Communications to the Editor

6-DEOXYILLUDIN M, A NEW ANTITUMOR ANTIBIOTIC: FERMENTATION, ISOLATION AND STRUCTURAL IDENTIFICATION

Sir:

A new antitumor antibiotic, 6-deoxyilludin M (**1**) was isolated from the culture broth of the Basidiomycetes, *Pleurotus japonicus*. This compound, which is structurally related to illudin M differing in the absence of the 6-OH group, is active against experimental murine leukemia P388. In this communication, we report the fermentation, isolation and structural identification of **1** and the coproduced 6-deoxyilludin S (**2**).

Agar slant cultures of *P. japonicus* ATCC 20195 were used to inoculate seed flasks containing 50-ml of a medium consisting of peptone (Kyokutou) 5 g, yeast extract 5 g, glucose 10 g, vegetative juice (V-8) 50 ml, $CaCO_3$ 3 g and malt extract 2 g per liter of deionized water. The inoculum was cultivated at 25°C for 2 days and added at the rate of 5% to the fermentation medium consisting of sucrose 50 g, soybean meal 20 g, $CaCO_3$ 5 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g and antiform agents LG-109 (Asahi Denka Kogyo) and KM-70 (Shinetsu

Table 1. Physico-chemical properties of **1** and **2**.

	1	**2**
Appearance	Pale yellow amorphous solid	Pale yellow amorphous solid
Molecular formula	$C_{15}H_{20}O_2$	$C_{15}H_{20}O_3$
MW (EI-MS, *m/z*)	232.1462	248.1411
$[\alpha]_D^{23}$ (*c* 1.0, MeOH)	−11°	−13°
UV λ_{max}^{MeOH} (nm)	248 (sh), 320	248, 320
IR ν_{max}^{KBr} (cm^{-1})	3480, 2950, 2920, 2850, 1690, 1600	3450, 2950, 2920, 2850, 1690, 1600
Rf value[a]	0.85	0.65
Solubility		
Soluble	MeOH, EtOAc, $CHCl_3$, Me_2CO	MeOH, EtOAc, $CHCl_3$, Me_2CO
Insoluble	Hexane, H_2O	Hexane, H_2O

[a] Silica gel TLC (Merck 5715), solvent; toluene - Me_2CO (7 : 3).

Table 2. ^1H NMR data of **1** and **2** (in DMSO-d_6).

