ISOLATION AND STRUCTURAL ELUCIDATION OF SEKOTHRIXIDE, A NEW MACROLIDE EFFECTIVE TO OVERCOME DRUG-RESISTANCE OF CANCER CELL

Sir:

The development of multidrug-resistance (MDR) is a major limiting factor to successful chemotherapy of cancer in humans. MDR is a form of drug-resistance characterized by decreased cellular sensitivity to a broad range of chemotherapeutic agents. This resistance is due at least in part to the *mdr*1 gene product, P-glycoprotein, and results in the failure of chemotherapeutic treatment[1].

During the screening for new pharmacologically active agents which will overcome MDR with colchicine-resistant KB cell line (KB-C2)[2] we isolated a new antibiotic stipiamide from *Myxococcus stipitatus*[3]. In this paper, we report the isolation and structural elucidation of a new macrolide, named sekothrixide, which exhibits cytocidal activity against KB-C2 in the presence of colchicine (1.5 μg/ml).

The producing microorganism, *Saccharothrixide* sp. CF24, was isolated from a soil sample collected at Yokose, Saitama, Japan†.

Fermentation was carried out for 3 days at 27°C in a 50-liter jar fermenter containing 30 liters of medium with agitation of 400 rpm and aeration of 30 liters/minute. The medium consisted of soluble starch 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ 0.4% (pH 6.2 before sterilization). The mycelium cake, harvested by centrifugation from the whole fermented broth, was stirred with 5 liters of acetone. The solvent extract was concd *in vacuo* to a small volume and the active material was extracted from the aqueous residue twice with 500 ml each of EtOAc. The separated organic layer was dried over Na_2SO_4 and concd *in vacuo*. The oily residue was applied to a column of silica gel (Wakogel C-200, 6 × 36 cm) packed with $CHCl_3$ and the column was developed with $CHCl_3$-MeOH (150:1). Active fractions were combined and concentrated to give a brownish oil. Further purification of the oil was achieved by silica gel preparative TLC (Merck, Kieselgel 60F_{254}, 0.5 mm) developed with $CHCl_3$ MeOH (19:1) followed by reversed phase chromatography (SEP-PAK C_{18}) developed with MeOH-H_2O (87:13) to yield 5 mg of sekothrixide as a colorless oil (wax appearance, mp 40.5°C). Its physico-chemical properties are summarized in Table 1.

Table 1. Physico-chemical properties of sekothrixide.

Appearance	Colorless oil
MP	40.5°C
Molecular formula	$C_{25}H_{50}O_6$
Solubility	
Soluble in	Benzene, $CHCl_3$, MeOH
Insoluble in	Hexane, H_2O
Rf value	0.75 ($CHCl_3$-MeOH, 19:1)
(Silica gel 60)	0.71 (Hexane-EtOAc, 2:3)
HRFAB-MS (*m/z*)	483.3665 $(M+H)^+$, Calcd: 483.3686
UV λ_{max}^{MeOH} nm (ε)	207 (6,370)
$\lambda_{max}^{MeOH+NaOH}$ nm (ε)	207 (20,000), 276 (5,450)
IR ν_{max} ($CHCl_3$) cm^{-1}	3460, 1729, 1706
$[\alpha]_D^{22}$ (*c* 1.0, MeOH)	−45.13°

