Potent Inhibitors of Histamine Release, Two Novel Triterpenoids from the Okinawan Marine Sponge Penares incrustans

Noboru Shoji, Akemi Umeyama, Setsuko Motoki, Shigenobu Arihara, Toshimasa Ishida, Kyosuke Nomoto, Jun'ichi Kobayashi, and Masao Takei

J. Nat. Prod., **1992**, 55 (11), 1682-1685• DOI:
10.1021/np50089a021 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 11, pp. 1682-1685, November 1992

POTENT INHIBITORS OF HISTAMINE RELEASE, TWO NOVEL TRITERPENOIDS FROM THE OKINAWAN MARINE SPONGE *PENARES INCRUSTANS*

NOBORU SHOJI, AKEMI UMEYAMA,* SETSUKO MOTOKI, SHIGENOBU ARIHARA,

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770, Japan

TOSHIMASA ISHIDA,

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

KYOSUKE NOMOTO,

Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

JUN'ICHI KOBAYASHI,

Faculty of Pharmaceutical Sciences, Hokkaido University, Kitaku, Sapporo 060, Japan

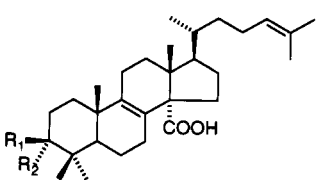
and MASAO TAKEI

La Jolla Institute for Allergy and Immunology, La Jolla, California 92937

ABSTRACT.—Two novel triterpenoids with an unusual 14-carboxyl group, penasterone [**1**] and acetylpenasterol [**2**], were isolated from the Okinawan marine sponge *Penares incrustans*. The structures of **1** and **2** were established mainly on the basis of nmr spectroscopic data. The relative stereochemistry of **1** was confirmed by single-crystal X-ray diffraction analysis. Compounds **1** and **2** potently inhibited histamine release from rat peritoneal mast cells induced by anti-IgE in a dose-dependent manner.

Numberous steroids have been isolated from marine organisms (1), but the isolation of tetracyclic triterpenoids, which are assumed to be biosynthetic precursors to steroids, is very rare. Penasterol [**3**] was the first and so far the only triterpenoid with a 14-carboxyl group obtained from marine organisms; it was isolated from *Penares* sp. by Cheng *et al.* in 1988 (2). In a continuation of our survey of sponges for pharmacologically active substances, we have focused our attention on the inhibitors of histamine release and have isolated two active metabolites from *Penares incrustans* Tanita (family Stellettidae) collected from Okinawa Island. We now report the isolation and structure elucidation of the two novel triterpenoids with a 14-carboxyl group, designated as penasterone [**1**] and acetylpenasterol [**2**].

The MeOH-toluene (3:1) extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was subjected to Si gel medium-pressure cc followed by Sephadex LH-20 cc to yield penasterone [**1**] and acetylpenasterol [**2**].

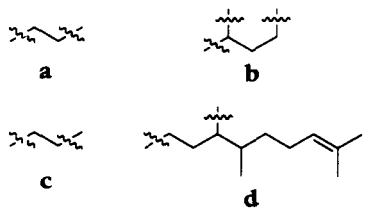


1 R_1, R_2=O
2 R_1=OAc, R_2=H
3 R_1=OH, R_2=H

Penasterone [**1**] was isolated as colorless crystals, mp 126–130°. The molecular formula $C_{30}H_{46}O_3$, determined by hreims (*m/z* calcd 454.3447, found 454.3446), required eight unsaturation equivalents. Of eight unsaturations, four are assigned to the tetracyclic triterpene ring system indicated by the positive Liebermann-Burchard reaction, one to a carbonyl group (δ_C 217.7; ir 1705 cm^{-1}), one to a carboxyl group (δ_C 181.7; ir 1720 cm^{-1}), and the other two to olefinic bonds (δ_C 139.1, 131.0, 128.3, and 125.1). The

^{1}H-nmr spectrum contained six methyl singlets at δ 0.80, 1.07, 1.09, 1.15, 1.59, 1.67, one methyl doublet at δ 0.93, and a signal at δ 5.07 (1H, t, J=7.3 Hz) assigned to the olefinic proton.