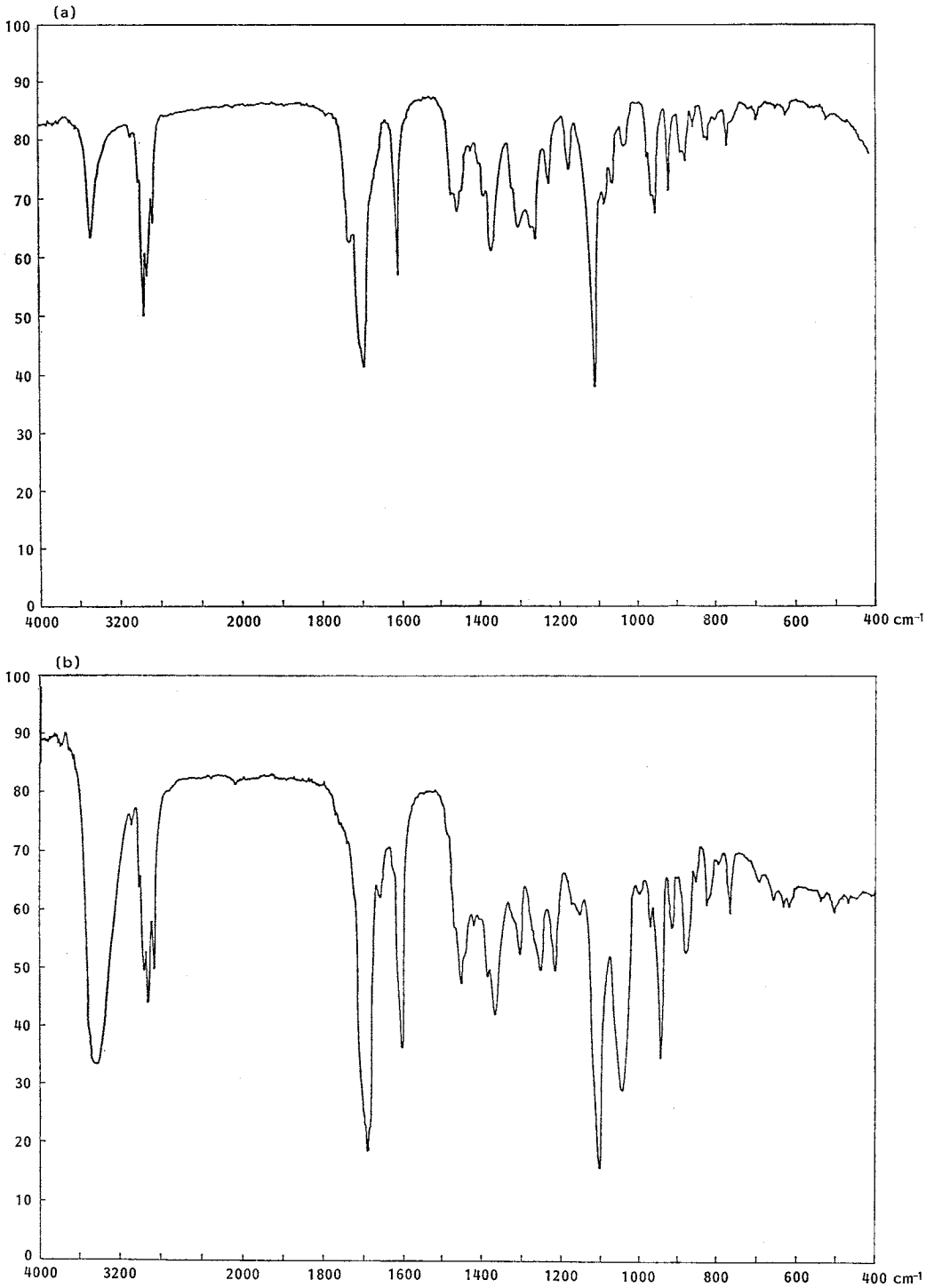
Proton No.	**1**	**2**
2-OH	4.90 s	4.86 s
6-CH_2	2.43 s	2.59 d (*J*=16.1 Hz), 2.23 d (*J*=16.1 Hz)
8-H	6.48 s	6.48 s
10-CH_3	1.19 s	1.20 s
11-CH_2	0.32 m, 0.63 m	0.31 m, 0.64 m
12-CH_2	0.80 m, 0.97 m	0.82 m, 1.03 m
13-CH_3	1.43 s	1.44 s
15-CH_3	1.14 s	1.10 s
14-CH_3	1.12 s	—
14-CH_2	—	3.30 m
14-OH	—	4.82 t (*J*=5.5 Hz)

Table 3. ^{13}C NMR data of **1** and **2** (in DMSO-d_6).

Carbon No.	**1**	**2**
C-1	199.8 s	201.8 s
C-2	75.9 s	77.7 s
C-3	31.5 s	32.5 s
C-4	136.0* s	139.1* s
C-5	126.6* s	128.4* s
C-6	42.9 t	39.4 t
C-7	44.1 s	51.6 s
C-8	147.2 d	145.8 d
C-9	135.9* s	137.5* s
C-10	28.7** q	24.9** q
C-11	5.0 t	5.8 t
C-12	7.6 t	8.4 t
C-13	14.5 q	14.9 q
C-14	24.5** q	69.7 t
C-15	27.9** q	24.1** q

*,** Assignment may be reversed.

Fig. 1. IR absorption spectra (KBr) of **1** (a) and **2** (b).



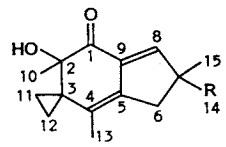
Kagaku) per liter of deionized water. The pH of medium was adjusted to 7.0 prior to sterilization. The jar fermentor was stirred at 300 rpm and aerated with 1 vol/vol/minute. At harvest (200 hours) the pH was 5.8 to 5.4. Total antibacterial activity reached a maximum at 180 hours measured by the paper-disc method on nutrient agar using *Bacillus subtilis* as the test organism.

