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METHYLPENDOLMYCIN, AN INDOLACTAM FROM A *NOCARDIOPSIS* SP.

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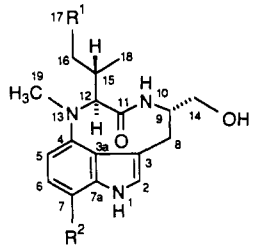
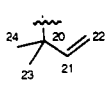
ABSTRACT.—Methylpendolmycin [**2**], a new indole alkaloid with an *N*-methylisoleucine moiety incorporated in the nine-membered indolactam ring, has been isolated from an actinomycete culture of *Nocardiopsis*. Methylpendolmycin exhibited inhibition of phorbol ester binding to protein kinase C. Its structure was assigned on the basis of spectroscopic data.

In the course of our screening for pharmacologically active agents from microbial sources, we found that several EtOAc extracts from actinomycete cultures were active in the phorbol dibutyrate (PDBu) receptor binding assay. The use of photodiode array coupled to hplc enabled us to detect active components having the characteristic indolactam uv chromophore (1). Subsequent lc-ms analysis with thermal spray suggested the presence of the teleocidins (2), known for their potent tumor-promoting activity (3,4). Teleocidins are generally distinguished structurally by a unique nine-membered indolactam ring with various linear or cyclic terpene moieties attached. Representative structures include teleocidin B (5–7), lyngbyatoxin A (8,9), and pendolmycin [**1**] (10).



1 R^1=H, R^2= 

2 R^1=Me, R^2= 

3 R^1=Me, R^2=H
4 R^1=R^2=H

Bioassay-guided fractionation of the extract from a *Nocardiopsis* strain (Thermonosporaceae) (11) led to the isolation of a new indolactam alkaloid, which we named methylpendolmycin [**2**], together with pendolmycin (**1**]. Methylpendolmycin inhibited PDBu binding to protein kinase C with an IC_{50} = 13.5 ng/ml (2). Its structure is closely related to pendolmycin. Methylpendolmycin contains an *N*-methylisoleucine moiety rather than the *N*-methylvaline moiety more commonly found in pendolmycin and other teleocidins. Irie *et al.* (12) recently reported the isolation of (−)-indolactam I [**3**], which also contains *N*-methylisoleucine, from *Streptoverticillium blastmyceticum*. We describe herein the isolation and structure elucidation of methylpendolmycin [**2**].

RESULTS AND DISCUSSION

The EtOAc extract of the culture broth of a strain of *Nocardiopsis* was found to be active in the PDBu receptor binding assay. Vacuum flash Si gel chromatography of the extract, followed by hplc, yielded a known compound, pendolmycin [**1**], and a pendolmycin homologue, methylpendolmycin [**2**]. The uv spectrum of **2** was typical of the teleocidins. Its molecular formula was determined to be $C_{23}H_{33}N_3O_2$ by high resolution fabms. The ^{1}H- and ^{13}C-nmr data of **2**, assigned on the basis of DEPT, COSY, HETCOR, COLOC, HMQC, and HMBC and summarized in Table 1,

TABLE 1. ^{1}H- and ^{13}C-nmr Data of Compound **2** in C_6D_6.

Position	^{1}H δ (multiplicity, *J* in Hz)	^{13}C δ (multiplicity[a])	HMBC (coupled ^{1}H)
1	8.07 (br s)	—	—
2	6.37 (br s)	121.3 (d)	—
3	—	114.6 (s)	H-1, H-2, H-5, H-8
3a	—	119.5 (s)	H-1, H-2, H-8
4	—	147.2 (s)	H-3, H-6, H-12, H-19
5	6.44 (d, 8.1)	106.6 (d)	—
6	7.01 (d, 8.1)	119.6 (d)	H-5
7	—	122.7 (s)	H-5, H-21, H-23, H24
7a	—	137.9 (s)	H-2, H-6
8	3.04 (dd, 17.4, 3.3)	34.0 (t)	—
	3.19 (br d)	—	—
9	4.40 (m)	56.6 (d)	H-8
10	8.79 (br s)	—	—
11	—	175.7 (s)	H-12
12	4.65 (d, 10.3)	69.7 (d)	H-18, H-19
14	3.60 (br dd, 10.1, 8.1)	65.1 (t)	H-8
	3.72 (br d, 10.1)	—	—
14-OH	4.33 (br m)	—	—
15	2.58 (m)	34.4 (d)	H-17, H-18
16	0.61 (m)	24.7 (t)	H-17, H-18
	1.45 (m)	—	—
17	0.51 (t, 7.2)	10.1 (q)	—
18	0.99 (d, 6.2)	17.2 (q)	H-12
19	2.78 (s)	33.0 (q)	H-12
20	—	40.0 (s)	H-6, H-21, H-22, H-23, H-24
21	5.91 (dd, 17.6, 10.5)	149.4 (d)	H-22, H-23, H-24
22	4.86 (dd, 10.5, 1.5)	111.3 (t)	—
	4.95 (dd, 17.6, 1.5)	—	—
23	1.35 (s)	26.7 (q)	H-21
24	1.36 (s)	27.1 (q)	H-21

[a]Deduced from DEPT experiment.

are similar to those reported for pendolmycin (10). The presence of a nine-membered indolactam ring, an *N*-methyl, a 3-methylbutenyl moiety at C-7, and a hydroxymethyl group at C-9 is consistent with the structural features of **1**, but **2** contains one more carbon. COSY and proton decoupling experiments showed that a methine at δ 2.58 (CH-15) is coupled with a methine at δ 4.65 (CH-12), a methyl at δ 0.99 (Me-18), and a methylene at δ 0.61 and 1.45 (CH_2-16), and this methylene is further coupled with a methyl at δ 0.51 (Me-17). Furthermore, both C-15 and C-16 were found to have cross peaks with Me-17 and Me-18, and C-12 was found to have cross peaks with Me-18 and Me-19 in the HMBC spectrum. These data established the presence of an *N*-methylisoleucine moiety in **2**, which is different from the *N*-methylvaline moiety found in **1**.

