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Eurypongiols: Ten New Highly Hydroxylated 9,11-Secosteroids with Antihistaminic Activity from the Sponge *Eurypongia* sp. Stereochemistry and Reduction.

[redacted]

Trans decalin 9,11-secosteroids have been found in the sponges *Aplysilla glacialis*[5] (glaciasterols A and B) and *Spongia officinalis*[6] both *trans* and Δ^5 decalin 9,11-secosteroids in the soft coral *Sclerophytum* sp;[7] and Δ^5-secosteroids in the gorgonian *Pseudopterogorgia americana*[3] and the soft coral *Sinularia* sp.[8,9] To date, the sole *cis* decalin system reported is herbasterol, found by Faulkner *et al.* from *Dysidea herbacea* .[10]

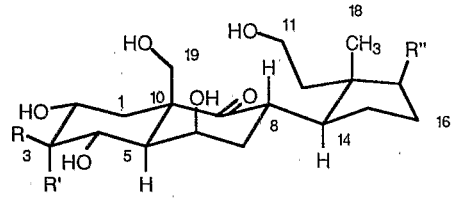
According to the degree and pattern of hydroxylation, the simplest compounds of this class are the 3,11-dihydroxy and the 3,6,11-trihydroxy-9,11-secosteroids.[3,6,7] Glaciasterols[5] have four oxygenated sites (3,5,6 and 11), with hydroxyl groups at C-3 and C-11 and an epoxide bridge at C-5, C-6. Herbasterol[10] which is pentahydroxylated at 2,3,6,11 and 19, has the highest degree of oxygenation hitherto reported in the secosteroid literature.

RESULTS AND DISCUSSION

In the course of our investigations on marine invertebrates we have now isolated ten hexahydroxylated 9,11-secosterols from the polar extracts of a new species of a marine sponge *Eurypongia* sp. (Porifera, Demospongiae, family Dysideidae), which was collected on the coast of New Caledonia and selected for study because of the cytotoxicity and strong antihistaminic activity of its extracts.

The structure of the new compounds which we have named eurypongiols (**1-10**, Fig. 1), were elucidated by spectral analysis and chemical transformations.

The freshly collected sponge (556.30 g) was extracted, defatted and chromatographed to afford a fraction containing the secosteroids, which were separated by reversed phase HPLC to yield compounds **1** (30 mg), **2** (32 mg), **3** (1.5 mg), **4** (1 mg), **5** (7 mg), **6** (17 mg), **7** (5 mg), **8** (1.5 mg), **9** (1.5 mg) and **10** (6 mg).



1 : R = OH, R' = H, R" = a
2 : R = OH, R' = H, R" = b
3 : R = OH, R' = H, R" = c
4 : R = OH, R' = H, R" = d
5 : R = OH, R' = H, R" = e
6 : R = H, R' = OH, R" = a
7 : R = H, R' = OH, R" = b
8 : R = H, R' = OH, R" = c
9 : R = H, R' = OH, R" = d
10: R = H, R' = OH, R" = e

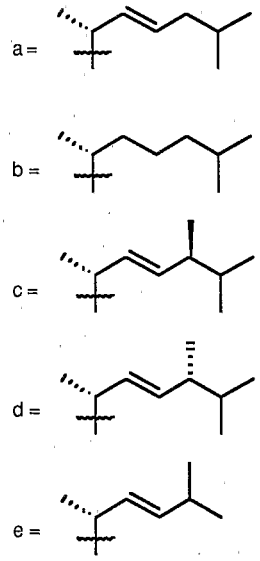


Figure 1. Structures of the 9,11-secosterols from *Eurypongia* sp.

These secosteroids can be classified in two series, eurypongiols A (compounds **1-5**) and eurypongiols B (compounds **6-10**), according to their stereochemistry at C-3. For the sake of simplicity, their structural

features will be presented in accordance with this classification.

Euryspongiol A1 (1). The molecular formula of the major component **1** was determined by high resolution EIMS as $C_{27}H_{46}O_7$ (5 unsaturations). EIMS showed main fragments at *m/z* 452 corresponding to $C_{26}H_{44}O_6$ [M-CH_2O]$^+$; 434 [M-CH_2O-H_2O]$^+$; 416 [M-CH_2O-2H_2O]$^+$ and 398 [M-CH_2O-3H_2O]$^+$. The presence of an unsaturated C_8H_{15} steroidal side chain was indicated by an ion at *m/z* 341 [M-CH_2O-C_8H_{15}]$^+$. Some of the most relevant fragments are shown in Figure 2.

Negative ion mode FAB MS produced a prominent fragment at *m/z* 451 [M-H-CH_2O]$^-$, while positive ion mode FAB MS in a glycerol matrix showed the expected [M+H]$^+$ ion at *m/z* 483 and also the loss of a molecule of water at *m/z* 465 [M+H-H_2O]$^+$, loss of five molecules of water at *m/z* 393 [M+H-5H_2O]$^+$, and loss of the side chain at *m/z* 354 [M+H-H_2O-C_8H_{15}]$^+$.