The culture liquor was filtered and the filtrate (15 liters) was applied to a column of Diaion HP-20, the column was washed with deionized water - MeOH (8 : 2) then eluted with MeOH. The active fractions were combined, and evaporated to dryness. Further purification was effected by two stages of silica gel chromatography using toluene - Me_2CO (20 : 1) and hexane - EtOAc (7 : 3) as eluents to yield 30 mg of **1** and 120 mg of **2**.

Physico-chemical properties of **1** and **2** are summarized in Table 1. ^1H and ^{13}C NMR data are shown in Tables 2 and 3, respectively. The molecular formula of **1** and **2** were deduced as $C_{15}H_{20}O_2$ (*m/z* 232.1462) and $C_{15}H_{20}O_3$ (*m/z* 248.1411) from electron ion mass spectrum (EI-MS). **1** and **2** have nearly identical UV spectra (MeOH), λ_{max} nm 248 (sh), 320 and λ_{max} nm 248, 320, suggesting the presence of cross-conjugated dienone. The characteristic absorptions attributed to OH and C=O were observed in IR spectra (Fig. 1). Evidence for the illudin-related structure of both compounds (Fig. 2) was obtained by spectroscopic analysis. The ^1H and ^{13}C NMR spectra of **1** are quite similar to that of illudin M[1~4] except that methylene resonances, δ 2.43 (^1H) and 42.9 (^{13}C) are observed for **1** instead of the methine resonance (C-6) of illudin M. The ^1H and ^{13}C NMR data of **2** are also quite similar to that of illudin S[1~4] except for the appearance of methylene resonances, δ 2.23, 2.59 (^1H) and 39.4 (^{13}C) instead of the methine resonance (C-6) of illudin S.

1 exhibited weak activity against *B. subtilis* (MIC; 50 μg/ml by agar dilution methods) but did not show antimicrobial activity against the following bacteria and fungi: *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Shigella sonnei*, *Salmonella typhosa*, *Klebsiella pneumoniae* and *Candida albicans*. **1** was effective against murine leukemia P388, showing significant increase of life span (ILS 24%) at a daily dose of 5 mg/kg for 5 days (ip). In contrast to this, **2** and illudin S were ineffective against murine leukemia P388 although illudin S is reported to exhibit antitumor activity against murine Ehrlich ascites tumor[5]. Detailed studies on the antitumor activity of **1** are in progress and will be published elsewhere.

Fig. 2. The structure of 6-deoxyilludin M (**1**) and 6-deoxyilludin S (**2**).



1 R = CH_3
2 R = CH_2OH

Acknowledgments

The authors are grateful to Dr. FUSAO TOMITA for his continuing interest and to Miss MITSUE AOYAGI for technical assistance.

MITSUNOBU HARA
MAYUMI YOSHIDA
MAKOTO MORIMOTO†
HIROFUMI NAKANO

Tokyo Research Laboratories,
Kyowa Hakko Kogyo Co., Ltd.,
Machida-shi, Tokyo, Japan
†Pharmaceutical Research Laboratories,
Kyowa Hakko Kogyo Co., Ltd.,
Nagaizumi-cho, Shizuoka, Japan

(Received May 9, 1987)

References

1) McMORRIS, T. C. & M. ANCHEL: Fungal metabolites. The structure of the novel sesquiterpenoid illudin-S and -M. J. Am. Chem. Soc. 87: 1594~1600, 1965
2) MATSUMOTO, T.; H. SHIRAHAMA, A. ICHIHARA, Y. FUKUOKA, Y. TAKAHASHI, Y. MORI & M. WATANABE: Structure of lampterol (illudin S). Tetrahedron 21: 2671~2676, 1965
3) NAKANISHI, K.; M. OHASHI, M. TADA & Y. YAMADA: Illudin S (lampterol). Tetrahedron 21: 1231~1246, 1965
4) PETER, A.; W. BRADSHAW, J. R. HANSON & I. H. SADLER: Studies in terpenoid biosynthesis.

Part 26. Application of ^{1}H and ^{13}C N.M.R. spectroscopy to the biosynthesis of the illudin sesquiterpenoids. J. Chem. Soc. Perkin Trans. I 1982: 2445~2448, 1982

5) SHINOZAWA, S.; K. TSUTSUI & T. ODA: Enhancement of the antitumor effect of illudin S by including it into liposomes. Experientia 35: 1102~1103, 1979