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ISOLATION OF A NEW INDOLE ALKALOID, PENDOLMYCIN, FROM *NOCARDIOPSIS*

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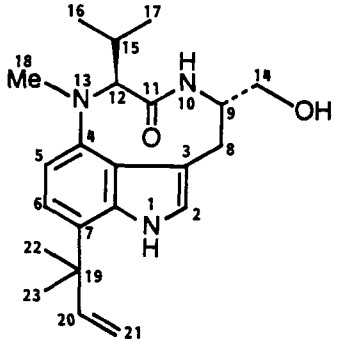
ABSTRACT.—A new indole alkaloid, pendolmycin [**1**], was isolated from *Nocardiopsis* strain SA1715 as an inhibitor of epidermal-growth-factor-induced phosphatidylinositol turnover in A431 cells. The structure of pendolmycin is closely related to that of teleocidin B [**2**] and of lyngbyatoxin A [**3**]. Pendolmycin may be a biosynthetic intermediate of these alkaloids.

In the course of screening for inhibitors of epidermal-growth-factor (EGF)-induced phosphatidylinositol turnover, we have isolated a new indole alkaloid from a strain of *Nocardiopsis*, which we named pendolmycin [**1**]. We report here the isolation, purification, and structure determination of this compound and show that it is structurally closely related to the indole alkaloids, teleocidin B [**2**] and lyngbyatoxin A [**3**].

Teleocidin B was isolated in 1960 from a *Streptomyces* species (1), and lyngbyatoxin A was isolated in 1979 from the lipid extract of an Hawaiian shallow-water variety of *Lyngbya majuscula* Gomont (2). Both teleocidin B and lyngbyatoxin A exhibit irritant toxicity, which is also seen with pendolmycin.



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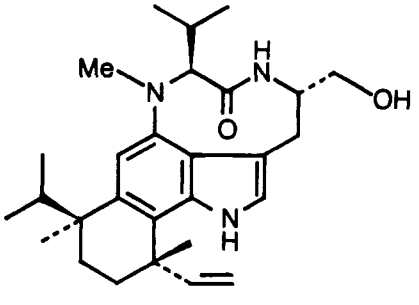

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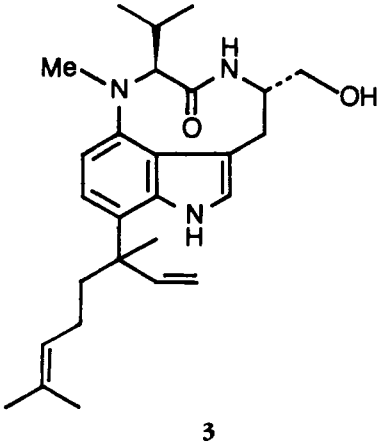

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RESULTS AND DISCUSSION

A culture filtrate of a strain of an actinomycete showed strong inhibition of EGF-induced phosphatidylinositol turnover in A431 cells. The microorganism was obtained from soil collected in a river near Shanghai. Taxonomic features indicated that the strain belonged to the genus *Nocardiopsis*. The culture filtrate contained two active components; one was found to be identical with psi-tectorigenin (3), and the other was further purified for structure determination. The active component was isolated and purified as described in the Experimental section and was named pendolmycin [**1**]. Pendolmycin inhibited epidermal-growth-factor (EGF)-induced phosphatidylinositol turnover with an IC_{50} value of about 1 ng/ml.

Purified pendolmycin was obtained as colorless crystals [mp 124–126°; fdms *m/z* $[M]^+$ 369; $[\alpha]_D$ −154° ($c = 0.1$, MeOH); uv λ max (ε): 214 (11800), 230 (19400), 285 (7900), 298 (7970)]. It is soluble in MeOH, Me_2CO, and $CHCl_3$ but insoluble in hexane and H_2O. The ir absorption of pendolmycin at 3380 cm^{-1} and 1655 cm^{-1} revealed the presence of an amide group. The uv spectrum of pendolmycin resembled the uv spectra of teleocidines and lyngbyatoxin A (2). The ^1H- and ^{13}C-nmr spectra of pendolmycin gave doubled signals which could be due to two conformations of the lactam

TABLE 1. ^1H-*nmr Spectrum* (400 MHz, $CDCl_3$) of Pendolmycin [**1**].

