Umabanol, a New Tetracyclic Diterpene from a Marine Sponge

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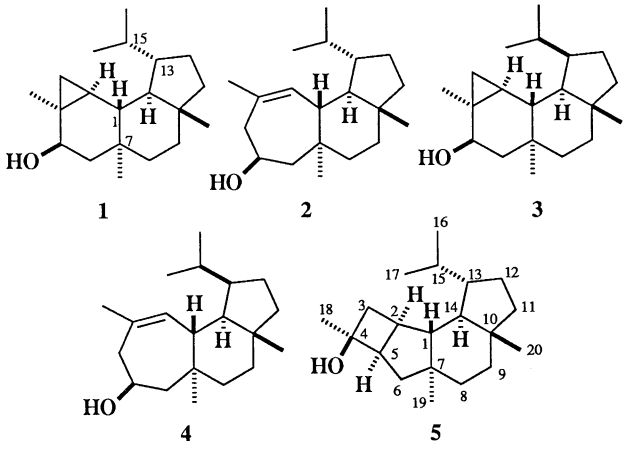
A new diterpene, umabanol, has been isolated from the sponge *Epipolasis kushimotoensis* and its structure determined by spectroscopic analysis. Umabanol possesses a new tetracyclic ring system related to verrucosane class diterpenes.

Verrucosane and related diterpenes have been known as characteristic metabolites of some species of terrestrial plants.[1] For example, Neoverrucosanol (**1**) has been reported from the liverwort *Mylia verrucosa*[2] and homoverrucosanol (**2**) from *Schistochila acuminata*.[3,4] Recently these two compounds have also been described as the constituents of a marine animal, the sponge *Axinyssa aplysinoides*.[5] It was the first report on the occurrence of verrucosane-class diterpenes in a marine organism.[13] In our search for bioactive compounds from marine organisms, we recently examined the constituents of the sponge *Epipolasis kushimotoensis*[6] and found, in addition to four known verrucosanes (**1-4**), a new related diterpene named umabanol (**5**) which was composed of a new tetracyclic ring system. In this paper we describe the isolation of these compounds and the structure elucidation of **5**.



A sample (430 g) of the sponge *E. kushimotoensis* was extracted by steeping in acetone. An ethyl acetate soluble portion of the extract was separated by vacuum flash chromatography (silica gel, hexane-EtOAc) followed by reverse phase HPLC (RP-8, MeCN) to give compounds **1** (366 mg), **2** (238 mg), **3** (132 mg), **4** (26 mg), and **5** (9 mg).[7] Compounds **1-4** were identified to be the known neoverrucosanol, homoverrucosanol, 13-*epi*-neoverrucosanol (**3**),[8] and 13-*epi*-homoverrucosanol (**4**),[9] respectively, by spectroscopic comparison with those reported.

The molecular formula $C_{20}H_{34}O$ of **5**, $[\alpha]_D$ -78° (c 0.10, $CHCl_3$), was determined by HREIMS (*m/z* 290.2632, Δ +2.4 mmu).[10] Four sites of unsaturation required by the formula and the absence of olefinic signals in both ^1H and ^{13}C NMR spectra indicated the compound to have four rings. The presence of a tertiary hydroxyl group was inferred from an IR (CCl_4) absorption band at 3620 cm^{-1} and a ^{13}C NMR signal at δ 71.1 (s). The gross structure was determined by 2D NMR connectivity study and comparison of the data with those of **1** and **2**. As a result, all the signals of **5** could be assigned as shown in Table 1. Nearly identical NMR data for the portion of C9-C17 and C20 with those of **1** and **2** suggested that **5** had the same partial structure including the 5- and 6-membered rings. Observation of COSY cross signals for H1/H14, H1/H2, H2/H3α, H2/H3β, H2/H5, H5/H6α, and H5/H6β together with HMBC results (Table 1) indicated that the remaining part of the structure was composed of another 5-membered ring and a 4-membered ring. The presence of the cyclobutane ring was further substantiated by typical coupling constants, i.e., a long range coupling (J = 1 Hz) between H3α and H5, and two *cis* couplings (J = 8 Hz) between H2 and H3α and between H2 and H5. The relative stereochemistry was established by difference NOEs observed for H1/H13, H1/H20, H2/H3α, H2/H5, H2/H14, H2/H15, H2/H19, H3α/H18, H5/H6α, H5/H18, H5/H19, and H14/H19. Biogenetically **5** may be derived from **1**, and *vice versa*. A question that **5** may be an artifact of the isolation is ruled out by the fact that acid treatment[11] of **1** gives **2** in quantitative yield, but no trace of **5**. This acid-catalyzed rearrangement has previously been reported by Matsuo *et al.*[2] The absolute configuration of **1** has been established by X-ray analysis.[2] Co-occurrence of **5** with **1** suggests that umabanol has the absolute configuration shown in the structure. The tetracyclic ring system of umabanol is new in diterpenes.

Homoverrucosanol (**2**) showed moderate cytotoxicity (IC_{50} 2.5-5 μg/mL) against P388, A549, and HT29 tumor cells, while that of neoverrucosanol (**1**) was weak (IC_{50} >10 μg/mL). No cytotoxicity test has been performed with compounds **3-5**.

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References and Notes

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6 The sponge was collected at -30 m by hand using SCUBA at Umabana, Yonaguni Island, Okinawa. Taxonomic identification was carried out by Dr. J. N. A. Hooper, Queensland Museum, South Brisbane, Australia, and a voucher specimen (G304924) is deposited at the museum.
7 **1**: mp 138-140 ℃ (acetone), $[\alpha]_D$ +2.6° (c 2.8, $CHCl_3$).

