Note

Isolation, Characterization and Synthesis of Pimprinine, Pimprinethine and Pimprinaphine, Metabolites of *Streptovercillium olivoreticuli*Yasumasa KOYAMA, Kazuteru YOKOSE
and Lloyd J. DOLBY*School of Pharmaceutical Science,
Toho University,

2-2-1, Miyama-cho, Funabashi 274, Japan

*Department of Chemistry,

University of Oregon,

Eugene, Oregon, 97403, U.S.A.

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Streptovercillium olivoreticuli (Arai, Nakada et Suzuki) Baldacci, Farina et Locci, ISP 5105, has been known as a producer of viomycin-like antibiotic,¹⁾ olivomycin²⁾ and BY-81 (an antibiotic).³⁾ Since no further investigation on the other metabolites has been reported, we reinvestigated this organism to find compounds with unique structures. In the present paper we wish to report the isolation, characterization and synthesis of three metabolites having 5-(3'-indolyl)-oxazole skelton.

For the production of these metabolites, fermentation was carried out in a jar-fermentor containing 10 liters of

the culture medium, composed of 0.5% meat extract, 1.0% polypeptone, 1.0% glucose and 0.3% NaCl. After 72 hr fermentation, the mycelia were harvested and extracted with acetone. The fermentation and extraction were also performed on a 100-fold scale. After evaporation of acetone from the extract, the resulting aqueous residue was extracted with chloroform. The chloroform extract was concentrated *in vacuo*, passed through an alumina column (5.5×28 cm) and eluted with chloroform (5 liters). The chloroform effluent was concentrated *in vacuo* and dissolved in benzene. The benzene solution was absorbed on a silica gel column (5.5×31 cm) and eluted with benzene (1 liter) and then benzene-2-butanone (10:1 v/v, 5 liters). The benzene-2-butanone effluent was concentrated *in vacuo*. Fractional recrystallization of the resulting syrup from chloroform and chloroform-methanol and preparative thin-layer chromatography on silica gel plates [solvent: benzene-2-butanone (10:1 v/v)] gave metabolites **I** (6 mg), **II** (480 mg) and **III** (201 mg). The physicochemical data of metabolites **I**, **II** and **III** are summarized in Table I.

Metabolite **I**, C₁₂H₁₀N₂O, was obtained as colorless needles (mp 204~5°C) and proved to be pimprinine (**I**). Pimprinine, which exhibits antiepileptic effect,⁴⁾ was isolated by Bhate *et al.* from *Streptomyces pimprina*⁵⁾ and synthesized by Joshi *et al.*⁶⁾ and by Oikawa *et al.*⁷⁾

Metabolite **II**, C₁₃H₁₂N₂O, was a new natural product which we have named pimprinethine. The UV absorption spectrum of pimprinethine was similar to that of **I**. Meanwhile, the NMR spectrum of pimprinethine indicated the presence of ethyl group [$\delta_{\text{CDCl}_3}^{\text{ppm}}$: 1.42 (3H, triplet, $J=8$ Hz), 2.85 (2H, quartet, $J=8$ Hz)] instead of

TABLE I. PHYSICOCHEMICAL PROPERTIES OF THE METABOLITES **I**, **II** AND **III**

	I (Pimprinine)	II (Pimprinethine)	III (Pimprinaphine)
Appearance	Colorless needle	Colorless needle	Colorless needle
Melting point (°C)	204~205	163~165	200~201
MS (m/z)	198 (M^+)	212 (M^+)	274 (M^+)
Anal. Found (%)	C, 72.65; H, 5.10; N, 14.13	C, 73.54; H, 5.80; N, 13.06	C, 78.75; H, 5.21; N, 10.14
Anal. Calcd. for	C ₁₂ H ₁₀ N ₂ O: C, 72.71; H, 5.09; N, 14.13	C ₁₃ H ₁₂ N ₂ O: C, 73.56; H, 5.70; N, 13.20	C ₁₈ H ₁₄ N ₂ O: C, 78.81; H, 5.14; N, 10.21
UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ)	225 (4.39), 267 (4.19), 284 (shoulder, 4.08), 300 (shoulder, 4.03)	225 (4.40), 268 (4.19), 284 (shoulder, 4.12), 302 (shoulder, 4.02)	225 (4.44), 272 (4.19), 286 (shoulder, 4.15), 302 (shoulder, 4.10)
NMR (in CDCl ₃) δ	1.42 (3H, triplet, $J=8$ Hz), 2.54 (3H, singlet), 7.10~7.98 (multiplet) 8.40 (1H, broad singlet)	1.42 (3H, triplet, $J=8$ Hz), 2.85 (2H, quartet, $J=8$ Hz), 7.18~7.94 (multiplet), 8.7 (1H, broad singlet)	4.18 (2H, singlet), 7.1~7.95 (multiplet), 8.55 (1H, broad singlet)
NMR (in CF ₃ COOH) δ		1.62 (3H, triplet, $J=8$ Hz), 2.85 (2H, quartet, $J=8$ Hz), 7.2~7.8 (6H, multiplet), 9.25 (1H, broad singlet)	4.56 (2H, singlet), 7.2~7.8 (11H, multiplet), 9.25 (1H, broad singlet)

methyl group in **I**. The analytical data and subsequent synthesis established structure **II** for this metabolite, the synthesis of which was recently achieved by Oikawa *et al.*⁷⁾ before the isolation from natural source.