Proton	Chemical shift[a]
1	8.48 (1H, br s)
2	6.84 (1H, br s)
5	6.48 (1H, d, $J = 8.0$ Hz)
6	7.00 (1H, d, $J = 8.0$ Hz)
8a	3.04 (1H, dd, $J = 4.0$ Hz, $J = 17.5$ Hz)
8b	3.15 (1H, br d, $J = 17.5$ Hz)
9	4.32 (1H, m)
10	7.50 (1H, br s)
12	4.34 (1H, d, $J = 10$ Hz)
14a	3.56 (1H, br dt, $J = 12.0$ Hz, $J = 3.0$ Hz)
14b	3.74 (1H, br dt, $J = 12.0$ Hz, $J = 7.0$ Hz)
14-OH	3.34 (1H, br)
15	2.59 (1H, m, $J = 7.0$ Hz, $J = 10.0$ Hz)
16	0.65 (3H, d, $J = 7.0$ Hz)
17	0.92 (3H, d, $J = 7.0$ Hz)
18	2.90 (3H, s)
20	6.19 (1H, dd, $J = 11.0$ Hz, $J = 17.5$ Hz)
21a	5.21 (1H, dd, $J = 1.5$ Hz, $J = 11.0$ Hz)
21b	5.31 (1H, dd, $J = 1.5$ Hz, $J = 17.5$ Hz)
22	1.47 (3H, s)
23	1.51 (3H, s)

[a]Chemical shifts in ppm downfield from TMS.

ring as reported in lyngbyatoxin A. The major resonances are listed in Tables 1 and 2. These spectra resemble those of indolactam V (4), having, instead of aromatic protons (δ 6.90 ppm), two methyls (δ 1.47 and 1.51 ppm, each a singlet) and three vinylic protons (δ 5.21, 5.32 and 6.91 ppm) attributable to a 3-methylbutenyl group at C-7. The structure was determined using data generated by long range ^1H-^{13}C correlation spectra and nOe experiments, as illustrated in Figure 1. The stereochemistry of pendolmycin was deduced to be 9*S*,12*S* by comparison of its cd spectrum $\{[\theta]_{303}$ +8900, $[\theta]_{294}$ 0, $[\theta]_{248}$ −33000, $[\theta])_{220}$ −44000, $[\theta]_{213}$ 0 ($c = 1.25 \times 10^{-3}$, MeOH)$\}$, recorded on a J20 spectro-polarimeter (Jasco Co.), with that of indolactam V (4).

TABLE 2. ^{13}C-nmr Spectrum (100 MHz, $CDCl_3$) of Pendolmycin [**1**].

Carbon	Chemical shift[a]
2	121.1 (d)
3	114.2 (s)
3a	118.7 (s)
4	146.5 (s)
5	106.4 (d)
6	119.1 (d)
7	122.8 (s)
7a	137.5 (s)
8	33.9 (t)
9	55.8 (d)
11	174.5 (s)
12	71.1 (d)
14	65.1 (t)
15	28.6 (d)
16	19.6 (q)
17	21.6 (q)
18	33.1 (q)
19	40.1 (s)
20	149.5 (d)
21	111.3 (t)
22	27.2 (q)
23	26.8 (q)

[a]Chemical shifts in ppm downfield from TMS.