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Neoverrucosanol (**1**), Homoverrucosanol (**2**), and Umabanol (**5**) in $CDCl_3$

	1		2		5		
C#	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	HMBC
1	47.6 d	1.02 dd, J = 4, 12 Hz	43.8 d	2.20 brdd, J = 5, 12 Hz	55.1 d	1.31 dd, J = 9, 12 Hz	C2,3,7,19
2	25.6 d	0.85 ddd, J = 4, 5, 8 Hz	131.7 d	5.29 brd, J = 5 Hz	30.9 d	2.03 ddt, J = 3, 9, 8 Hz	C1,5,14
3	19.6 t	0.28 t, J = 5 Hz	131.3 s	-	41.6 t	1.57 m	
		0.56 dd, J = 5, 8 Hz				2.23 ddd, J = 1, 8, 13 Hz	C1,2,5,18
4	22.0 s	-	42.6 t	2.03 m	71.1 s	-	
				2.54 ddd, J = 2, 11, 13 Hz			
5	71.2 d	4.03 brdd, J = 7, 11 Hz	65.6 d	3.60 ddt, J = 2, 3, 11 Hz	48.2 d	2.61 brq, J = 8 Hz	C1,2,3,6,18
6	47.2 t	0.68 dd, J = 11, 13 Hz	58.9 t	1.47 dd, J = 11, 13 Hz	34.3 t	1.45 m	
		1.69 dd, J = 7, 13 Hz		1.89 ddd, J = 2, 3, 13 Hz		1.54 m	
7	37.1 s	-	38.0 s	-	46.7 s	-	
8	35.3 t	1.10 ddd, J = 2, 4, 13 Hz	39.0 t	1.23 dt, J = 14, 3 Hz	38.8 t	1.47 m	
		1.29 dt, J = 4, 13 Hz		1.54 dt, J = 4, 14 Hz		1.57 m	
9	34.6 t	1.35 m	35.1 t	1.32 dt, J = 4, 13 Hz	35.1 t	1.33 m	
		1.42 m		1.40 m		1.43 m	
10	44.0 s	-	42.6 s	-	43.5 s	-	
11	39.2 t	1.05 m	38.8 t	1.03 brq, J = 11 Hz	39.2 t	1.07 brq, J = 10 Hz	C9,10,12,20
		1.37 m		1.37 m		1.44 m	
12	21.6 t	1.40 m,	21.4 t	1.38 m	20.8 t	1.37 m	
		1.60 dq, J = 13, 10 Hz		1.60 dq, J = 13, 11 Hz		1.52 m	
13	45.2 d	1.90 dddd, J = 4, 7, 10, 12 Hz	46.8 d	1.71 ddt, J = 3, 7, 11 Hz	44.2 d	1.74 m	C1,14,16
14	46.9 d	1.21 t, J = 12 Hz	47.8 d	1.16 t, J = 11 Hz	47.6 d	1.13 t, J = 12 Hz	C1,7,10,13
15	28.7 d	2.15 m	28.0 d	2.05 m	27.4 d	1.67 m	C12
16	14.9 q	0.82 d, J = 7 Hz	14.9 q	0.83 d, J = 7 Hz	15.2 q	0.75 d, J = 7 Hz	C13,15,17
17	22.7 q	0.91 d, J = 7 Hz	22.9 q	0.86 d, J = 7 Hz	22.8 q	0.85 d, J = 7 Hz	C13,15,16
18	25.8 q	1.19 s	25.8 q	1.76 s	31.3 q	1.36 s	C3,4,5
19	17.2 q	0.83 s	20.1 q	0.86 s	19.3 q	0.66 s	C1,6,7,8
20	18.6 q	0.74 s	18.1 q	0.78 s	18.6 q	0.78 s	C9,10,11,14

Since the sign of the optical rotation of this sample was opposite to that (-10°) reported,[2] we determined the absolute configuration of our sample by Modified Mosher's method[12] and found that it was identical with that reported by X-ray analysis. **2**: mp 135-136 °C (MeOH), $[\alpha]_D$ +21° (c 0.59, $CHCl_3$). **3**: mp 133-135 °C (MeOH), $[\alpha]_D$ +57° (c 0.62, $CHCl_3$). **4**: $[\alpha]_D$ +43°. Since full NMR data for **1** and **2** have not been reported, these data are listed in Table 1 along with those of **5** for comparison. In addition to diterpenes **1**-**5**, five known sesquiterpenes, (1*S*,6*S*,7*R*,10*R*)-10-isothiocyanato-4-cadinene (356 mg), (1*S**,4*S**,5*R**,6*S**,7*S**,10*S**)-1-isothiocyanatoaromadendrane (50 mg), epipolasin A (14 mg), β-cubebene (11 mg), and (1*S**,4*R**,5*S**,10*S**)-10-isothiocyanatoguaia-6-ene (6 mg), were isolated from other fractions.

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10 LREIMS *m/z* 290 (M^+, 25), 272 (70), 191 (100 rel %).

11 A solution of **1** (20.2 mg) in acetone was treated with 0.5 N H_2SO_4 as described[2,9] previously to give 19.0 mg of **2** as a single product which was identical with a sample isolated from the sponge.

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13 A referee informed us that the following abstract of a paper presented at a meeting described the isolation of **1**-**3** from the sponge *Halichondria panicea* (Pallas) collected in Okinawa: H. Nakamura, S. Tou, M. Takamatsu, J. Kobayashi, Y. Ohizumi, T. Kondo, and Y. Hirata, Abstracts of Papers, Annual Meeting of Chem. Soc. Jpn., April, 1990, p.1197.