Pergamon

PII: S0031-9422(97)00382-8

Phytochemistry, Vol. 46, No. 5, pp. 959–961, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

A LANOSTANE-TYPE TRITERPENE FROM A MUSHROOM *DAEDALEA DICKINSII*

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(*Received in revised form* 4 *April* 1997)

Key Word Index—*Daedalea dickinsii*; Polyporaceae; collagenase inhibitor; lanostane; triterpene; polyporenic acid C.

Abstract—A novel lanostane-type triterpene and a known compound, polyporenic acid C, were isolated as collagenase inhibitors from the mushroom *Daedalea dickinsii*. © 1997 Elsevier Science Ltd

INTRODUCTION

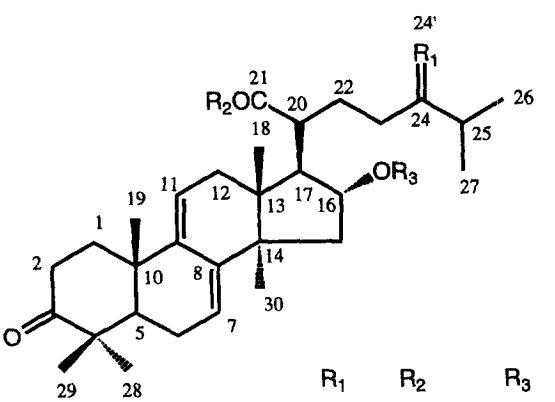
Collagenase inhibitors are candidates of the medicines for the treatment of rheumatism, metastasis, or periodontal disease [1–9]. In the course of screening for the inhibitory activity of some mushroom extracts, we found a strong inhibitory activity of the extract of *Daedalea dickinsii*. We describe now the isolation, structure and biological activity of a new lanostane-type triterpene and a known one.

RESULTS AND DISCUSSION

Fresh fruiting bodies of *Daedalea dickinsii* were extracted with 85% ethanol, and the extract was concentrated and partitioned between ethyl acetate and water. Repeated silica gel chromatography and HPLC of the ethyl acetate extract gave **1** and **2**.

Compound **1** showed an ion peak at *m/z* 483 $[M+H]^+$ by FAB mass spectrometry. The NMR data are shown in Table 1. All the data of **1** agreed with those of polyporenic acid C [10, 11].

The molecular formula $C_{30}H_{44}O_5$ of **2** was assigned by HRFAB mass spectrometry from the $[M-H+2Na]^+$ ion peak at *m/z* 529.2941 (529.2906, calcd $C_{30}H_{43}O_5Na_2$). The NMR data of **2** were very similar to those of **1**. However, **2** had an additional carbonyl group (δ 214.4 in ^{13}C NMR) instead of the exomethylene signals (^{13}C NMR: δ 107.9 and 154.9; ^1H NMR: δ 4.71 *s*, 4.76 *s*) of **1**. These data indicated that **1** was 20-carboxy-16-hydroxy-21-nor-5α-7,9(11)-lanostadien-3,24-dione. The structure was confirmed by HMBC experiments. The cross peaks observed were as follows; H-1/C-2, H-1/C-10, H-1/C-19, H-2/C-1, H-2/C-3, H-5/C-4, H-5/C-10, H-5/C-28, H-5/C-29, H-6/C-7, H-6/C-10, H-7/C-9, H-11/C-8, H-12/C-13, H-12/C-14, H-12/C-18, H-15/C-13, H-15/C-14, H-15/C-17, H-15/C-30, H-16/C-14, H-16/C-20, H-17/C-12, H-17/C-13, H-17/C-14, H-17/C-18, H-17/C-20, H-17/C-21, H-18/C-12, H-18/C-13, H-18/C-14, H-18/C-17, H-19/C-1, H-19/C-5, H-19/C-9, H-19/C-10, H-20/C-17, H-20/C-21, H-22/C-20, H-22/C-21, H-22/C-23, H-23/C-20, H-23/C-22, H-23/C-24, H-25/C-24, H-25/C-26, H-25/C-27, H-26/C-24, H-26/C-25, H-



	R_1	R_2	R_3
1	CH_2	OH	H
2	O	OH	H
3	CH_2	OH	Ac
4	CH_2	NHOH	H
5	CH_2	NHOH	Ac

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Table 1. NMR data for compounds **1** and **2** (500 MHz in $CDCl_3$)