It is interesting to note that a number of ^{1}H-nmr and ^{13}C-nmr signals of **2** were doubled. Similar data were also reported in lyngbyatoxin (8), indolactam V (13), and pendolmycin (10). As reported (13), the indolactam ring existed as two conformers in solution. In our hands ^{1}H-nmr spectra of **2** obtained in deuterated C_6H_6, $CHCl_3$, and MeOH all yielded the doubled signals but in different ratios of 4:1, 2:1, 1:1, respectively, also supporting two conformations.

A cd dispersion curve of **2** was in good agreement with those of (−)-indolactam V [**4**] and (−)-indolactam I [**3**], whose structures were confirmed by total syn-

thesis (14–16) and biosynthesis (12), respectively. This indicated that the stereochemistry of three chiral centers in **2** is 9*S*, 12*S*, and 15*S* and suggested that methylpendolmycin [**2**], like the teleocidins, is also derived biosynthetically from L-amino acids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on Varian Gemini 300 and Jeol GSX-270 spectrometers. Chemical shifts are reported as δ values in ppm relative to TMS (solvent peaks used as references). Cd curves were measured on a JASCO J-600 spectropolarimeter. Uv traces were recorded on a Hewlett Packard 8450A diode array spectrophorometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Ir spectra were recorded on a Nicolet IBM IR/3X spectrometer. Ms were recorded using a Finnigan MAT TSQ 70 mass spectrometer and a VG analytical ZAB 2-SE high field mass spectrometer. Photodiode array hplc was performed using a Waters system including 990 photodiode array detector, 510 pump, 715 ultra WISP, automated gradient controller, and Powermate 386/20. YMC silica (A-023) and ODS (A-323) columns were used.

FERMENTATION.—The microbial culture *Norcardiopsis*, collected from rhizospheres of wax jambo (17) grown in T'ou Cheng, Taiwan, has been deposited in the Sterling Culture Collection at Sterling Research Group in Malvern, Pennsylvania as SC 0037. The organism forms chains of spores developed from irregularly flexuous aerial hyphae. The culture cell wall contains meso-DAP (diaminopimelic acid), small amounts of rhamnose, and type PIII phospholipids (11). The frozen stock culture was inoculated into a seed medium containing 2.0% glucose, 1.5% Pharmamedia, 0.3% $(NH_4)_2SO_4$, 0.003% ZnSo·$7H_2O$, and 0.4% $CaCO_3$. The seed was grown for 2 days at 27° on a shaker at 220 rpm, then transferred into 1-liter Fernback flasks containing 500 ml of production medium. The production medium contained 2.0% glucose, 2.0% glycerol, 0.5% monosodium glutamate, 0.2% K_2HPO_4, 0.1% yeast extract, 2% $CaCO_3$, and 1% of a stock solution of trace elements containing 6.1% $MgSO_4·7H_2O$, 0.144% $ZnSO_4·7H_2$, 0.111% $MnSO_4·H_2O$, and 0.025% $CuSO_4·5H_2O$. The fermentation was carried out on a shaker at 220 rpm at 27° for 4 days.

ISOLATION OF METHYLPENDOLMYCIN [**2**].—The whole broth (2 liters) was extracted with EtOAc (2 liters × 2). The residue (590 mg) obtained from the extract was subjected to vacuum flash chromatography using a 35 × 30 mm Li-Chroprep Si 60 column. Fractions were eluted sequentially with hexane-EtOAc (5:1), hexane-EtOAc (1:1), EtOAC, and MeOH. A mixture containing pendolmycin [**1**] and methylpendolmycin [**2**] was eluted with hexane-EtOAc (1:1). Further hplc on a silica column [hexane-EtOAc (5:95)] with photodiode array detection yielded 10 mg of **1** (1.69% of crude extract) and 14 mg of **2** (2.37%) as amorphous solids. Separation of **1** and **2** was also achieved on an ODS hplc column by eluting with MeCN-H_2O (4:1). The physical and spectroscopic data of **1** including [α]D, cd, ms, and ^{1}H- and ^{13}C-nmr were in excellent agreement with the published values (10). Compound **2**: mp 174–178°; [α]D −76° (C = 0.57, MeOH); cd $\{\theta\}_{328}$ 0, $\{\theta\}_{305}$ +5600, $\{\theta\}_{293}$ 0, $\{\theta\}_{254}$ −17,000, $\{\theta\}_{240}$ −16,400, $\{\theta\}_{221}$ −13,800, $\{\theta\}_{212}$ 0 ($C = 6.1 \times 10^{-3}$, MeOH); uv λ max (MeOH) 230 (18,000), 289 (6200), 299 nm (6500); hrfabms $[M]^+$ 383.2574 (calcd 383.2573 for $C_{23}H_{33}N_3O_2$); ir ν max (neat) 3450, 3380, 2970, 2930, 2870, 2810, 1655, 1650, 1610, 1508, 1470, 1410, 1370, 1350, 1050, 910, 750 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1.

PHORBOL ESTER BINDING ASSAY.—The method used was based on a procedure originally described by Dunphy *et al.* (18). Briefly, the procedure used rat forebrain homogenate as a source of receptor and PDBu as the radioligand. The positive reference control used for these tests was 4-*O*-methylphorbol 12-myristate 13-acetate obtained from LC Services Company, Woburn, MA (K_i = 0.5–0.6 uM).

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