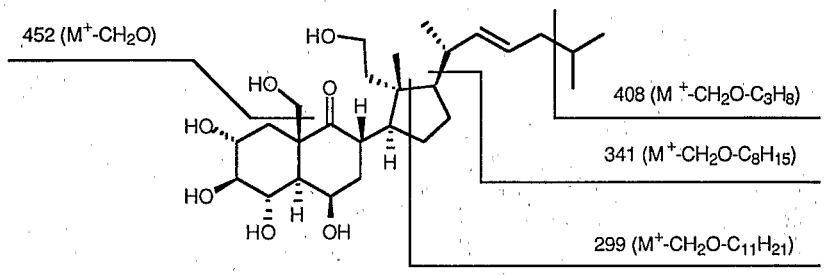


Figure 2. Fragments observed in EIMS of compound **1**.

The ^{13}C NMR spectrum of **1** contained signals for all twenty seven carbon atoms. A ketone signal at δ 215.4 (C-9) and a pair of sp^2 carbons at δ 138.4 and δ 128.4 (C-22/23) indicated the presence of a carbonyl group and a disubstituted double bond, so only three rings (as against four in a normal steroid) are present to justify the unsaturation number, suggesting a secosteroid structure. Six resonances between δ 82.8 and 59.2 supported the existence of six sites with a heteroatom (oxygen) substituent. On the basis of DEPT experiments, two of these signals (δ 63.9 and 59.2) were assigned to hydroxymethylene carbons and the other four (δ 82.8, 71.1, 70.5 and 64.7) to hydroxymethines (see Table 1).

Due to the polyhydroxylated structure of **1**, pyridine-induced deshielding[11] was prominent and the ^1H NMR pattern varied greatly as compared with the spectrum taken in CD_3OD (see Table 2). In pyridine-d_5, compound **1** showed signals for four of the five methyl groups of a C-27 sterol at δ 0.84 (6H, d, H-26/27), 0.91 (3H, s, H-18), 1.03 (3H, d, H-21).

The singlet that one would expect for the C-19 methyl protons is absent from the spectrum of **1**, and instead doublets were observed at δ 5.73 and 4.40 ppm (1H each, J = 11.7 Hz) corresponding to an isolated C-19 hydroxymethylene group. A second hydroxymethylene group gave rise to signals at δ 4.20 (1H) and 4.10 (1H) that agree well with the C-11 protons of a 9,11-secosterol.

In spite of the information provided by the pyridine-induced effects, the overlapping of signals was too extensive for complete analysis of the relevant spin systems and identification of signals by 2D correlations. However, a combination of the NMR data (COSY and HETCOR) for **1** and various derivatives allowed complete signal assignment and stereochemical analysis, as follows.

The mutually coupled signals observed in the COSY spectrum at δ 4.48 (1H, dd, J = 8.7, 4.7 Hz), 3.95

(1H, t, J = 8.7 Hz) and 4.58 (1H, dd, J = 11.2, 8.7 Hz) were taken to correspond to the three axial hydroxymethine protons of a 2α,3β,4α-trihydroxysterol.

Table 1. ^{13}C NMR Spectral data for the secosterols **1**, **1a** and **7**

Carbon No.	**1** δ, DEPT (CD_3OD)	**1a** δ, DEPT (CD_3OD)	**7** δ, DEPT (CD_3OD)
1	35.5, t	37.0, t	30.6, t
2	71.1, d	70.8, d	68.4, d
3	82.8, d	83.4[b], d	74.3, d
4	70.5, d	70.6, d	68.2, d
5	56.3, d		50.2, d
6	64.7, d	64.8, d	65.1, d
7	41.3, t	41.3[a], t	41.4, t
8	39.7, d	39.7, d	39.9, d
9	215.4, s	83.2[b], d	215.0, s
10	56.2, s		56.1, s
11	59.2, t	59.1, t	59.2, t
12	41.3, t		41.2, t
13	46.6, s	47.0, s	46.7, s
14	43.1, d		42.9, d
15	23.9, t	22.7, t	23.8, t
16	26.7, t	27.3, t	26.8, t
17	51.0, d		50.8, d
18	18.1, q	18.6, q	17.8, q
19	63.9, t	63.6, t	63.5, t
20	39.7, d	40.0[a], d	35.6, d
21	22.3, q	22.7, q	19.8, q
22	138.4, d	138.6, d	36.7, t
23	128.4, d	128.3, d	25.6, t
24	43.2, t		40.6, t
25	29.7, d	29.7, d	29.1, d
26	22.7, q	22.2, q	22.9, q
27	22.7, q	22.2, q	22.9, q

[a,b] Assignments may be interchanged

The HETCOR spectrum showed correlation of the peak at δ 82.8 attributed to C-3 with the triplet at 3.95 (H-3) and correlation of the signals at δ 71.1 and 70.5 with the multiplets at δ 4.48 and 4.58 assigned to H-2 and H-4. The remaining signal at δ 5.05 (1H) was then attributed to an equatorial hydroxymethine proton α H-6 by analysis of the C-4/C-5/C-6 spin system (*vide infra*).

