Mycgranol, a New Diterpene from the Marine Sponge *Mycale* aff. *graveleyi*

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Mycgranol, a new isocopalane diterpene (methyl 1β-hydroxy-2-oxoisocopalanoate) was isolated from the Kenyan sponge *Mycale* (*Aegogropila*) aff. *graveleyi*. The structure of the compound was elucidated by interpretation of MS, COSY, HMQC, HMBC, and ROESY data. The absolute configuration of mycgranol was determined to be the same as that of the nudibranch isocopalane diterpenes rather than that of the *ent*-compounds known from sponges of the genus *Spongia*.

As part of our ongoing efforts to isolate biologically active compounds from marine invertebrates,[1,2] the constituents of the Kenyan long-digitated tubular orange sponge *Mycale* (*Aegogropila*) aff. *graveleyi*, Burton (Demospongiae, Poecilosclerida, Mycalidae), collected in the Shimoni Channel, Kenya, were examined and a new diterpene designated as mycgranol (**1**) was isolated. Although more than 20 subspecies of *Mycale* were investigated resulting in many unique secondary metabolites,[3−5] *M. graveleyi* has thus far not been explored and no tricyclic diterpene was reported.

Diterpenes are well known from marine invertebrates, and a large variety of skeletons have been reported.[3−5] Among those, *ent*-isocopalane diterpenoids are widely present in marine sponges;[6,7] for example, from the Mediterranean *Spongia zimocca* were isolated several oxygenated *ent*-isocopalanes such as 15-hydroxy-*ent*-isocopal-12-en-16-al.[7] The enantiomeric isocopalane diterpenoids were mainly isolated from nudibranches as part of acylglycerols.[8] The carbon skeleton of these diterpenoids is rather rare in nature. The ethyl acetate/CH_3OH (1:1) extract of the freeze-dried sponge was subjected to solvent partition,[9] and the petroleum ether fraction was chromatographed on Sephadex LH-20 and silica gel columns to afford mycgranol (**1**) (40 mg, 0.8% dry weight). The EI mass spectrum of mycgranol (**1**) exhibited a molecular ion M^+ at *m/z* 348. The ^{13}C NMR, ^1H NMR, and 2D spectra (Table 1) revealed the presence of the following moieties: (a) a ketone (δ_C 212.0), (b) an external double bond (δ_C 143.1 C, 108.6 CH_2), (c) a methyl ester (δ_C 171.8 C, 50.9 CH_3), and (d) a hydroxyl group (δ_C 79.1 CH). On the basis of this analysis, the molecular formula was determined to be $C_{21}H_{32}O_4$, and to fulfill the six degrees of unsaturation, **1** has to be tricyclic. First, the strong HMBC correlations of the four methyl groups and the methylene with their neighboring C atoms were analyzed, and the resulting units were then extended by COSY correlations (Figure 1). Correlations in the HMBC spectrum from H-1 and H-3 to the ketone carbonyl group and from the OCH_3 (H-21) and H-14 to the ester CO_2 group established the position of the carbonyl (C-2) and the methoxycarbonyl (C-15) groups. The downfield resonances of the AB system of H_2-3 (2.85 and 2.03 d) and the singlet of H-14 (2.89 s) confirmed the position of the carbonyl group. Additional HMBC correlations (Table 1) corroborated the suggested planar structure of **1**.

The relative stereochemistry of mycgranol was determined from the coupling constants and correlations observed in the ROESY spectrum (Figure 2). Thus, H-5 and H-9 have to be both axial according to large $J_{ax,ax}$ values, with H-6ax and with H-11ax, respectively (Table 1).