Teleocidin B (5) and pendolmycin[2] do not inhibit binding of EGF to its receptor in A431 cells. Thus, pendolmycin should not inhibit EGF-induced phosphatidylinositol turnover by inhibiting EGF binding. Teleocidin B and lyngbyatoxin A are known to induce inflammation and tumor promotion in mouse skin (6). Pendolmycin likewise induced erythema in human skin. It showed weak antibacterial activity with the following MIC values: *Staphylococcus aureus* (25 μg/ml), *Micrococcus luteus* (25 μg/ml), *Bacillus anthracis* (50 μg/ml), *Bacillus subtilis* (50 μg/ml), *Bacillus cereus* (50 μg/ml),

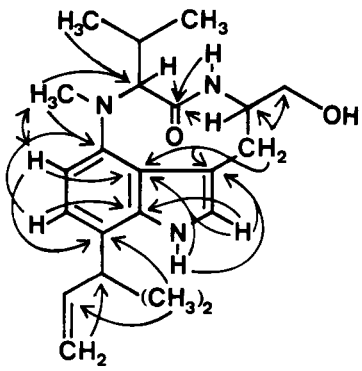

FIGURE 1. Structure of pendolmycin demonstrated by long range ^{1}H-^{13}C correlation spectrum and nOe.
: Long range ^{1}H-^{13}C correlation
: NOe

[2]K. Umezawa *et al.*, manuscript in preparation.

Corynebacterium bovis (50 μg/ml), *Mycobacterium smegmatis* (50 μg/ml), and *Klebsiella pneumoniae* (100 μg/ml).

Both teleocidin B [**2**] and lyngbyatoxin A [**3**] contain two isoprene units on the indolactam structure, while pendolmycin [**1**] carries only one such unit. Indolactams without an isoprene unit have also been isolated from actinomycetes (7). Therefore, it is possible that pendolmycin is a biosynthetic intermediate of teleocidin B and lyngbyatoxin A.

EXPERIMENTAL

ISOLATION.—*Nocardiopsis* strain SA1715 has been deposited with the Fermentation Research Institute of the Agency of Industrial Science and Technology, Tsukuba, Japan under the collection number FERM P-10278. The organism was cultured in a 500-ml Erlenmeyer flask containing 110 ml of a medium consisting of 2% sucrose, 1% corn steep liquor, and 0.4% $CaCO_3$ in 2-fold diluted artificial sea water (Jamarin Laboratory), pH 7.4, on a rotary shaker at 27° for 2 days. Then, 3.0 ml of the cultured broth was inoculated into a 500-ml flask containing 110 ml of the same medium. The fermentation (36 liters) was carried out at 27° for 4 days. The broth filtrate was extracted with an equal volume of EtOAc, and the mycelial cake was extracted with Me_2CO. The Me_2CO extract was dried, dissolved in H_2O, and extracted with EtOAc. The EtOAc extracts were combined and concentrated in vacuo. The dried material was applied to a Si gel column (40 × 100 mm), and the active fraction was eluted with $CHCl_3$-MeOH (100:1). The eluate was dried to give a yellow powder (81.6 mg), which was dissolved in MeOH and applied to a Toyopearl HW-40 column (50 × 100 mm) to obtain purified material (40.8 mg). Further purification was carried out using reversed-phase hplc (Nucleosil $_5C_{18}$, Nagel, 20 × 300 mm) with 50% MeCN/H_2O as eluent, yielding 8.8 mg of pendolmycin [**1**].

PHOSPHATIDYLINOSITOL TURNOVER ASSAY.—Human epidermoid carcinoma A431 cells (3×10^5 cells) pre-labeled with ^{3}H-myoinositol (1 μCi) for 30 min were incubated in Hepes-buffered saline (HBS) at 37° with samples and EGF (400 ng/ml) for 60 min (8). The medium was then removed, and 0.5 ml of 10% trichloroacetic acid (TCA) containing 0.01 M sodium pyrophosphate was added to the cells. Thereafter, the adherent cells were washed 3 times with ice-cold TCA solution and the residue dissolved in 0.5 N NaOH. Finally, TCA-insoluble radioactivity was determined in a liquid scintillation counter.

ACKNOWLEDGMENTS

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