ISOLATION AND STRUCTURE OF A NEW ANTIBIOTIC, INDOLIZOMYCIN, PRODUCED BY A STRAIN SK2-52 OBTAINED BY INTERSPECIES FUSION TREATMENT

Sir:

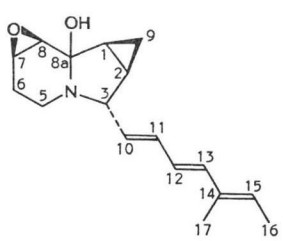
As reported in our separate papers,[1,2] we obtained the strain SK2-52 by protoplast fusion treatment between non-antibiotic-producing mutants of *Streptomyces griseus* and *S. tenjimariensis* and this produced a new antibiotic. In this communication, the isolation, characterization and structural elucidation of the antibiotic, indolizomycin are reported.

The strain SK2-52 was cultured at 27°C for 37 hours on a rotatory shaker (180 rpm) in a 500-ml flask containing 110 ml of a medium [2.0% dextrin, 2.0% glycerol, 0.3% yeast extract, 1.0% Bacto-Soytone (Difco), 0.2% $(NH_4)_2SO_4$ and 0.2% $CaCO_3$, pH 7.4]. Vegetative inoculum (0.5 ml) grown at 27°C for 28 hours in a Tryptic Soy Broth (Difco) medium was transferred into each flask. The cultured broth in 101 flasks was collected and filtered (11.6 liters, 88 μg/ml of indolizomycin) using Hyflo Super-Cel (Johns-Manville) as the filter aid. The concentration of the antibiotic was determined by the usual paper disc (8 mm, thin) method against *Bacillus subtilis* PCI219, using a pure sample of indolizomycin as the assay standard.

The antibiotic in the filtrate was adsorbed on a column of Amberlite XAD-2 (Rohm & Haas, 500 ml) and eluted with 30% aqueous acetone. The active eluate (1.7 liters) was concentrated to 150 ml (5,576 μg/ml) and lyophilized to give a crude powder (4.3 g, 48 μg/mg). The crude powder (500 mg) was treated with methanol (13 ml×10) and the methanol solution (130 ml) was concentrated to afford a residue which was purified by preparative TLC on silica gel plates with chloroform - methanol (1: 1). After the elution from the plate with methanol, the eluate was concentrated to give an oily residue, which was extracted with ethyl acetate to yield a pure antibiotic (20.4 mg, 1,000 μg/mg).

Indolizomycin (**1**) was obtained as a pale yellow thick oil, $[\alpha]_D^{20}$ −28.6° (*c* 0.5, MeOH). FD-MS: *m/z* 273 (M^+). *Anal* Calcd for $C_{17}H_{23}NO_2$: C 74.69, H 8.48, N 5.12, O 11.70. Found: C 74.52, H 8.54, N 5.11, O 11.59. The UV spectrum showed a typical absorption of triene, λ_{max}^{MeOH} nm (ε): 260 (sh, 29,100), 268 (33,400), 277 (sh, 27,300). The IR spectrum of **1** is shown in Fig. 1. By high-voltage paper electrophoresis under 3,500 V for 25 minutes in formic acid - acetic acid - water (1: 3: 36), the antibiotic moved to the cathode with Rm (relative mobility to alanine) 0.80. Rf value on silica gel TLC with chloroform - methanol (1: 1) was 0.2. The antibiotic is labile even in the freezer. On storage at −30°C the antibiotic lost 13% of its activity in one week and 28% of its activity in two weeks. The ^1H NMR and ^{13}C NMR chemical shifts of **1** are represented in Tables 1 and 2, respectively. In the ^1H NMR spectrum the signals due to a cyclopropyl methylene group were readily assignable at δ 0.42 and 0.58. The ^{13}C NMR spectrum of **1** strongly suggested that **1** had an epoxy group at C-7 (δ 50.5, $^1J_{C-H}$ 176.0 Hz) and C-8 (δ 55.4, $^1J_{C-H}$ 180.0 Hz).