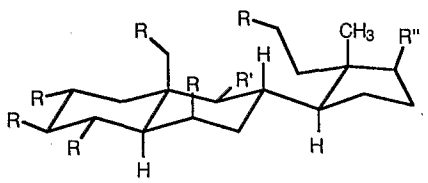
Table 2. ^{1}H NMR Spectral data for the secosterol **1**, **1a** and **7**

Proton No.	**1** δ, mult, J(Hz) (C_5D_5N)	**1** δ, mult, J(Hz) (CD_3OD)	**1a** δ, mult, J(Hz) (CD_3OD)	**7** δ, mult, J(Hz) (CD_3OD)
1α	2.20	1.45	0.99 t, 12.7	1.64
1β	3.12 dd, 13.3, 4.7	2.34 dd, 13.7, 4.8	2.65 dd, 12.7, 4.6	2.00 dd, 13.2, 4.15
2	4.48 dd, 8.7, 4.7,	3.69	3.66	3.82
3	3.95 t, 8.7	3.10 t, 8.9	3.14 t, 8.9	3.93 dd, 2.9, 2.4
4	4.58 dd, 11.2, 8.7	3.64	3.60	3.80 dd, 11.4, 2.4
5	2.05 dd, 11.2, 2.7	1.49 dd, 11.3, 2.3	1.20 dd, 11.3, 2.3	1.86 dd, 11.4, 2.4
6	5.05	4.32 dd, 2.4, 2.4	4.15 dd, 2.4, 2.4	4.28 dd, 2.4, 2.4
7α	2.45	2.32	1.92	2.28
7β	1.70	1.56	1.29	1.52
8	4.20	3.46 ddd, 13.6, 4.6, 4.1	1.65	3.47 ddd, 13.2, 4.6, 4.1
9			2.98 d, 10.8	
11	4.10	3.66	3.70	3.60
11'	4.20	3.66	3.70	3.60
12	2.00	1.64	1.78	1.67
14	3.00	2.49	2.32	2.47
15	1.55	1.37	1.48	1.37
16	1.48	1.36	1.44	1.37
17	1.70	1.60	1.59	1.53
18	0.91 s	0.81 s	0.82 s	0.77 s
19	4.40 d, 11.7	3.70	3.64 d, 12.1	3.70 d, 11.5
19'	5.73 d, 11.7	4.85	4.43 d, 12.1	4.78 d, 11.5
20	2.10	2.24	2.16	1.42
21	1.03 d, 6.7	1.07 d, 6.8	1.06 d, 6.8	1.00 d, 6.4
22	5.28	5.36	5.33	
23	5.28	5.36	5.33	
24	1.80	1.88 dd, 9.4, 3.3	1.86	1.10
25	1.50	1.53	1.61	1.54
26	0.84 d, 6.6	0.91[a] d, 6.6	0.87 d, 6.6	0.87 d, 6.7
27	0.84 d, 6.6	0.89[a] d, 6.6	0.87 d, 6.6	0.87 d, 6.7

[a] Assignments may be interchanged

The coupling constants corresponding to H-5 (δ 2.05, dd, J = 11.2, 2.7 Hz) are indicative of its *trans* diaxial relationship with H-4 and axial-equatorial relationship with H-6. The *trans* A/B ring fusion in **1** can be deduced by comparison of the C-13 chemical shifts, (especially that of the C-19 hydroxymethyl group) with the calculated values for *cis* and *trans* decalines. Addition of the standard -OH group α effect (+49 ppm) to the chemical shift of the methyl group in *trans* (15.7 ppm) and *cis* (28.2 ppm) 9-methyldecalin predicts shifts of

64.7 ppm and 77.2 ppm for the corresponding *trans* and *cis* 9-hydroxymethyl decalines respectively; thus the experimental value of 63.9 ppm for **1** clearly implies *trans* fusion.[12] Furthermore, direct experimental evidence of *trans* fusion came from a NOE experiment run on derivative **1a** (Fig. 3), which showed NOEs among H-5 (ax) and H-3 (ax) and H-9 (ax) and H-7 (ax) (Table 3).



1 a	R = OH	R' = OH	R" = a
1 b	R = OAc	R' = OAc	R" = a
2 a	R = OAc	R' = O	R" = b
2 b	R = OAc	R' = OH	R" = b
2 c	R = OAc	R' = OAc	R" = b

Figure 3

The signal due to H-8 is especially difficult to identify in this type of secosteroids, not only because of the complexity of the spectra and the rotation around the C-8/C-14 bond, but also because these compounds are not structurally stable under basic treatment,[11] which in consequence cannot be used to simplify the spectra by deuterium exchange. In C_5D_5N, and due to overlapping, the coupling constants of H-8 with H-7/7´ and H-14 could not be measured. In CD_3OD, they could be obtained (J = 13.6, 4.6, 4.1 Hz), and were assigned taking into account the *trans* diaxial relationship between H-8 and H-9 (10.8 Hz in **1a**). Thus H-8 lies in axial position, with coupling constants of 13.6 Hz with αH-7 (ax) and 4.6 and 4.1 Hz with βH-7 (eq) and H-14 respectively.

Comparison of the ^1H and ^{13}C NMR data for the side chain with those of the literature[13] suggested the presence of an E Δ^{22} double bond in **1**.

Taken together, the above evidence indicates that compound **1**, named eurypongiol A1, is 2α,3β,4α,6β,11,19-hexahydroxy-9,11-secocholest-22(E)-en-9-one.

Table 3. NOEs observed in compound **1a**

Irradiated proton	NOE				
H-3	H-5	H-1α	-----	-----	-----
H-9	H-14	H-5	H-1α	-----	-----
H-5	H-6	H-3	H-9	H-7α	H-1α
H-1α	H-3	H-9	H-1β	H-5	-----

while compound **5**, eurypongiol A5, had twenty six. Comparison of their ^{1}H and ^{13}C-NMR data with those of **1** and **2** indicate that all the secosteroids of the A series share the same skeleton, substitution pattern and regio; and stereochemistry, and differ only in their side chain structures.