Table 1. NMR Data for Mycgranol (**1**) in $CDCl_3$ (500 MHz for ^1H and 100 MHz for ^{13}C)[a]

C#	δ_C		δ_H (m, *J* in Hz)	HMBC (C to H)[b]
1	79.1	CH	3.58 s	9, 20
2	212.0	C		1, 3a, 3b
3	50.8	CH_2	2.85 d (12.8) 2.03 d (12.8)	18, 19
4	39.0	C		3a, 3b, 5, 18, 19
5	47.9	CH	1.75 dd (13.9, 2.0)	3a, 3b, 7a, 18, 19, 20
6	18.8	CH_2	1.60 m 1.45 m	5, 7a, 7b
7	39.3	CH_2	1.63 m 1.32 m	5, 6a, 9, 17
8	39.6	C		9, 14, 17
9	48.7	CH	1.89 dd (12.0, 2.8)	14, 17, 20
10	45.0	C		5, 20
11	22.2	CH_2	1.48 m 1.42 m	9
12	35.6	CH_2	2.40 ddd (12.8, 4.1, 2.0) 2.10 dt (12.8, 2.0)	14, 16a, 16b
13	143.1	C		12a, 12b, 14, 16a, 16b
14	63.1	CH	2.89 s	9, 12a, 16a, 16b, 17
15	171.8	C		14, 21
16	108.6	CH_2	4.82 s, 4.66 s	12a, 12b, 14
17	14.9	CH_3	1.07 s	7a, 7b, 14
18	23.2	CH_3	0.87 s	3a, 3b, 5, 19
19	32.9	CH_3	1.03 s	3a, 3b, 5, 18
20	16.1	CH_3	0.86 s	1, 9
21	50.9	CH_3	3.62 s	

[a] Multiplicities of the carbon signals were determined by DEPT, and all assignments were confirmed from 2D experiments. [b] a and b denote downfield and upfield resonances within a geminal pair.

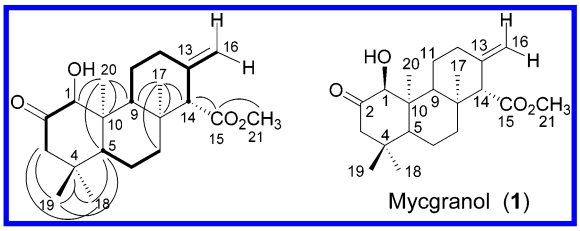

Figure 1. COSY (—) and key HMBC (∩) correlations for **1**.

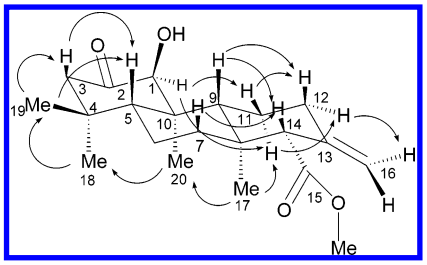

Figure 2. Key NOEs for **1**.

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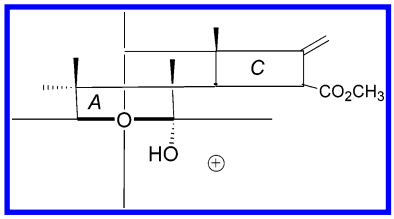

Figure 3. Octants projection for **1**.

ROESY correlations between methyls 17 and 20 and between 18 and 20 established the α-axial position of all three. The proposed configuration also agrees with their relative upfield resonances (δ_C 15−23 ppm) due to γ-effects. The all-*trans* structure was confirmed from several NOEs on the β-side of **1** as shown in Figure 2.

The absolute configuration of compound **1** was determined from its Cotton effect measured from the CD curve ($\Delta\epsilon$ +0.25). The n→π* excitation of the C-2 carbonyl group is expected to be mainly perturbed by the neighboring 1β-axial-hydroxyl group which is in the first sphere of the CO group.[10,11] The axial OH group (against equatorial groups which are on the nodal plane and have little effect) causes a bathochromic shift to λ_{max} 307 mμ and an increase of the Cotton effect. According to the octant rule, the hydroxyl group has to be in the rear lower-right octant as depicted in Figure 3. Ring C (Figure 3) with its substituents is in the fourth sphere[10] and expected to have a weak negative contribution to the Cotton effect, if there is any. Hence, the 1*S* configuration was determined for **1**, and accordingly, the absolute configuration of **1** is as in the anisodorin-isocopalane diterpenes isolated from the mantle of the dorid nudibranch *Anisodoris fontaini*.[8] The proposed configuration is enantiomeric to the *ent*-diterpenes isolated from sponges of the genus *Spongia*.[7] Mycgranol (methyl 1β-hydroxy-1-oxoisocopalanoate) is the first example of an isocopalane-diterpene from a sponge.

