

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7) The sponge was collected in course of the Campaign MUSORSTOM IV of the ORSTOM-CNRS Programme "Substances Marine d'Interet Biologiques" (SMIB) in the North of New Caledonia at a depth of 225 m. A sample is kept at the ORSTOM Centre de Nouméa under the reference R 1369. The sponge has been identified by C.Lévi as a new genus; C.Lévi and P.Lévi, *Bull. Mus. Natn. Hist. Nat.* Paris IV series, 5, 101 **1983.**

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9) ^{13}C nmr data ($CDCl_3$) of jereisterol A (**1**); C-1: 36.0, C-2: 27.0, C-3: 79.5, C-4: 33.0, C-5: 37.8, C-6: 34.8, C-7:106.8*, C-11: 107.2*, C-12: 38.3, C-13: 44.5, C-14: 59.5, C-15: 22.2, C-16: 27.9, C-17: 53.6, C-18: 11.9, C-19:17.5, C-20: 36.0, C-21: 19.7, C-22: 33.8, C-23: 31.0, C-24: 39.1, C-25: 32.5, C-26 and C-27: 18.3 and 20.2, C-28: 15.5 OCH3; 55.6 ppm. Signals for quaternary C-8, C-9 and C-10 confused in the noise.

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11) Spectral data of jereisterol B (**2**): IR ($CHCl_3$): 2950, 2936, 2870, 1727, 1700, 1460, 1371, 1213, 1029 cm^{-1}; ^1H-nmr ($CDCl_3$): 0.61 (3H, s, CH_3-18), 0.81, 0.82, 0.87 (each 3H, d, J= 6.5 Hz, 7.0 and 7.0 Hz, CH_3-26, 27 and 28). 0.86 (3H, s, CH3-19), 1.08 (3H, d, J= 6.5 Hz, CH_3-21), 2.36 (1H, m, CH), 3.20 (1H, m, H-3), 3.36 (3H, s, OCH_3); ^{13}C nmr ($CDCl_3$); C-1: 36.6, C-2: 27.2, C-3: 79.5, C-4: 32.4, C-5: 43.8, C-6: 30.1, C-7: 42.1, C-8: 211.0, C-9: 63.4, C-10: 41.6, C-11: 17.7, C-12: 37.6, C-13: 52.6, C-14: 224.0, C-15: 37.9, C-16: 23.6, C-17: 47.1, C-18: 18.4, C-19:12.4, C-20: 34.5, C-21: 18.5, C-22: 34.0, C-23: 31.0, C-24: 39.1, C-25: 32.5, C-26 : 20.4, C-27: 18.4, C-28: 15.5, OCH_3: 55.7 ppm .

12) prepared from ergosterol acetate by hydrogenation at 3 atm for 20 h at room temperature in the presence of platinum oxide according to W.H.Lee, B.N.Lutsky and G.J.Shroepfer, *J. Biol. Chem.* **244**, 5440 (1969).

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14) Spectral data of the synthetic 3β-acetoxy-8,14-secoergostane-8,14-dione:
^1H-nmr ($CDCl_3$): 0.63 (3H, s, CH_3-18), 0.81 (6H, d, J= 6.7 Hz), 0.87 (3H, d, J= 7.0 Hz, CH_3-26, 27 and 28), 0.86 (3H, s, CH_3-19), 1.08 (3H, d, J= 6.5 Hz, CH_3-21), 2.03 (3H, s, $\underline{CH_3}CO$), 2.35 (1H, m, CH), 4.75 (1H, m, 3-H); ^{13}C nmr ($CDCl_3$); C-1: 36.5, C-2: 26.9, C-3: 73.0, C-4: 32.4, C-5: 43.6, C-6: 30.1, C-7: 41.9, C-8: 210.7, C-9: 63.0, C-10: 41.6, C-11: 17.6, C-12: 37.6, C-13: 52.5, C-14: 223.9, C-15: 37.8, C-16: 23.5, C-17: 47.1, C-18: 18.4, C-19:12.4, C-20: 34.8, C-21: 18.6, C-22: 33.5, C-23: 31.3, C-24: 39.3, C-25: 31.7, C-26: 20.4, C-27: 17.8, C-28: 15.5, CH_3-CO: 21.2 and 170.1 ppm .

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