Communications to the Editor

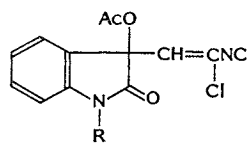
A NEW ANTIBIOTIC INDISOCIN AND *N*-METHYLINDISOCIN

Sir:

A new antibiotic indisocin was isolated from the culture filtrate of actinomycete strain MG323-hF2 which was isolated from the soil sample collected at Shinagawa-ku, Tokyo, Japan. The strain has been classified as *Nocardia blackwellii* MG323-hF2 by taxonomic studies and assigned as accession number FERM P-6606. In this paper we report the fermentation and isolation procedures, physico-chemical properties, structure determination and biological properties of indisocin (**1**) and *N*-methylindisocin (**2**) (Fig. 1).

The stock culture of strain MG323-hF2 was inoculated into 110 ml of a seed medium consisting of glucose 1%, glycerol 1%, sucrose 1%, soybean flour (Prorich) 2%, dried yeast 1%, Casamino acids 0.5%, oatmeal 0.5%, $CaCO_3$ 1% and one drop of silicone oil in a 500-ml Erlenmeyer flask and incubated at 30°C for 72 hours on a rotary shaker (the medium was adjusted to pH 7.0 before sterilization). Three ml portions of the above seed culture were transferred to 125 ml of a production medium composed of maltose 0.375%, yeast extract 0.075%, NZ-amine (Sheffield Co.) 0.25% and NaCl 0.75% in each 500-ml Sakaguchi flasks and cultured at 27°C for 40 hours with reciprocal shaking. Indisocin was isolated from the culture filtrate using silica gel and Sephadex LH-20 chromatography as shown in Fig. 2 and finally obtained in a methanol solution. Indisocin is too unstable to be isolated as the intact substance but is stable in organic solvents such as methanol, ethyl acetate and chloroform.

Fig. 1. Structures of indisocin and *N*-methylindisocin.



Indisocin (**1**) R = H

N-Methylindisocin (**2**) R = CH_3

Fig. 2. Isolation and purification of indisocin.

Culture filtrate 14.3 liters
↓ extracted with EtOAc at pH 6.6
Organic layer 14 liters (84.5%*)
↓ evaporated *in vacuo*
↓ silica gel column chromatography (benzene - EtOAc, 12 : 1)
Active fraction 504 ml (60%*)
↓ evaporated *in vacuo*
↓ Sephadex LH-20 column chromatography (MeOH)
Active fraction 10 ml (40%*)

* The yields of each steps were determined by paper-disk assay method using *Comamonas terrigena* IFO 12685 as test organism.

As listed in Table 1, the IR spectrum in carbon tetrachloride exhibited characteristic absorption at 2105 cm^{-1} attributed to an isonitrile group. The ^1H NMR spectrum (in $CDCl_3$) indicated four aromatic protons [δ 7.45~7.30 (2H), 7.10 (1H) and 6.91 (1H)], one amide proton (δ 7.57), one olefinic proton (δ 6.03) and one acetyl group (δ 2.14). Indisocin was converted by reaction with diazomethane into the *N*-methyl compound (**2**), which, in the ^1H NMR, showed one methyl signal at δ 3.27 ppm with disappearance of the amide proton signal. The high resolution mass spectra (HR-MS) of the *N*-methyl derivative (**2**) showed molecular ions at *m/z* 290.0485 and 292.0457 (calcd for $C_{14}H_{11}{}^{35}ClN_2O_3$, 290.0459 and $C_{14}H_{11}{}^{37}ClN_2O_3$, 292.0429), indicating the molecular formula of **1** to be $C_{13}H_9ClN_2O_3$. Catalytic hydrogenation ($Pd/BaSO_4$ in MeOH) of **1** gave a basic product, which showed the disappearance of the *O*-acetyl group in the ^1H NMR spectrum, followed by acetylation (Ac_2O-pyridine) to give a neutral compound (**3**) [electron impact mass spectra (EI-MS) *m/z* 232 (M^+)] (Fig. 3). The ^1H NMR spectrum of **3** showed the presence of a newly induced *N*-acetyl group (δ 2.0), an *N*-methyl group (δ 3.0) and a methine group (δ 3.8) attached to an ethyl chain (δ 2.1 and 3.4). These results indicated the presence of a readily reducible allylic acetoxyl group and vinylic chloride. Furthermore the UV spectrum

Table 1. Physico-chemical properties of indisocin and *N*-methylindisocin.