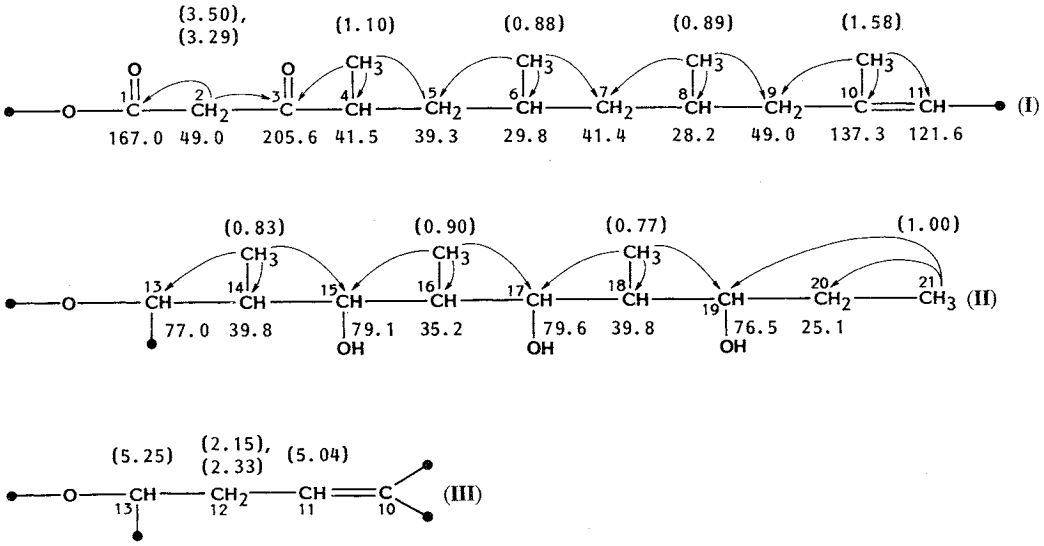
Table 2. ^{13}C and ^1H NMR signals of sekothrixide taken in $CDCl_3$.

No.	δ_C	δ_H
1	167.0	
2	49.0	3.50 (1H, d, *J*=13 Hz), 3.29 (1H, d, *J*=13 Hz)
3	205.6	
4	41.5	2.91 (1H, m)
5	39.3	0.94 (1H, m), 1.88 (1H, m)
6	29.8	1.55 (1H, m)
7	41.4	0.51 (1H, ddd, *J*=7 Hz), 1.30 (1H, ddd, *J*=7 Hz)
8	28.2	1.61 (1H, m)
9	49.0	2.00 (1H, dd, *J*=6, 13 Hz), 1.69 (1H, dd, *J*=6, 13 Hz)
10	137.3	
11	121.6	5.04 (1H, dd, *J*=7 Hz)
12	27.1	2.15 (1H, br d, *J*=13 Hz), 2.33 (1H, m)
13	77.0	5.25 (1H, d, *J*=11 Hz)
14	39.8	2.03 (1H, m)
15	79.1	3.62 (1H, d, *J*=10 Hz)
16	35.2	1.67 (1H, m)
17	79.6	3.84 (1H, d, *J*=10 Hz)
18	39.8	1.88 (1H, m)
19	76.5	3.72 (1H, br s)
20	25.1	1.53 (2H, m)
21	11.1	1.00 (3H, t, *J*=7.5 Hz)
4-CH_3	19.3	1.10 (3H, d, *J*=7 Hz)
6-CH_3	23.3	0.88 (3H, d, *J*=7.5 Hz)
8-CH_3	21.1	0.89 (3H, d, *J*=6 Hz)
10-CH_3	16.6	1.58 (3H, s)
14-CH_3	10.8	0.83 (3H, d, *J*=7.5 Hz)
16-CH_3	3.9	0.90 (3H, d, *J*=7.5 Hz)
18-CH_3	12.0	0.77 (3H, d, *J*=7.5 Hz)

† The paper pertaining to the taxonomy and isolation of this strain will be published elsewhere.

Fig. 1. Partial structures of sekothrixide revealed by HMBC (**I** and **II**) and ^{1}H-^{1}H COSY (**III**).

Values and those in parentheses show ^{13}C- and ^{1}H-chemical shifts in ppm, respectively. Arrows mean long range ^{13}C-^{1}H couplings.



The molecular formula of sekothrixide was determined to be $C_{28}H_{50}O_6$ by HRFAB-MS data (*m/z* 483.3665 $(M+H)^+$, Calcd: 483.3686). The ^{13}C and ^{1}H NMR spectra data of sekothrixide are tabulated in Table 2. The signals in the ^{13}C NMR spectrum at δ_C 167.0 and 205.6 were assigned to an ester and a ketone due to IR absorption bands at 1729 and 1706 cm^{-1}, respectively.