Both **3** and **4** have the same molecular formula, $C_{28}H_{48}O_7$, showing the presence of a disubstituted double bond (δ 137.7, C-23; δ 135.2, C-22) and one more methyl group than in **1** and **2** (**3**, δ 0.94, d, J = 6.9 Hz, Me-28; **4**, δ 0.96, d, J = 6.9 Hz, Me-28). Both these features are located in their side chains, and make them (22E)-24-methyl- Δ^{22}-sterols.[14] Under (-)FAB MS, both compounds had the same $[M-H]^-$ ion (*m/z* 495) and the same fragmentation pattern, suggesting that these two isomers are epimers at C-24. Their EIMS showed ions at *m/z* 466 $[M-CH_2O]^+$ and *m/z* 341 $[M-CH_2O-C_9H_{17}]^+$, confirming the presence of the unsaturated C_9H_{17} side chains. Their stereochemistry at C-24 was deduced by comparison with known 24R and 24S

epimers of (22E)-24-methyl- Δ^{22}-sterols, using the proton chemical shift of the Me-21 protons as diagnostic signal (the 24R epimer signal lies downfield of the 24S signal.[14,15] Since Me-21 resonates at δ 1.06 (d, J = 6.9 Hz) in **3** at δ 1.07 (d, J = 6.8 Hz) in **4**, compound **3** is 2α,3β,4α,6β,11,19-hexahydroxy-9,11-secocholest-(22E,24S)-24-methyl-en-9-one and compound **4** 2α,3β,4α,6β,11,19-hexahydroxy-9,11-secocholest-(22E,24R)-24-methyl-en-9-one.

Secosterol **5**, eurypongiol A5, has the molecular formula $C_{26}H_{44}O_7$, corresponding to a norsecosteroid.

eurypongiol B3 is 2α,3α,4α,6β,11,19-hexahydroxy-9,11-secocholest-(22E,24S)-24-methyl-en-9-one. Compound **9**, eurypongiol B4 is 2α,3α,4α,6β,11,19-hexahydroxy-9,11-secocholest-(22E,24R)-24-methyl-en-9-one. Compound **10**, eurypongiol B5 is 2α,3α,4α,6β,11,19-hexahydroxy-9,11-seconorcholest-(22E)-en-9-one.

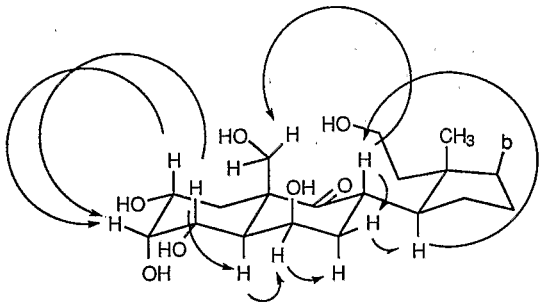


Figure 4. Selected NOEs for compound **7**.

Conformation and absolute stereochemistry. In addition to coupling constant values, ROESY experiments gave information on rotation about the C-8/C-14 bond and the presence of a conformational preference. The coupling constant between H-8 and H-14 about 4 Hz, (see Table 2) suggested a gauche-like arrangement of these hydrogens, and this was confirmed by the ROESY spectrum. Strong NOEs were observed between H-8 and H-14; H-8 and H-7(β, eq); H-14 and H-7(β, eq); and between Me-18 and H-7(β, eq). Thus, the preferred conformation is as depicted in Figure 5.

Molecular Mechanics calculations (MM2 Force Field) confirmed the existence of an energy minimum when the dihedral angle between H-8 and H-14 was 50°, in which conformation their calculated coupling constant was 4.55 Hz. MM2 calculations also confirmed the dihedral angles and general geometry of the A/B

Hydride reduction of the 9 keto group of 9,11-secosteroids. The reduction of the C-9 carbonyl group of **1** with $NaBH_4$/MeOH yielded exclusively the heptahydroxylated 9,11-secosteroid **1a** (δ

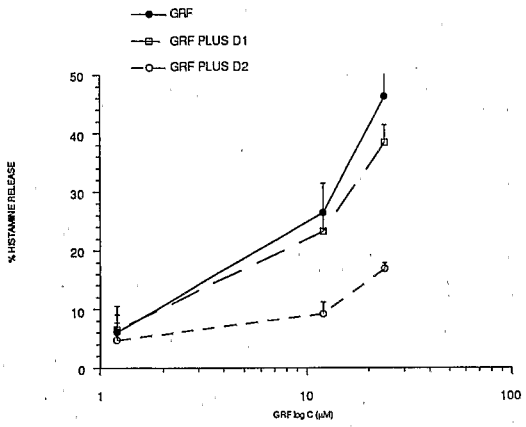


Figure 8

The similarity of action of **1** and **2** with that of disodium cromoglycate (DSCG),[19] a well known antihistaminic agent, suggest a common mode of action.

CONCLUSIONS

The genus *Euryspongia* is known to be an important source of furan and pyran-based terpenoids but this is the first report of secosteroids in that genus. Structurally, euryspogiols A (**1-5**) and euryspongiols B (**6-10**) constitute two series epimeric at C-3 and represent the most highly hydroxylated secosterols isolated so far and the first hydroxylated at C-4. The use of **1** as a model for the study of the stereoselective hydride reduction of the carbonyl group is noteworthy due to the high number of hydroxylic groups and their location to produce intramolecular assistence.