Position		δ ppm (multiplicity, J in Hz) **1**		**2**
1	36.6	1.72 (*ddd*, 14.03, 13.74, 4.58), 2.20(*m*)	36.3	1.72 (*ddd*, 14.03, 13.88, 4.58), 2.22(*m*)
2	34.7	2.32 (*ddd*, 14.65, 4.58, 3.30)	34.7	2.33 (*ddd*, 14.65, 4.42, 3.36)
		2.74 (*ddd*, 14.65, 14.03, 5.79)		2.74 (*ddd*, 14.65, 14.03, 5.80)
3	216.4		216.7	
4	47.4		47.5	
5	50.6	1.52 (*dd*, 11.90, 3.66)	50.6	1.52 (*dd*, 11.90, 3.66)
6	23.6	2.04 (*m*)	23.6	2.05 (*ddd*, 13.66, 6.56, 3.66)
		2.20 (*m*)		2.08 (*dd*, 13.66, 11.90)
7	120.8	5.48 (*br d*, 6.72)	120.8	5.49 (*d*, 6.56)
8	141.8		141.7	
9	144.3		144.4	
10	37.3		37.2*	
11	116.7	5.33 (*br d*, 6.41)	116.5	5.33 (*d*, 6.11)
12	35.4	1.81 (*dd*, 17.39, 6.41)	35.5	1.77 (*dd*, 17.40, 6.11)
		2.27 (*br d*, 17.39)		2.28 (*m*)
13	44.7		44.6	
14	48.7		48.7	
15	43.4	1.47 (*br d*, 13.73), 2.22 (*m*)	43.3	1.48 (*br d*, 13.42), 2.22 (*m*)
16	76.5	4.11 (*dd*, 6.41, 7.93)	76.5	4.21 (*dd*, 6.41, 7.94)
17	57.0	2.19 (*m*)	56.3	2.22 (*m*)
18	17.2	0.62 (*s*)	17.3	0.60 (*s*)
19	22.0	1.15(*s*)	22.0	1.15 (*s*)
20	46.3	2.48 (*ddd*, 11.30, 10.98, 3.36)	45.4	2.46 (*ddd*, 11.14, 10.83, 3.51)
21	180.2		179.8	
22	30.4	1.86 (*m*), 2.12 (*m*)	25.3	1.85 (*m*)
23	32.1	2.01 (*m*), 2.07 (*m*)	37.3*	2.55 (*m*)
24	154.9		214.4	
25	33.7	2.22 (*m*)	41.0	2.57 (*m*)
26	21.7*	0.99 (*d*, 6.71)*	18.2†	1.07 (*d*, 6.86)*
27	21.9*	1.01 (*d*, 7.41)*	18.3†	1.08 (*d*, 7.07)*
28	22.4†	1.07 (*s*)†	22.4‡	1.08 (*s*)†
29	25.4†	1.10(*s*)†	25.3‡	1.10 (*s*)†
30	26.0	1.08 (*s*)	26.0	1.07 (*s*)
24′	107.1	4.71 (*s*), 476 (*s*)		

*, †, ‡ Between the same character, the data are interchangable.

26/C-27, H-28/C-3, H-28/C-4, H-28/C-29, H-29/C-3, H-29/C-4, H-29/C-28, H-30/C-8, H-30/C-13, H-30/C-14, H-30/C-15.

The two compounds showed inhibitory activity against human collagenase; IC_{50} of **1** was 126 μM. The inhibitory activity of **2** (14.7% at 1.5 mM) was much weaker than that of **1**. In order to examine the relationship between structure and biological activity, derivatives **3** to **5** were prepared from **1**. The activity of **4** (IC_{50} = 30.7 μM) was stronger than those of **1**, **3** (143 μM), and **5** (more than 200 μM). Since **1** and **2** did not inhibit other metallo-proteinases such as collagenase from *Clostridium* or angiotensin converting enzyme, the inhibitory activity of the compounds was not due to the chelation of metal cations.

EXPERIMENTAL

Mp: uncorr. ^{1}H NMR: 400 or 250 MHz as int. standard. ^{13}C NMR: 100 MHz.

The matured fruiting bodies of *Daedalea dickinsii* (Berk. ex Cke.) Yasuda were collected at Tochigi prefecture, Japan, in September, 1993, and identified by Dr. S. Inoue.

Extraction and Isolation. The whole fresh fruiting bodies of *D. indusiata* (7.36 kg) were extracted with 85% EtOH (30 l × 3) and the solvent was concd under red. pres. and partitioned between EtOAc and H_2O. The residue (234 g) obtained after removing EtOAc, was fractionated by repeated silica gel CC and HPLC to give **1** (6.0 mg, mp 273–275°) as white crystals and **2** (2.5 mg) as colourless amorphous solid.

Compound **1**. $[\alpha]_D^{25}$ +17° (c = 0.50, $CHCl_3$). IR ν_{max} cm^{-1}: 3405, 1720, 1683.

Compound **2**. $[\alpha]_D^{25}$ +3.7 (c = 0.50, MeOH). IR ν_{max} cm^{-1}: 3349, 1708, 1677.

Collagenase (MMP-1) inhibitory assay. Inhibitory activity of the compounds against MMP-1 was assessed by a modification of the collagen fibril assay using fluorescein isothiocyanate (FITC)-labelled soluble type-I collagen [12]. Soluble type-I collagen was labelled with FITC as described by Terato *et al.* [13].

Each compound (1.0 mg) was dissolved in 20 μl of dimethyl sulphoxide and diluted 1:250 with a 50 mM Tris–HCl buffer (pH 7.5) containing 5 mM $CaCl_2$, 0.05% Briji 35, and 0.02% NaN_3. The diluted sample soln (50 μl) was mixed with 50 μl of trypsin-activated MMP-1 (0.38 Uml^{-1}). After 100 μl of FITC-labelled soluble type-I collagen soln (0.5 mg ml^{-1}) was gently added, the mixt. was incubated at 35° for 16 hr. The reaction was terminated by addition of 600 μl of a cold buffered EtOH soln (7 parts of EtOH to 8 of 0.1 M Tris–HCL, pH 8.8, containing 0.4 M NaCl and 3 mM $CaCl_2$). The reaction mixt. was vigorously vortexed and centrifuged at 6000 *g* for 3 min. The enzyme activity was estimated by measuring the fluorescence intensity of the supernatant at 520 nm on excitation at 495 nm. The inhibitory activity was expressed as the extract concn required for 50% inhibition (IC_{50}) or percentage of the inhibition to control.

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