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1010 polarimeter. Circular dichroism measurements were performed on an AVIV 202 spectrometer. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and Avance-400 spectrometers. ^{1}H, ^{13}C, COSY, HMQC, and HMBC were recorded using standard Bruker pulse sequences. EIMS and HREIMS measurements were recorded on a Fisons, Autospec Q instrument.

Biological Material. *Mycale* (*Aegogropila*) aff.*graveleyi*, Burton, a long-digitated tubular orange sponge, was collected in the Shimoni Channel off Mombasa, Kenya, by scuba at a depth of 8−11 m in February 2003. A voucher sample, ZMAPOR 17808, is deposited at the Zoological Museum, Tel Aviv University.

Extraction and Isolation. Freeze-dried sponge (5 g) was homogenized and successively extracted with ethyl acetate/CH_3OH (1:1). The extract (900 mg) was subjected to partition by the method of Kupchan et al.[9] to afford five fractions (petroleum ether, CCl_4, $CHCl_3$, n-BuOH, and water). The petroleum ether fraction (200 mg), containing **1** with sterols and glycerides, was repeatedly chromatographed on a Sephadex LH-20 column, eluting with a mixture of hexane/$CHCl_3$/CH_3OH (2:1:1), followed by a silica gel column [hexane/ethyl acetate (7:3)] to afford mycgranol (**1**) (40 mg, 0.8% dry weight).

Compound 1: colorless oil; $[\alpha]_D$ +2.5 (*c* 2.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2950, 1735, 1717, 1649 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; CIMS *m/z* (%) 348 (100), 331 (21), 317 (37), 299 (18), 289 (11); HREIMS obsd [M$^+$] *m/z* 348.2305 (calcd for $C_{21}H_{32}O_4$, 348.2301).

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References and Notes

(1) Chill, L.; Rudi, A.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *Org. Lett.* **2004**, *6*, 755−758.
(2) Rudi, A.; Shalom, H.; Schleyer, M.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **2004**, *67*, 106−109.
(3) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and previous reports in this series.
(4) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48; **2004**, *21*, 1−49.
(5) Blunt, J. W.; Munro, M. H. G. "marinlit" Database 2004, University of Canterbury, Christchurch, New Zealand.
(6) de Miranda, D. S.; Brendolan, G.; Imamura, P. M.; Sierra, M. G.; Marsaioli, A. J.; Ruveda, E. A. *J. Org. Chem.* **1981**, *46*, 4851−4856.
(7) Zubia, E.; Gavagnin, M.; Scognamiglio, G.; Cimino, G. *J. Nat. Prod.* **1994**, *57*, 725−731.
(8) Gavagnin, M.; Ungur, N.; castelluccio, F.; Muniain, C.; Cimino. G. *J. Nat. Prod.* **1999**, *62*, 269−274.
(9) Kupchan, S. M.; Komoda, Y.; Branfman, A. R.; Sneden, A. T.; Court, W. A.; Thomas, G. J.; Hintz, H. P. J.; Smith, R. M.; Karim, A.; Howie, G. A.; Verma, A. K.; Nagao, Y.; Dailey, R. G., Jr.; Zimmerly, V. A.; Summer, W. C., Jr. *J. Org. Chem.* **1977**, *42*, 2349−2357.
(10) Snatzke, G., Ed. *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*; Heyden and Son Ltd.: London, 1967.
(11) Eliel, L. E.; Wilen, S. H.; Mander L. N. *Stereochemistry of Organic Compounds*; John Wiley and Sons Inc.: New York, 1994; p 991.

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