The antihistaminic activity of these compounds is interesting because it might be an indication of their potential use as antiallergic and in the treatment of asthma. Certain polyhydroxylated steroids have been recently

a JASCO DIP-370 digital polarimeter. Circular dichroism spectra were recorded on a Jobin-Ivon instrument in absolute MeOH. Column chromatography was performed on Amberlite XAD-2 from Sigma (20-60 mesh) and Sephadex LH-20 (20-100 mm). Thin layer chromatography (TLC) analyses were performed on Merck GF-254 precoated silicagel. Reversed phase HPLC was performed with a Waters Associates 590 pump, a μ-Bondapak C_{18} column (7.8 mm x 30 cm) and a differential refractometer as detector. Compound 48/80 was purchased from Sigma Chemical Co.

Eluents: compounds **1**, **2** and **7**, 7:3 MeOH/H_2O at 3 mL. min $^{-1}$; compound **5**, 7:3 MeOH/H_2O at 2 mL. min $^{-1}$; compounds **3**, **4**, **6**, **8**, **9** and **10**, 6:4 MeOH/H_2O at 4 mL.min $^{-1}$.

Collection and extraction of the sponge: *Eurypongia* sp. (reference R1225), was collected by divers using scuba from St. 184, Point Bovis, New Caledonia on 3/12/1979 at a depth of 22 m on coral abutting fine sand. Other sponges and soft coral were in association. A voucher specimen will be deposited in the Museum of Natural History, Paris and in Museum Orstom in Nouméa.

The specimen is 10 cm high, 15 cm wide, thrown in to low lobes along the crest of wich large oscules lie flush with the surface. The colour is a brilliant lilac purple (Munsell RP^6/6) mottled with whitish conules which mark the point of intersection of primary fibres with the translucent, finely reticulated pinacoderm. The skeleton is a strong reticulum in which the primary, ascending fibres are clear of debris but contain scattered bacterial-type particles. The secondary connecting fibres form an irregular, relatively dense reticulum. The choanocyte chambers are large, oval and eurypylous. The sponge, given these characteristics, falls into the genus *Eurypongia* (Family Dysideiidae, Order Dictyoceratida) and is distinguished from other members of that genus in colour, and in the peculiar nature of the coring material in the fibres. Should subsequent electron microscopy study on appropriately fixed material confirm the nature of the inclusions, it could be that a new genus within the Family Dysideiidae will have to be established for the species. A full description will be included in a forthcoming publication on the Dictyoceratida of New Caledonia.

The freshly collected sponge was cut into pieces and extracted at room temperature with methanol. The solvent was removed from the methanolic extracts by evaporation in vacuo, and the residue was partitioned successively between hexane and 10 % aqueous methanol, between Cl_2CH_2 and 20 % aqueous methanol, and between n-BuOH and 40 % aqueous methanol; evaporation of the solvents gave a hexane extract (9.3 g), a Cl_2CH_2 extract (9.9 g) and an n-BuOH extract (5.0 g). The n-BuOH extract was added to a column of Amberlite XAD-2, and the column was washed with H_2O (3L at 2 mL.min^{-1}) and then eluted with MeOH (2L at 10 mL.min^{-1}).

The MeOH eluates were concentrated in vacuo to give a residue (2.2 g) that was chromatographed on a Sephadex LH-20 column eluted with 2:1 MeOH/H_2O. Fractions of 10 mL each were collected and analysed by TLC on SiO_2 in 12:3:5 n-BuOH/AcOH/H_2O.

Fractions D (208 mg) and E (125 mg), which contained the secosterols, were separated by semipreparative reversed phase HPLC on a C_{18} μ-Bondapak column, eluted with MeOH/H_2O. This afforded pure samples of **1** (30 mg, R_t 44.01 min), **2** (32 mg, R_t 70.00 min), **3** (1.5 mg, R_t 120.11 min), **4** (1 mg, R_t 120.90 min), **5** (7 mg, R_t 30.00 min), **6** (17 mg, R_t 60.37 min), **7** (5 mg, R_t 60.22 min), **8** (1.5 mg, R_t 120.00 min), **9** (1.5 mg, R_t 120.50 min) and **10** (6 mg, R_t 46.00 min).

Eurypongiol A1 (1): (30 mg); $[\alpha]^{21}_D$ -42° (*c* 0.001, MeOH); IR spectrum: υ_{max}(KBr dis) 3450 (br), 2950, 1700 cm^{-1}; ^{1}H NMR (CD_3OD, C_5D_5N, 250 Mz) see Table 2; ^{13}C NMR (CD_3OD, 250 Mz) see Table 1;

HR EIMS: obsd 482.3217, calcd for $C_{27}H_{46}O_7$ 482.3243; EIMS *m/z* (%) 482 (1), 452 (4), 434 (3), 416 (2), 408 (2), 390 (2), 299 (12), 281 (7), 218 (5), 147 (8), 125 (76), 67 (34), 54 (100 rel); FAB MS (+, glycerol matrix) *m/z* 483, 465, 393, 354, 316, 224; FAB MS (-, glycerol matrix) *m/z* 451, 387, 275, 257, 183, 127, 91.