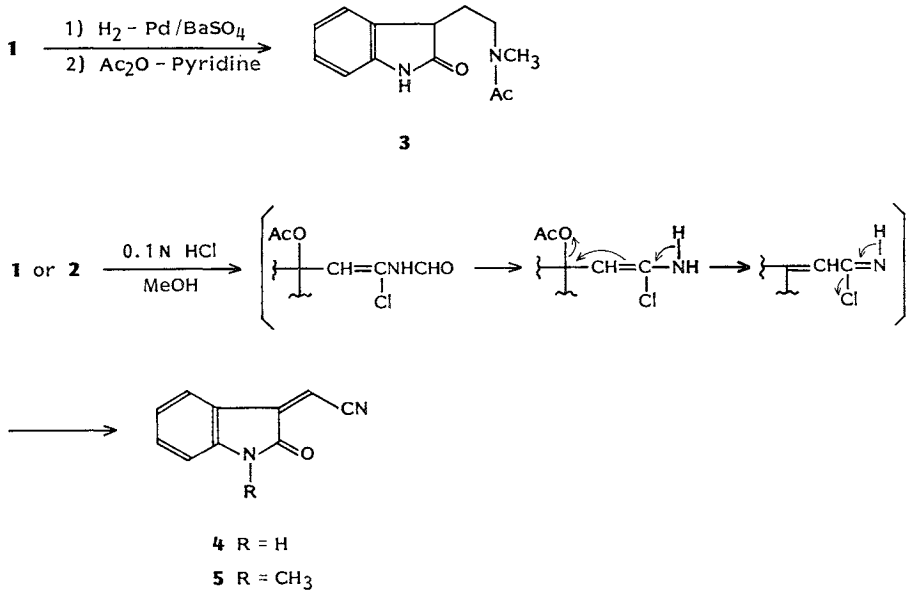
	Indisocin	*N*-Methylindisocin
$[\alpha]_D^{25}$ [a]	+20±5° (*c* 0.03, MeOH)	+22±5° (*c* 0.04, MeOH)
UV[b] λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	208 (1,120), 225 (sh, 730), 255 (sh, 340), 290 (sh, 80)	208 (950), 225 (sh, 660), 260 (sh, 260), 295 (sh, 60)
(FT)-IR $\nu_{max}^{CCl_4}$ cm^{-1}	3445, 2960, 2925, 2855, 2105, 1760, 1740, 1620, 1475, 1370, 1225, 910	2965, 2925, 2865, 2105, 1760, 1745, 1615, 1470, 1370, 1225, 910
Formula	$C_{13}H_9ClN_2O_3$	$C_{14}H_{11}ClN_2O_3$
HR-MS (*m/z*)	Obtained no useful data	290.0485 ($C_{14}H_{11}{}^{35}ClN_2O_3$), 292.0457 ($C_{14}H_{11}{}^{37}ClN_2O_3$)
^1H NMR δ ppm ($CDCl_3$)	7.57 (1H, br), 7.45~7.30 (2H, m), 7.10 (1H, br t, *J*=8.0 Hz), 6.91 (1H, br d, *J*=8.0 Hz), 6.03 (1H, s), 2.14 (3H, s)	7.41 (1H, br t, *J*=8.0 Hz), 7.34 (1H, br d, *J*=8.0 Hz), 7.11 (1H, br t, *J*=8.0 Hz), 6.89 (1H, br d, *J*=8.0 Hz), 6.08 (1H, s), 3.27 (3H, s), 2.12 (3H, s)

[a] Concentrations of indisocin and *N*-methylindisocin in MeOH solutions were determined by their UV spectra after acid hydrolysis by addition of 1 N HCl.