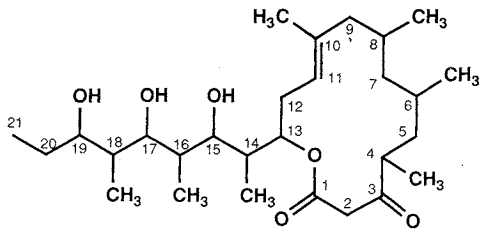
Functional groups in sekothrixide were classified as $CH_3 \times 8$, $CH_2 \times 6$, $CH \times 6$, $CH{-}O \times 4$, $CH{=} \times 1$, $C{=} \times 1$, $COO \times 1$ and $CO \times 1$. These accounted for three of the four degrees of unsaturation required by the molecular formula indicating sekothrixide to be monocyclic.

Detailed NMR spectral analysis of sekothrixide was aided by the use of heteronuclear multiple-bond correlation (HMBC)[4,5], since it is a particularly effective method for the structural elucidation of complicated molecules with many methyl groups[6]. The partial structure **I** in Fig. 1 was determined through the analysis of ^{1}H-^{13}C long range couplings observed with methyl groups. A methyl proton doublet at δ_H 1.10 (4-CH_3) was coupled to a ketone (δ_C 205.6, C-3), a non-oxygenated methine (δ_C 41.5, C-4) and a methylene (δ_C 39.3, C-5). Another methyl doublet at δ_H 0.88 (6-CH_3) was coupled to a methylene (δ_C 41.4, C-7), a methine (δ_C 29.8, C-6) and the methylene just explained (C-5). These results provided the connectivity of $-C3(=O)-C4H(CH_3)-C5H_2-C6H(CH_3)-C7H_2-$. By repeating the same procedure, this partial connectivity was extended to C-11. In addition, non-equivalent methylene proton doublets at δ_H 3.50 and 3.29 (2-H) showed cross peaks with an ester carbonyl signal at δ_C 167.0 (C-1) and the ketone signal at δ_C 205.6 (C-3). The partial structure thus established as shown in Fig. 1 (**I**) contained a β-ketoester group, whose system was supported by the UV absorption of sekothrixide at 276 nm in alkali solution. Further HMBC spectral analysis reveraled the remaining connectivity of the molecule from C-13 to C-21. The cross peaks between methyl proton signals (14-CH_3, 16-CH_3, 18-CH_3 and C-21) and oxygenated methines (C-15, C-17 and C-19), non-oxygenated methines (C-14, C-16 and C-18) or a methylene (C-20) enabled to determine the partial carbon skeleton from C-13 to C-21 (Fig. 1, **II**).

These partial structures **I** and **II** were connected through a non-equivalent methylene (12-H) at δ_H 2.15 and 2.33, which showed ^{1}H-^{1}H couplings to 13-H and 11-H (Fig. 1, **III**).

The formation of a macrolide ring at C-13 in sekothrixide was indicated by the low field chemical shift of 13-H at 5.25 ppm. The geometry of the doublet bond (C-10 and C-11) was determined to be *E* based on the high field shift of 10-CH_3 (δ_C 16.6)[7]. Sekothrixide was thus demonstrated to be a 14-membered β-ketolactone with a side chain (Fig. 2). Its relative and absolute configurations remain to be established. Unlike known 14-membered

Fig. 2. Planar structure of sekothrixide.



macrolide antibiotics having a β-ketoester system such as rustmicin[8], neorustmicin[9] and pikromycin[10], sekothrixide is unique in possessing a polyoxygenated long side chain.

Sekothrixide exhibited neither antibacterial nor antifungal activity at a concentration of 1,000 μg/ml. It inhibited in a dose dependent fashion, the growth of KB cells and its colchicine-resistant cells (KB-C2) with an IC_{50} of 6.5 μg/ml in EAGLE's minimum essential medium (EMEM) without colchicine. In the medium containing colchicine (1.5 μg/ml), sekothrixide showed synergistic activity against KB-C2 with an IC_{50} of 1.0 μg/ml. Further studies on the stereochemistry and action mechanism of sekothrixide are in progress.

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