The reduction of **1** with $NaBH_4$ in methanol at room temperature for five minutes gave a deoxygenated product **2**, $[\alpha]_D^{27}$ −12.6° (*c* 1, MeOH). The molecular formula of **2** was derived from the high-resolution MS spectrum, Found: *m/z* 257.1782, Calcd for $C_{17}H_{23}NO$: *m/z* 257.1778. On the other hand a treatment of **1** with $NaBD_4$ under similar conditions as **2**, afforded a deuterated compound **3**, $[\alpha]_D^{24}$ −12.9° (*c* 1, MeOH), EI-MS: 258 (M^+). NMR data of **2** and **3** are summarized in Tables 1 and 2. The singlet carbon



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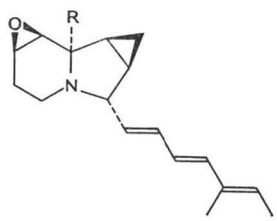

2 R = H

3 R = D

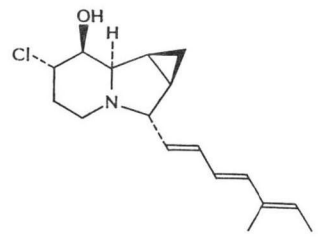

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Fig. 1. IR spectrum of indolizomycin (Neat).

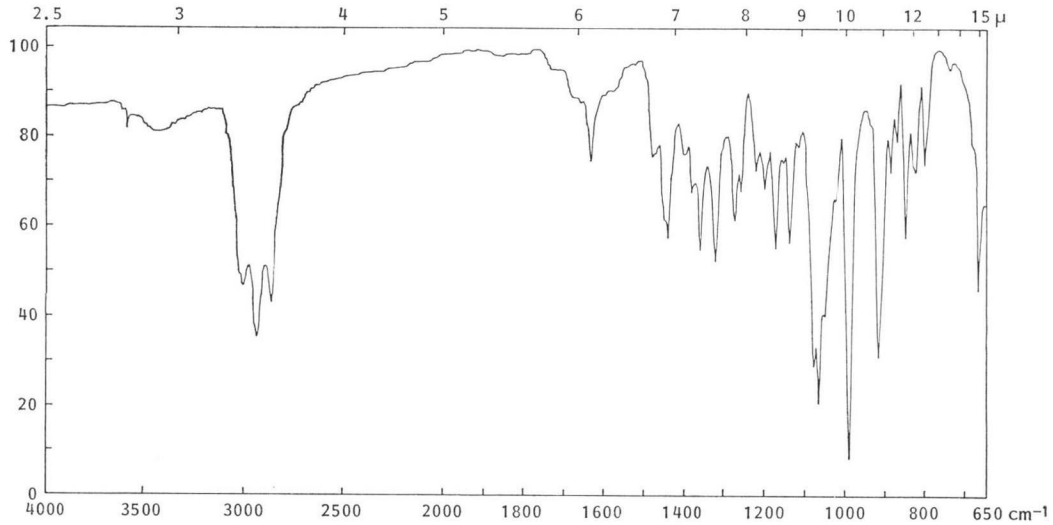


Table 1. ^{1}H NMR data of **1**, **2**, **3** and **4**.

Proton	**1** (in MeOH-d_4)		**2** (in $CDCl_3$)		**3** (in $CDCl_3$)		**4** (in $CDCl_3$)	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
1	1.74	8.0, 6.0, 4.1	1.59	7.5, 6.5, 3.8, 3.5	1.59	7.5, 6.5, 4.0	1.44	7.6, 6.8, 4.4, 3.5
2	1.28	7.8, 6.0, 4.1, 0	1.19	7.5, 6.5, 4.0, 0	1.19	7.5, 6.5, 4.0, 0	1.21	7.6, 6.8, 4.0, 0
3	3.33	8.2, 0	3.47	9.0, 0	3.47	9.0, 0	3.51	9.8, 0
5	2.69	11.5, 10.2, 4.9	2.39	11.2, 9.0, 5.5	2.40	11.2, 9.0, 5.5	2.50	11.8, 5.0, 2.0, 1
5′	2.80	10.2, 5.6, 2.2	2.67	11.2, 7.0, 3.5	2.67	11.2, 7.0, 4.0	2.68	11.8, 11.8, 3.0
6	2.03	14.6, 4.9, 3.0, 2.2	1.82	15.0, 5.5, 4.0, 3.5	1.82	15.0, 5.5, 4.0, 4.0	1.74	15.5, 3.0, 3.0, 2.0
6′	2.09	14.6, 11.5, 5.6, 1	2.02	15.0, 9.0, 7.0, 1.0	2.02	15.0, 9.0, 7.0, 1.0	2.21	15.5, 11.8, 5.0, 3.0
7	3.25	4.0, 3.0, 1	3.11	4.5, 4.0, 1.0	3.12	4.5, 4.0, 1.0	4.17	3.0, 3.0, 3.0, 1
8	3.27	4.0	3.30	4.5, 1	3.30	4.5	4.02	3.0, 1, (broadened by 8-OH)
8a	—		3.21	3.5, 1, 1	—		3.38	3.5, 1, 1
9	0.58	8.0, 7.8, 5.0	0.44	7.5, 7.5, 4.5	0.44	7.5, 7.5, 4.5	0.53	7.6, 7.6, 4.6
9′	0.42	5.0, 4.1, 4.1	0.91	4.5, 4.0, 3.8, 1	0.90	4.5, 4.0, 4.0	0.93	4.6, 4.4, 4.0, 1
10	5.64	14.4, 8.2	5.62	14.0, 9.0	5.63	14.5, 9.0	5.69	14.0, 9.8
11 12 13	6.08 ~6.22		6.06 ~6.27		6.08 ~6.24		6.12 ~6.27	
15	5.55	6.8, 1	5.57	7.0, 1	5.57	7.0, 1	5.59	7.0, 1
16	1.73	6.8	1.73	7.0	1.74	7.0	1.75	7.0
17	1.74	1	1.75	1	1.75	1	1.76 1.57	1 (8-OH)