Eurysponglol A2 (2): (32 mg); $[\alpha]^{21}_D$ -22° (*c* 0.001, MeOH); CD (MeOH) $(\theta)_{295}$ - 6796°; UV λ_{max} (MeOH) 208 nm; HR EIMS: obsd 484.3419, calcd for $C_{27}H_{48}O_7$ 484.3399; EIMS *m/z* (%) 484 [M^+, 1], 454 [M-CH_2O^+, 1], 436 [M-CH_2O-H_2O^+, 5], 418 [M-CH_2O-$2H_2O^+$, 3], 410 [M-CH_2O-$C_3H_8^+$, 1], 400 [M-CH_2O-$3H_2O^+$, 1], 382 [M-CH_2O-$4H_2O^+$, 1], 364 [M-CH_2O-$5H_2O^+$, 1], 323 [M-CH_2O-H_2O-$C_8H_{17}^+$, 2]; FAB MS (-, glycerol matrix) *m/z*: 483, 465, 453, 433, 386, 350, 325, 311, 273, 243, 219, 181, 165, 151, 121, 97, 93; ^{1}H NMR (C_5D_5N, 250 Mz) δ 0.91 (3H, s, H-18), 0.98 (3H, d, J = 6.4 Hz, H-21), 1.74 (1H, H-7β), 2.31 (1H, m, H-1α), 2.51 (1H, H-7α), 3.34 (1H, dd, J = 13.2, 4.7 Hz, H-1β), 3.97 (1H, t, J = 8.8 Hz, H-3α), 4.23 (1H, H-8), 4.44 (1H, d, J = 11.2 Hz, H-19), 4.58 (1H, m, H-2α), 4.62 (1H, m, H-4α), 5.13 (1H, s, H-6β), 5.78 (1H, d, J = 11.2 Hz, H-11'); ^{13}C NMR (C_5D_5N, 250 Mz) δ 17.4 (C-18), 19.4 (C-21), 22.5[b] (C-26), 22.7[b] (C-26), 23.0 (C-15), 24.5 (C-23), 26.1 (C-16), 27.9 (C-25), 34.5 (C-20), 35.8[a] (C-22), 35.6[a] (C-1), 38.7 (C-8), 39.5 (C-24), 41.1 (C-12), 41.9 (C-14), 42.0 (C-7), 45.9 (C-13), 49.7 (C-17), 55.9 (C-10), 56.3 (C-5), 58.1 (C-11), 63.7 (C-19), 64.0 (C-6), 70.1 (C-4), 70.7 (C-2), 83.2 (C-3), 215.4 (C-9).

Eurysponglol A3 (3): (1.5 mg); HR EIMS obsd 496.3401, calcd for $C_{28}H_{48}O_7$ 496.3400; EIMS *m/z* (%) 496 [M^+, 1], 466 [M-CH_2O^+, 7]; FAB MS (-, glycerol matrix) *m/z* 495 [M-H]$^-$; ^{1}H NMR (CD_3OD, 250 Mz) δ 0.79 (3H, s, H-18), 0.87 (6H, d, J = 6.9 Hz, H-26, 27), 0.94 (3H, d, J = 6.9 Hz, H-28), 1.06 (3H, d, J = 6.9 Hz, H-21), 1.34 (2H, m, H-16), 1.36 (2H, m, H-15), 1.46 (1H, H-1α), 1.49 (1H, H-5), 1.57 (1H, H-7β), 2.20 (1H, H-7α), 2.34 (1H, dd, J = 13.6, 4.9 Hz, H-1β), 2.49 (1H, H-14), 3.07 (1H, t, J = 9.0 Hz, H-3), 3.47 (1H, H-8), 3.62 (1H, H-4), 3.68 (2H, H-11), 3.68 (1H, d, J = 11.5 Hz, H-19), 3.70 (1H, H-2), 4.30 (1H, H-6), 4.85 (1H, d, J = 11.5 Hz, H-19), 5.25 (2H, m, H-22, 23); ^{13}C NMR (CD_3OD, 250 Mz) δ 18.1 (C-18), 19.6 (C-28), 23.5 (C-21), 23.9 (C-15), 24.2 (C-26, 27), 26.7 (C-16), 35.5 (C-1), 35.7 (C-25), 39.7 (C-8), 41.3 (C-7), 41.3 (C-12), 41.7 (C-20), 43.1 (C-14), 45.8 (C-24), 46.6 (C-13), 51.0 (C-17), 56.2 (C-10), 56.3 (C-5), 59.2 (C-11), 63.9 (C-19), 64.7 (C-6), 70.5 (C-4), 71.1 (C-2), 82.8 (C-3), 135.2 (C-22), 137.7 (C-23), 215.4 (C-9).