^{1}H-^{1}H COSY, COLOC, and HMBC experiments enabled us to construct the carbon skeleton. ^{1}H-^{1}H connectivity from COSY suggested the subunits **a–d**. The subunits **a–d** except for C-8 and C-14 could be connected from the COLOC and HMBC experiments (Figure 1). Finally, C-8 connected to C-14 from the consideration of the molecular formula. Penasterone [**1**] was thus represented by structure **1**.



The stereochemistry of penasterone [**1**] was established unequivocally by a chemical reaction and an X-ray analysis. Reduction of **1** with $NaBH_4$ gave penasterol [**3**] as a major product (2). The spectral data obtained for the reduced product were in complete agreement with those of penasterol [**3**].

Furthermore, an X-ray crystal structure confirmed the structural assignments based on nmr spectra.

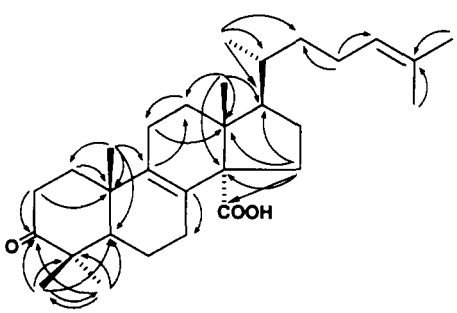


FIGURE 1. The HMBC and COLOC correlations for penasterone [**1**].

Acetylpenasterol [**2**] was obtained as colorless crystals and gave a parent ion in the hreims at *m/z* 498.3693 consistent with a molecular formula of $C_{32}H_{50}O_4$ (calcd 498.3709), differing from the molecular formula of **1** by addition of C_2H_4O. Examination of the ^{13}C-nmr and ^{1}H-nmr data obtained for acetylpenasterol [**2**] revealed that it differed from **1** only by having an acetoxyl group at the C-3 position. The ^{13}C-nmr spectrum of **2** showed an ester carbonyl at δ 171.0 instead of a carbonyl at δ 217.7 in **1**, and the ^{1}H-nmr spectrum of **2** an acetyl group at δ 2.05. The β-acetoxyl configuration could be assigned from the coupling constant of the H-3 proton at δ 4.51 (1H, dd, J=11.7, 4.4 Hz) and the ^{13}C-nmr chemical shift of C-3 (δ 80.7). The correlation of ^{1}H-^{1}H COSY, COLOC, and HMBC experiments for **2** was very similar to that obtained for **1**. Deacetylation of **2** with NaOMe gave a product **3**, which was identified as penasterol from a comparison of physicochemical properties.

Penasterone [**1**] and acetylpenasterol [**2**] potently inhibited histamine release from rat peritoneal mast cells induced by anti-IgE in a dose-dependent manner (3). The IC_{50} values for **1** and **2** were 1.5 μM and 10 μM, respectively. Compound **1** was about 17 times more potent than disodium cromoglycate (DSCG), a well-known antiallergy drug. Detailed studies of the pharmacological properties of **1** and **2** are in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: Yanagimoto micro melting point apparatus, Shimadzu IR-27G photometer (ir), JASCO DIP-360 polarimeter (optical rotation), JEOL JMS-HX-100 mass spectrometer (hrms), JEOL JNM-GX-400FT NMR spectrometer (^{1}H and ^{13}C nmr), and Rigaku AFC-5 computer-controlled diffractometer.

COLLECTION, EXTRACTION, AND ISOLATION PROCEDURES.—The sponge *P. incrustans* (1.2 kg wet wt) was collected by netting at Unten Bay (−70 m), Okinawa Island, in June 1988, and immediately frozen (−20°). A voucher specimen (US011) is deposited in the Herbarium of the

Department of Pharmacognosy, Tokushima Bunri University. The frozen sample (1.2 kg) was lyophilized and exhaustively extracted with MeOH-toluene (3:1) (2 liters×4) at room temperature for 1 d. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 ml×3). The EtOAc-soluble portion (10.7 g) was subjected to Si gel medium pressure cc [hexane-EtOAc (3:2)] followed by Sephadex LH-20 cc [MeOH-EtOAc (1:1)] to give **1** (380 mg, 0.032 % wet wt of the sponge) and **2** (175 mg, 0.015%).