Metabolite **III**, $C_{18}H_{14}N_2O$, was a new natural product which we have named pimprinaphine. The UV absorption spectrum of pimprinaphine resembled those of **I** and **II**. The NMR (in CF_3COOH) spectrum of pimprinaphine

showed a singlet at $\delta 4.56$ (2H) due to methylene protons, signals at $\delta 7.2 \sim 7.8$ due to eleven aromatic protons and a broad singlet at $\delta 9.25$ due to an indolyl NH proton. From these data, pimprinaphine was identified as 2-benzyl-5-(3'-indolyl)-oxazole (**III**) and confirmed by synthesis. Recently, Oikawa *et al.*⁷⁾ also synthesized **III**.

Our synthetic approach to these metabolites is outlined in Chart 1.

CHART 1.

The reaction of ethyl indole-3-carboxylate (**IV**) with isocyanomethyl lithium in tetrahydrofuran at low temperature (-60 to $-30^\circ C$) for eleven hours followed by neutralization with acetic acid and addition of water gave the desired product. Chromatographic purification of the product on silica gel column afforded pure compound (**V**), mp $178 \sim 180.5^\circ C$ (colorless plates), in 53% yield. The analytical values (*Anal.* Found: C, 71.48; H, 4.39; N, 15.28) of the product corresponded to $C_{11}H_8N_2O$ (*Anal.* Calcd.: C, 71.48; H, 4.38; N, 15.21) and its UV absorption spectrum [λ_{max}^{EtOH} nm (log ϵ): 223 (4.42), 265 (4.16), 280 (4.12), 300 (4.05)] closely resembled that of **I**. The NMR (in $CDCl_3$) spectrum of the product has signals at

$\delta 7.2 \sim 7.95$ due to aromatic protons and a broad singlet at $\delta 8.55$ (1H) due to an indolyl NH proton. From these data, it was concluded that the product was the expected 5-(3'-indolyl)-oxazole (**V**). The synthesis probably proceeds *via* the β -ketoisocyanide which enolizes and cyclizes during isolation.⁸⁾

The product (**V**) was refluxed with acetic anhydride in acetic acid and successive chromatographic separation afforded the desired product, mp $204 \sim 206^\circ C$, in 13% yield. The elemental analysis and the spectral data indicated that the product was identical with pimprinine (**I**) obtained from natural sources. Similarly, reaction of **V** with propionic anhydride in propionic acid gave

pimprinethine (II), mp 163~165°C, in 19% yield.

Without further separation of the reaction mixture, meanwhile, the reaction product of V with appropriate acid anhydride (VIa: R=CH₃; VIb: R=C₂H₅; VIc: R=benzyl) in corresponding carboxylic acid (VIIa: R=CH₃; VIIb: R=C₂H₅; VIIc: R=benzyl) was subjected to hydrolysis with potassium hydroxide in refluxing methanol to give the corresponding 3-acylamidoacetylindoles (VIIIa: R=CH₃; VIIIb: R=C₂H₅; VIIIc: R=benzyl). Thus, 3-acetamidoacetylindole (VIIIa, mp 220~221°C, *Anal.* Found: C, 66.58; H, 5.93; N, 12.88. Calcd. for C₁₂H₁₂N₂O₂: C, 66.65; H, 5.59; N, 12.96), 3-propionamidoacetylindole (VIIIb, mp 219~221°C, *Anal.* Found: C, 67.94; H, 6.15; N, 12.04. Calcd. for C₁₃H₁₄N₂O₂: C, 67.81; H, 6.13; N, 12.17) and 3-phenylacetamidoacetylindole (VIIIc, mp 222~223°C, *Anal.* Found: C, 73.92; H, 5.54; N, 9.38. Calcd. for C₁₈H₁₆N₂O₂: C, 73.96; H, 5.52; N, 9.58) were obtained in 87, 79 and 20% yield, respectively.

3-Acetamidoacetylindole (VIIIa) was refluxed in phosphorous oxychloride for 4 hours and the crude product was purified by silica gel column chromatography and subsequent recrystallization to give pure pimprinine (I) in 84% yield. Similarly, VIIIb gave pimprinethine (II) in

84% yield. And finally VIIIc was converted to pimprinaphine (III) in 92% yield.

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