Eurysponglol A4 (4): (1 mg); HR EIMS obsd 496.3401, calcd for $C_{28}H_{48}O_7$ 496.3400; EIMS *m/z* (%) 496 [M^+, 1], 466 [M-CH_2O^+, 7]; FAB MS (-, glycerol matrix) *m/z* 495 [M-H]$^-$; ^{1}H NMR (CD_3OD, 250 Mz) δ 0.81 (3H, s, H-18), 0.89 (6H, d, J = 6.6 Hz, H-26, 27), 0.96 (3H, d, J = 6.9 Hz, H-28), 1.07 (3H, d, J = 6.8 Hz, H-21), 1.36 (2H, m, H-16), 1.37 (2H, m, H-15), 1.46 (1H, H-1α), 1.49 (1H, H-5), 1.58 (1H, H-7β), 1.67 (2H, H-12), 2.24 (1H, H-7α), 2.32 (1H, dd, J = 13.6, 4.8 Hz, H-1β), 2.47 (1H, H-14), 3.10 (1H, t, J = 8.9 Hz, H-3), 3.46 (1H, H-8), 3.64 (1H, H-4), 3.64 (2H, m, H-11), 3.66 (1H, H-2), 3.75 (1H, d, J = 11.5 Hz, H-19), 4.33 (1H, H-6), 4.85 (1H, d, J = 11.5 Hz, H-19), 5.20 (2H, m, H-22, 23); ^{13}C NMR (CD_3OD, 250 Mz) δ 18.1 (C-18), 19.6 (C-28), 23.5 (C-21), 23.9 (C-15), 24.2 (C-26, 27), 26.7 (C-16), 35.5 (C-1), 35.7 (C-25), 39.7 (C-8, C-20), 41.3 (C-7), 41.3 (C-12), 43.1 (C-14), 45.8 (C-24), 46.6 (C-13), 51.0 (C-17), 56.2 (C-10), 56.3 (C-5), 59.2 (C-11), 63.9 (C-19), 64.7 (C-6), 70.5 (C-4), 71.1 (C-2), 82.8 (C-3), 135.2 (C-22), 137.7 (C-23), 215.4 (C-9).

Eurypongiol A5 (5): (7 mg); $[\alpha]^{21}_D$ -48° (*c* 0.0001, MeOH); HR EIMS: obsd 468.3090, calcd for $C_{26}H_{44}O_7$ 468.3087; FAB MS (+, NaCl+glycerol matrix) *m/z* 491 $[M+Na]^+$, 394 $[M+Na-C_7H_{13}]^+$; ^1H NMR (CD_3OD, 250 Mz) δ 0.78 (3H, s, H-18), 0.96 (6H, d, J = 6.7 Hz, H-25, 26), 1.03 (3H, d, J = 6.8 Hz, H-21), 1.34 (2H, m, H-16), 1.38 (2H, m, H-15), 1.45 (1H, H-1α), 1.45 (1H, dd, J = 11.7, 2.5 Hz, H-5), 1.53 (1H, H-7β), 1.64 (2H, m, H-12), 2.21 (1H, H-24), 2.22 (1H, H-7α), 2.30 (1H, dd, J = 13.8, 5.1 Hz, H-1β), 2.44 (1H, H-14), 3.07 (1H, t, J = 9.0 Hz, H-3), 3.46 (1H, H-8), 3.61 (2H, m, H-11), 3.65 (1H, H-4), 3.69 (1H, H-2), 3.72 (1H, d, J = 11.6 Hz, H-19), 4.31 (1H, d, J = 2.5 Hz, H-6), 4.87 (1H, d, J = 11.8 Hz, H-19), 5.30 (2H, m, H-22, 23); ^{13}C NMR (CD_3OD, 250 Mz) δ 18.1 (C-18), 22.2 (C-21), 23.9 (C-15), 26.3 (C-16), 32.3 (C-24), 35.5 (C-1), 39.4 (C-20), 39.8 (C-8), 41.3 (C-7,12), 43.1 (C-14), 46.7 (C-13), 51.0 (C-17), 56.2 (C-10), 56.3 (C-5), 59.2 (C-11), 64.0 (C-19), 64.7 (C-6), 70.5 (C-4), 71.1 (C-2), 82.8 (C-3), 134.0 (C-23), 136.0 (C-22), 214.8 (C-9).

Eurypongiol B1 (6): (17 mg); $[\alpha]^{21}_D$ -39° (*c* 0.003, MeOH); HR EIMS: obsd 482.3239, calcd for

135.2 (C-22), 137.7 (C-23), 215.4 (C-9).

Eurypongiol B4 (9): (1.5 mg); HR EIMS: obsd 496.3405, calcd for $C_{28}H_{48}O_7$ 496.3400; EIMS *m/z* (%) 496 [M^+, 1] 466 [M-CH_2O^+, 6], 305 [M-CH_2O-$2H_2O$-$C_9H_{17}^+$, 8]; FAB MS (+, NaCl+KCl+glycerol matrix) *m/z* 535 [M+K]$^+$, 519 [M+Na]$^+$, 371 [M-C_9H_{17}]$^+$; ^{1}H NMR (CD_3OD, 500 Mz) δ 0.79 (3H, s, H-18), 0.87 (6H, d, J = 6.5 Hz, H-26, 27), 0.93 (3H, d, J = 6.5 Hz, H-28), 1.06 (3H, d, J = 7.0 Hz, H-21), 1.38 (2H, m, H-16), 1.51 (1H, H-7β), 1.52 (2H, m, H-15), 1.58 (2H, H-12), 1.64 (1H, H-25), 1.65 (1H, H-1α), 1.85 (1H, dd, J = 11.9, 3.0 Hz, H-5), 2.00 (1H, dd, J = 13.5, 3.9 Hz, H-1β), 2.18 (1H, H-20), 2.23 (1H, H-7α), 2.48 (1H, H-14), 3.48 (1H, H-8), 3.59 (2H, H-11), 3.72 (1H, d, J = 11.5 Hz, H-19), 3.80 (1H, dd, J = 11.9, 3.0 Hz, H-4), 3.82 (1H, H-2), 3.93 (1H, t, J = 3.0 Hz, H-3), 4.28 (1H, H-6), 4.85 (1H, d, J = 11.5 Hz, H-19), 5.24 (2H, dd, J = 15.0, 7.5 Hz, H-22), 5.28 (1H, dd, J = 15.0, 8.0 Hz, H-23); ^{13}C NMR (CD_3OD, 500 Mz) δ 19.0 (C-18), 19.6 (C-28), 23.5 (C-21), 24.2 (C-26, 27), 25.1 (C-15), 28.1 (C-16), 31.7 (C-1), 35.7 (C-25), 41.0 (C-8), 41.1 (C-20), 42.2 (C-12), 42.8 (C-7), 44.1 (C-14), 45.8 (C-24), 47.9 (C-13), 51.3 (C-5), 57.3 (C-10), 60.5 (C-11), 64.5 (C-19), 66.2 (C-6), 69.6 (C-4), 69.6 (C-2), 75.4 (C-3), 135.2 (C-22), 137.7 (C-23), 215.0 (C-9).

Eurypongiol B5 (10): (6 mg); $[\alpha]^{21}_D$ -45° (*c* 0.002, MeOH); HR EIMS: obsd 468.3092, calcd for $C_{26}H_{44}O_7$ 468.3087; FAB MS (+, NaCl+glycerol matrix) *m/z* 491 [M+Na]$^+$; ^{1}H NMR (CD_3OD, 250 Mz) δ 0.78 (3H, s, H-18), 0.97 (6H, d, J = 6.7 Hz, H-25, 26), 1.03 (3H, d, J = 6.8 Hz, H-21), 1.54 (1H, H-7β), 1.61 (2H, H-12), 1.65 (1H, H-1α), 1.83 (1H, dd, J = 11.5, 2.8 Hz, H-5), 2.02 (1H, dd, J = 13.3, 4.6 Hz, H-1β), 2.22 (1H, H-7α), 2.47 (1H, H-14), 3.43 (1H, H-8), 3.63 (2H, H-11), 3.69 (1H, d, J = 11.8 Hz, H-19), 3.83 (1H, dd, J = 11.5, 2.6 Hz, H-4), 3.85 (1H, H-2), 3.86 (1H, dd, J = 4.2, 2.6 Hz, H-3), 4.28 (1H, H-6), 4.83 (1H, d, J = 11.8 Hz, H-19), 5.29 (2H, m, H-22, 23); ^{13}C NMR (CD_3OD, 250 Mz) δ 18.1 (C-18), 22.2 (C-21), 22.9 (C-26, 27), 30.7 (C-1), 32.3 (C-24), 39.3 (C-20), 40.1 (C-8), 41.5 (C-7), 41.5 (C-12), 43.2 (C-14), 46.7 (C-13), 51.2 (C-5), 56.1 (C-10), 59.2 (C-11), 63.5 (C-19), 65.1 (C-6), 68.2 (C-4), 68.5 (C-2), 74.3 (C-3), 134.0 (C-23), 137.0 (C-22), 215.0 (C-9).

$NaBH_4$ Reduction of eurypongiol A1 (1). $NaBH_4$ (2 mg) was added to **1** (12 mg) in MeOH (2 mL). The mixture was stirred 7 h, dilute HCl (10 %, 1 mL) was added, and the solvent was evaporated. Purification of the product by preparative HPLC on μ-Bondapak C_{18} (6:4 MeOH/H_2O) yielded **1a** (7.2 mg, R_t 80.09 min): $[\alpha]^{21}_D$ -35° (*c* 0.001, MeOH); UV λ_{max} (MeOH) 206 nm; HR EIMS: obsd 484.3410, calcd for $C_{27}H_{48}O_7$ 484.3400; EIMS *m/z* (%) 484 (1), 454 (3), 422 (4), 355 (5), 338 (9), 320 (10), 306 (19), 289 (11), 235 (36), 191 (16), 147 (27), 95 (61), 69 (100); ^{1}H NMR (CD_3OD) δ see Table 2; ^{13}C NMR (CD_3OD) δ see Table 1.

Acetylation of 1a to 1b. A mixture of **1a** (2 mg) and excess Ac_2O in 0.5 ml of dry pyridine was kept at room temperature for 12 h, after which removal of the excess reagents in vacuo yielded **1b** (4 mg): $[\alpha]^{21}_D$ -50° (*c* 0.0001, Cl_2CH_2); HR EIMS: obsd 778.4150, calcd for $C_{41}H_{62}O_{14}$ 778.4140; ^{1}H NMR (CD_3OD, 250 Mz) δ 0.77 (3H, s, H-18), 0.90 (6H, d, J = 6.6 Hz, H-26, 27), 1.08 (3H, d, J = 6.8 Hz, H-21), 2.01, 2.01, 2.03, 2.07, 2.10, 2.18 and 2.18 (each 3H, s, OAc).

Acetylation of 2 to 2a. A crystal of 4-dimethylaminopyridine was left overnight at room temperature

in a mixture of **2** (13 mg) and excess of Ac_2O in 1 mL of dry pyridine. Removal of excess reagents in vacuo afforded the acetylated compound **2a** (23 mg): $[\alpha]^{21}_D$ -29° (*c* 0.002, Cl_2CH_2); UV λ_{max} (Cl_3CH) 244 nm; HR

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