Penasterone [**1**].—Colorless crystals: mp 126–130° (from MeOH), $[\alpha]^{25}D$ −18.2° (c=0.60, $CHCl_3$); ν max (KBr) 3650–2500, 2980, 1720, 1705, 1465, 1380, 1190, 1115 cm^{-1}; ^1H nmr ($CDCl_3$) see Table 1; ^{13}C nmr ($CDCl_3$) see Table 1; hreims m/z $[M]^+$ 454.3446 (calcd for $C_{30}H_{46}O_3$ Δ

TABLE 1. ^1H- and ^{13}C-nmr Data for Compounds **1** and **2**.[a]

Position	Compound			
	1		**2**	
	^1H	^{13}C	^1H	^{13}C
1	1.75 2.00	35.6 (t)	1.45 1.78	35.0 (t)
2	2.43 2.60	34.5 (t)	1.60 1.72	24.1 (t)
3		217.7 (s)	4.51	80.7 (d)
4		47.4 (s)		37.9 (s)
5	1.68	50.7 (d)	1.23	50.1 (d)
6	1.63	19.5 (t)	1.58 1.67	18.1 (t)
7	2.14	27.5 (t)	2.07	27.7 (t)
8		128.3 (s)		128.4 (s)
9		139.1 (s)		140.6 (s)
10		37.4 (s)		37.5 (s)
11	2.17	22.3 (t)	2.12 2.20	22.3 (t)
12	1.76 2.12	31.5 (t)	1.74 2.11	31.5 (t)
13		47.3 (s)		47.3 (s)
14		62.9 (s)		63.1 (s)
15	1.56 2.07	27.9 (t)	1.54 2.07	27.8 (t)
16	1.41 2.18	29.3 (t)	1.38 2.15	29.4 (t)
17	1.54	50.8 (d)	1.53	50.7 (d)
18	0.80 (3H, s)	17.7 (q)	0.78 (3H, s)	17.5 (q)
19	1.15 (3H, s)	19.0 (q)	1.05 (3H, s)	19.5 (q)
20	1.43	35.9 (d)	1.42	35.8 (d)
21	0.93 (3H, d, 5.9)	18.4 (q)	0.93 (3H, d, 6.6)	18.5 (q)
22	1.06	36.1 (t)	1.06 1.42	36.1 (t)
23	1.86 2.02	24.9 (t)	1.86 2.01	24.9 (t)
24	5.07 (1H, t, 7.3)	125.1 (d)	5.09 (1H, t, 7.3)	125.1 (d)
25		131.0 (s)		131.0 (s)
26	1.59 (3H, s)	17.6 (q)	1.59 (3H, s)	17.6 (q)
27	1.67 (3H, s)	25.7 (q)	1.68 (3H, s)	25.7 (q)
28	1.07 (3H, s)	21.3 (q)	0.89 (3H, s)	16.6 (q)
29	1.09 (3H, s)	26.2 (q)	0.88 (3H, s)	27.9 (q)
30		181.7 (s)		181.6 (s)
-OC(=O)Me				171.0 (s)
-OC(=O)*Me*			2.05 (3H, s)	21.3 (q)

[a]Chemical shifts (ppm) from $CDCl_3$ (multiplicity, J in Hz).

−0.1 mmu); eims 454 $[M]^+$ (48), 410 (92), 409 (100), 297 (27), 257 (75).

REDUCTION OF COMPOUND **1**.—To an MeOH solution of compound **1** (60 mg) was added an excess of $NaBH_4$, and the mixture was stirred under reflux for 2 h. After workup, the residue was passed through a short Si gel column (2% MeOH in $CHCl_3$) to furnish compound **3** (35 mg): hreims *m/z* $[M]^+$ 456.3596 (calcd for $C_{30}H_{48}O_3$ Δ −0.8 mmu); ^1H nmr [$(CD_3)_2SO$] δ 0.69 (3H, s), 0.70 (3H, s), 0.88 (3H, d, J=7.0 Hz), 0.89 (3H, s), 0.95 (3H, s), 1.55 (3H, s), 1.63 (3H, s), 2.99 (1H, dd, J=5.7, 10.3 Hz), 5.06 (1H, t, J=7.0 Hz); ^{13}C nmr ($CDCl_3$) δ 179.9 (s), 140.7 (s), 131.0 (s), 128.2 (s), 125.1 (d), 78.9 (d), 63.0 (s), 50.7 (d), 50.1 (d), 47.2 (s), 38.9 (s), 37.6 (s), 36.1 (t), 35.8 (d), 35.3 (t), 31.5 (t), 29.3 (t), 27.9 (q), 27.8 (t), 27.7 (t), 27.7 (t), 25.7 (q), 24.8 (t), 22.3 (t), 19.4 (q), 18.4 (q), 18.2 (t), 17.7 (q), 17.5 (q), 15.5 (q).