[b] The UV spectra of compounds **4** and **5** were 257 nm (ε 24,500) and 258 nm (ε 28,000), respectively.

FT: Fourier transformation.

Fig. 3. Reactions of indisocin.



of **1** showed the olefinic bond was not conjugated with the 2-oxoindolin ring system[1]. From above findings the structure of indisocin was proposed as shown in Fig. 1. Mild acid hydrolysis (0.1 N HCl - 90% aq MeOH) of **1** or **2** quantitatively gave (*Z*)-2-oxoindolin-3-ylideneacetonitrile (**4**)[2] or (*Z*)-1-methyl-2-oxoindolin-3-ylideneacetonitrile (**5**)[2], respectively (the mechanism is illustrated as in Fig. 3). For formation of the nitrile, the reaction was considered to take a course *via* imino-chloride[3] [C(Cl)=NH]. Hence this intermediate restricted the position of

Table 2. Antimicrobial activities of indisocin and *N*-methylindisocin.

Test organisms	MIC (μg/ml)	
	Indisocin	*N*-Methyl-indisocin
Bacillus anthracis	0.2	1.56
B. cereus ATCC 10702	12.5	12.5
B. subtilis NRRL B-558	6.25	3.12
B. subtilis PCI 219	0.1	0.39
Corynebacterium bovis 1810	<0.025	0.05
Micrococcus luteus FDA 16	0.2	0.2
M. luteus PCI 1001	0.39	0.1
Staphylococcus aureus 209P	0.05	0.1
S. aureus MS8710	0.05	0.1
S. aureus MS9610	0.1	0.2
S. aureus Smith	0.05	0.2
Escherichia coli K-12	0.2	0.78
E. coli ML 1629	1.56	3.12
E. coli NIHJ	<0.025	0.05
Klebsiella pneumoniae PCI 602	0.2	0.39
Proteus rettgeri GN311	0.78	3.12
P. rettgeri GN466	0.39	1.56
P. vulgaris OX19	0.78	3.12
Pseudomonas aeruginosa A3	25	25
Salmonella enteritidis 1891	0.05	0.2
S. typhi T-63	0.78	3.12
Serratia marcescens	0.39	3.12
Shigella dysenteriae JS11910	0.2	0.39
S. flexneri 4bJS11811	0.78	3.12
S. sonnei JS11746	3.12	12.5
Mycobacterium smegmatis ATCC 607	0.1	3.12
Aspergillus niger F-16	10	NT
Candida albicans 3147	10	NT
C. krusei F-5	>10	NT
C. pseudotropicalis F-2	10	NT
C. tropicalis F-1	>10	NT
Cryptococcus neoformans F-10	5	NT
Helminthosporium oryzae	>10	NT
Pellicularia sasakii	5	NT
Pyricularia oryzae	10	NT
Saccharomyces cerevisiae F-7	2.5	NT
Trichophyton asteroides 429	1.25	NT
T. mentagrophytes	1.25	NT
Xanthomonas citri	2.5	NT
X. oryzae	0.08	NT

NT: Not tested.

chlorine atom and supported the structure of indisocin to be 2-(3-acetoxy-2-oxoindolin-3-yl)-1-chlorovinylisocyanide (1). Among known isonitrile-containing antibiotics[4,5], indisocin resembles to B371[6]. Indisocin differs, however, form B371 in the point of having oxindole skeleton, chlorine atom and acetoxyl group.

The antimicrobial spectra of indisocin and *N*-methylindisocin were determined by an agar dilution method using Mueller-Hinton agar (Difco) for bacteria and nutrient agar with glucose 1% for fungi. As shown in Table 2, indisocin and its *N*-methyl derivative have strong antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. The mouse servived when 0.5 mg of indisocin was administered by intraperitoneal injection (but died at a dose of 1.0 mg).

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