Table 2. ^{13}C NMR data of **1** and **2** (100 MHz).

Carbon	**1** (in MeOH-d_4)	**2** (in $CDCl_3$)
	δ (m)	δ (m)
1	29.0 d	18.9 d
2	18.8 d	18.1 d
3	72.3 d	65.9 d
5	43.6 t	43.9 t
6	24.7 t	24.0 t
7	50.5 d	49.1 d
8	55.4 d	53.5 d
8a	95.1 s	57.8 d
9	7.5 t	6.3 t
10	135.9 d	131.8 d
11	131.6 d	131.9 d
12	126.7 d	125.4 d
13	138.2 d	137.2 d
14	135.9 s	134.6 s
15	127.8 d	127.3 d
16	14.0 q	14.0 q
17	12.1 q	11.9 q

m: Muliplicity.

at δ 95.1 in the ^{13}C NMR spectrum of **1** shifted to the doublet at δ 57.8 in the spectrum of **2**. This suggested that the carbon at 8a in **1** was of the carbinolamine type. From the NMR spectral data, **1** has the 7,8-epoxy-8a-hydroxy-1,2-methyleneindolizidine skeleton with the 5-methylheptatrienyl side chain at C-3.

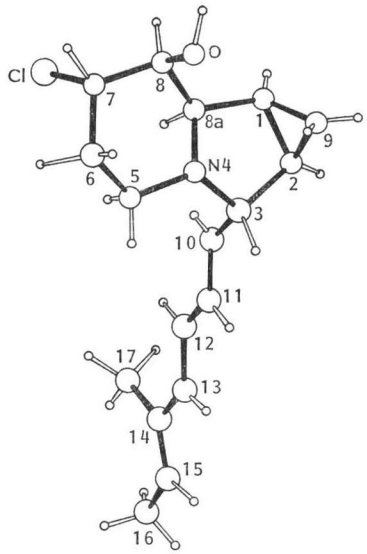
The acidic treatment of **2** with two equivalents of hydrochloric acid in methanol at 50°C for 15 minutes followed by column chromatography on silica gel with hexane - ethyl acetate (3: 1) gave a crystalline chlorohydrin derivative **4**. Recrystallization gave prisms from ethyl acetate - hexane, mp 157~158°C (dec), $[\alpha]_D^{27}$ −116° (*c* 1, MeOH), EI-MS: *m/z* 293 (M^+), 295 (M^++2). The ^1H NMR spectrum of **4** (Table 1) showed that the epoxide ring of **2** opened in the *trans*-diaxial fashion.

The absolute structure of **4** was confirmed by X-ray crystallographic analysis to be (1*S*,2*R*,3*S*, 7*S*, 8*S*, 8a*R*)-7-chloro-8-hydroxy-1,2-methylene-3-[(1*E*,3*E*,5*E*) - 5 - methyl - 1,3,5 - heptatrienyl]octahydroindolizine. A well developed prismatic crystal of **4** was cut into a small X-ray specimen with approximate dimensions 0.15×0.3×0.3 mm and was mounted on a Philips diffractometer using graphite monochromated CuKα radiation. Crystal data: **4**, $C_{17}H_{24}NOCl$, MW 293.8, orthorhombic, space group $P2_12_12_1$, a=15.329(8), b=17.354(8), c=6.260(3) Å, U=1665 Å^3, Z=4, D_{calc}=1.172 g cm^{-3}, μ for CuKα=20.0 cm^{-1}. Out of 1,977 theoretically possible reflections, 1,740 hkl reflections were measured within the 2θ range of 6° through 156°, and 461 Friedel reflections with indices $\bar{h}k\bar{l}$ were also measured at the same time. The structure was solved by the direct method using MULTAN[3] and refined by the least-squares calculations with block-diagonal-matrix approximations. The hydrogen atoms were located on the difference electron-density map and the R factor was reduced to 0.056 including all the 24 hydrogen atoms. The absolute configuration was determined by taking into account the anomalous dispersion effect of the chlorine atom for CuKα radiation (f′=0.348, f″=0.702). Of 164 Friedel pairs for which the

Table 3. The antibacterial spectrum.

Test organism	MIC (μg/ml)
Staphylococcus aureus Terajima	25
S. aureus Smith	12.5
S. aureus MS353	25
Micrococcus flavus FDA16	12.5
M. lysodeikticus IFO3333	12.5
M. luteus ATCC9341	12.5
Bacillus anthracis	12.5
B. subtilis NRRL B-558	25
B. subtilis ATCC6633	12.5
B. cereus ATCC10702	25
Corynebacterium bovis 1810	12.5
Mycobacterium smegmatis ATCC607	100
Escherichia coli NIHJ	12.5
E. coli K-12	25
E. coli K-12 C600	50
E. coli K-12 ML1629	100
Shigella dysenteriae JS11910	12.5
S. flexneri 4b JS11811	25
S. sonnei JS11746	100
Salmonella typhimurium IFO971	100
S. typhi 901	100
S. schotmuelleri 8006	25
S. enteritidis G14	100
S. paratyphi 1015	25
Pseudomonas aeruginosa A3	100
Klebsiella pneumoniae PCI602	12.5
Enterobacter aerogenes ATCC13048	100
E. cloacae 963	100
Serratia marcescens IAM1184	50
Proteus morganii IFO3848	50
P. rettgeri IFO3850	50
P. vulgaris OX19	50
P. mirabilis IFO3849	100
Candida albicans 3147	50

Fig. 2. A perspective view of chlorohydrin derivative **4**.



observed intensity ratios between the Friedel reflections ($|F_0(hkl)|/|F_0(\bar{h}\bar{k}\bar{l})|$) were greater than 1.03 or less than 0.97, 134 pairs agree with the calculated intensity ratios assuming the absolute configuration shown in Fig. 2.* The side-chain atoms of the conjugated double bonds are planar with a fully extended conformation which nearly bisects the angle N4–C3–C2. The torsion angles, N4–C3–C10–C11 and C2–C3–C10–C11 are −117.3(2)° and 125.4(2)°, respectively. The six and five membered rings of the indolizidine group take the chair and envelope conformations, respectively. An intermolecular hydrogen bond, O-H ··· N4 of 2.903(4) Å was observed in the crystal structure.

In accordance with these data, the absolute structure of indolizomycin (**1**) except for the configuration at C-8a was determined to be (1*S*, 2*R*,3*S*,7*R*,8*R*)-7,8-epoxy-8a-hydroxy-1,2-methylene-3-[(1*E*,3*E*,5*E*)-5-methyl-1,3,5-heptatrienyl]-octahydroindolizine.

Indolizomycin (**1**) exhibited a weak anti bacterial activity as shown in Table 3. The intraperitoneal acute LD_{50} in mice was 12.5~25 mg/kg.

* The lists of final atomic parameters, bound lengths and angles have been sent to the Cambridge Crystallographic Data Center. Table of observed and calculated structure factors may be obtained from one of the authors (H. NAKAMURA) upon request.

Among known antibiotics, cyclizidine[4] isolated from the culture of *Streptomyces* sp. has been reported to have the oxireno[*g*]indolizine skeleton.

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SHUICHI GOMI
DAISHIRO IKEDA
HIKARU NAKAMURA
HIROSHI NAGANAWA
FUMIO YAMASHITA
KUNIMOTO HOTTA
SHINICHI KONDO
YOSHIRO OKAMI
HAMAO UMEZAWA
Institute of Microbial Chemistry
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

YOICHI IITAKA
Faculty of Pharmaceutical Sciences,
University of Tokyo
Hongo, Bunkyo-ku, Tokyo 115,
Japan

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