CRYSTAL DATA FOR **1**.—$C_{30}H_{46}O_3$, M=470.694, orthorhombic, space group $P2_12_12$, a=11.547 (3), b=29.951 (9), c=7.935 (1) Å, U=2744.2 Å, Z=4, D_c=1.139 $g \cdot cm^{-3}$, F(000)=1032, μ(CuKα)=0.6861 cm^{-1}. The final R and Rw factors were 0.0801 and 0.0998 (w=1.0), respectively.[1]

Acetylpenasterol [**2**].—Colorless crystals: mp 185–187°; $[\alpha]^{25}D$ −44.7° (c=0.59, $CHCl_3$); ν max (KBr) 3650–2500, 2980, 2960, 2890, 1740, 1700, 1460, 1375, 1250, 1030, 980 cm^{-1}; ^1H nmr ($CDCl_3$) see Table 1; ^{13}C-nmr ($CDCl_3$) see Table 1; eims $[M]^+$ 498 (rel. % 23), 453 (100), 423 (4), 393 (57); hreims $[M]^+$ 498.3693 (calcd for $C_{32}H_{50}O_4$, Δ −1.6 mmu).

DEACETYLATION OF COMPOUND **2**.—To a solution of compound **2** (30 mg) in MeOH-CH_2Cl_2 (1:1) was added NaOMe (3% solution, 0.2 ml) under N_2 atmosphere. The reaction mixture was stirred at room temperature for 48 h, diluted with EtOAc, and extracted with 10% HOAc. The organic extracts were washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure and purification of the residue on a Si gel column (30% EtOAc in hexane) afforded compound **3** (10 mg): ^1H nmr [$(CD_3)_2SO$] 0.69 (3H, s), 0.70 (3H, s), 0.88 (3H, d, J=7.0 Hz), 0.89 (3H, s), 0.95 (3H, s), 1.55 (3H, s), 1.63 (3H, s), 2.99 (1H, dd, J=5.7, 10.3 Hz), 5.06 (1H, t, J=7.0 Hz); ^{13}C nmr ($CDCl_3$) δ 179.9 (s), 140.7 (s), 131.0 (s), 128.2 (s), 125.1 (d), 78.9 (d), 63.0 (s), 50.7 (d), 50.1 (d), 47.2 (s), 38.9 (s), 37.6 (s), 36.1 (t), 35.8 (d), 35.3 (t), 31.5 (t), 29.3 (t), 27.9 (q), 27.8 (t), 27.7 (t), 27.7 (t), 25.7 (q), 24.8 (t), 22.3 (t), 19.4 (q), 18.4 (q), 18.2 (t), 17.7 (q), 17.5 (q), 15.5 (q).

BIOASSAY METHOD.—Rat peritoneal mast cells were purified using a method of Nemeth and Rohlich (4). Viability of the cells was 97% as assessed by trypan blue exclusion. Passively sensitized rat mast cells were prepared as described previously (5).

Assay method of histamine release in vitro has been previously reported (6).

ACKNOWLEDGMENTS

We thank Prof. P.R. Bergquist, University of Auckland, for her kind identification of the marine sponge and Mr. Z. Nagahama for help with collections. We are grateful to Dr. Z. Taira for his helpful advice. We are indebted to Dr. M. Toyota and Ms. I. Okamoto for measurements of mass spectra.

LITERATURE CITED

1. R.G. Kerr and B.J. Baker, *Nat. Prod. Rep.*, **8**, 465 (1991).
2. J.F. Cheng, J. Kobayashi, H. Nakamura, Y. Ohizumi, Y. Hirata, and T. Sasaki, *J. Chem. Soc., Perkin Trans. 1*, 2403 (1988).
3. K. Saeki, *Jpn. J. Pharmacol.*, **14**, 375 (1964).
4. A. Nemeth and P. Rohlich, *Eur. J. Cell. Biol.*, **20**, 272 (1980).
5. M. Takei, T. Matsumoto, K. Endo, and M. Muramatsu, *Agents Actions*, **25**, 17 (1988).
6. A. Umeyama, N. Shoji, M. Takei, K. Endo and S. Arihara, *J. Pharm. Sci.*, **81**, 661 (1992).

Received 5 June 1992

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK.