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Physostigmine and N^8 - Norphysostigmine, Insecticidal Compounds, from Streptomyces sp.

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Note

Physostigmine and N^8-Norphysostigmine, Insecticidal Compounds, from *Streptomyces* sp.

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Several insecticides have been found among the metabolites of microorganisms,[1～5] but only tetranactin[6] is practically used as a miticidal antibiotic. We screened microbes for insecticides, and successfully obtained a strain showing insecticidal activity from a sample of soil collected in Sakai city. This strain, *Streptomyces* sp. AH-4, showed marked activity toward silkworms on oral administration. This paper describes the isolation and identification of the insecticidal compounds involved together with their activities.

Streptomyces sp. AH-4 was cultivated at 28°C for 30 hr in a 30-liter jar fermentor, which contained 20 liters of a medium consisting of 1.0% glucose, 2.0% meat extract, 2.0% Polypepton and 0.5% NaCl (pH 7.0). The culture filtrate was adjusted to pH 8.5 and then extracted with ethyl acetate. The active ethyl acetate extract (5.1 g) was subjected to alumina column chromatography with a solvent system of hexane–ethyl acetate. The insecticidal activity was found in the fractions eluted with 40～60% and 80～100% ethyl acetate in hexane. These fractions were further chromatographed on silica gel with chloroform–methanol to afford two insecticidal compounds, **1** (530 mg) and **2** (65 mg), in pure forms, respectively.

Compound **1** was a colorless oil, $[\alpha]_D^{21}$ −69.3° (c=0.57, MeOH); Mass m/z: 275.1635 (M^+, $C_{15}H_{21}N_3O_2$, calcd. 275.1633), 218, 174 and 160; UV λ_{max}^{MeOH} nm (ε): 251 (7400) and 309 (1800); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 2990, 1750, 1620, 1495 and 1208. The spectral data together with the ^{1}H-NMR spectrum (Table I) for **1** coincided very well with those reported for physostigmine.[7] Furthermore, the sulfate of **1**, a white powder, mp 145～147°C, was identified as physostigmine sulfate by comparing its physicochemical properties (mp, $[\alpha]_D$, IR, UV, ^{1}H-NMR) with those of authentic physostigmine sulfate (Wako Pure Chemicals), allowing us to positively identify insecticidal compound **1** as physostigmine.

Compound **2** was obtained as a white powder from ether, mp 151.5°C (lit.,[6] 151°C), $[\alpha]_D^{30}$ −64.4° (c=0.45, MeOH); $C_{14}H_{19}N_3O_2$ (M^+, 261.1491, calcd. 261.1477); λ_{max}^{MeOH} nm (ε): 245 (4900) and 303 (1500); ν_{max}^{KBr} cm^{-1}: 3350, 2960, 1717, 1620, 1500 and 1265. These data indicated that **2** was an analogue of **1**. The ^{1}H-NMR spectrum (Table I) for **2** showed the same signal features, except that a methyl signal assigned to a methyl at N-8 of **1** was replaced by a signal due to a NH proton. Methylation of **2** with methyl iodide in alkaline methanol gave **1**. Thus **2** was identified as N^8-norphysostigmine.

Physostigmine was first isolated in 1864 from the seeds of *Physostigma venenosum* (Calabar beans),[8] and has recently been obtained from the culture filtrate of *Streptomyces pseudogriseolus* subsp. *iriomotensis* subsp. nov.[9] N^8-Norphysostigmine is also an alkaloid present in the bean,[6] but the production of this compound by microorganisms is, so far as we know, reported for the first time in this paper. Besides these two compounds, there has been only one report of a plant-derived alkaloid being obtained from a microorganism. Namely, maytanacine, which had been isolated from *Putterlickia verrucosa* (Celastraceae),[10] was obtained from fermented broth of

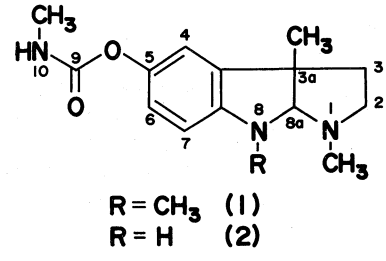

FIG. 1. Chemical Structures of Physostigmine (**1**) and N^8-Norphysostigmine (**2**).

TABLE I. ^{1}H-NMR CHEMICAL SHIFT DATA

Spectra were obtained for $CDCl_3$ solutions at 90 MHz. Chemical shifts are expressed in δ relative to tetramethylsilane.

Proton	Compound **1**	Compound **2**
1-N-CH_3	2.53 (3H, s)	2.39 (3H, s)
2-H_2	2.67 (2H, t, J=8 Hz)	2.62 (2H, t, J=7 Hz)
3-H_2	1.95 (2H, t, J=8 Hz)	1.93 (2H, t, J=7 Hz)
3a-CH_3	1.45 (3H, s)	1.36 (3H, s)
4-H	6.77 (1H, s)	6.76 (1H, s)
6-H	6.78 (1H, d, J=9 Hz)	6.72 (1H, d, J=9 Hz)
7-H	6.33 (1H, d, J=9 Hz)	6.48 (1H, d, J=9 Hz)
8-N-CH_3	2.90 (3H, s)	
8-N-H		3.40 (1H, br. s)
8a-H	4.10 (1H, s)	4.31 (1H, s)
10-N-CH_3	2.80 (3H, d, J=5 Hz)	2.82 (3H, d, J=5 Hz)
10-N-H	5.06 (1H, br. s)	4.98 (1H, br. s)

TABLE II. INSECTICIDAL ACTIVITY OF PHYSOSTIGMINE (1) AND N^8-NORPHYSOSTIGMINE (2) TOWARD SILKWORM

Silkworm larvae were fed on an artificial diet after hatching. A definite amount of each compound was added to the diet and orally administered to the 3rd larvae. Thirty larvae were treated at each dosage. The mortality rate was determined 24 hr after the initiation of the administration.

Compound	Dosage (μg/g diet)	Mortality rate (%)
Physostigmine	100	100
	30	97
	10	77
	3	20
	1	0
N^8-Norphysostigmine	100	100
	30	77
	10	10
	3	0
Control		0

Nocardia sp.[11] Therefore, our finding strongly suggests that plant-derived alkaloids with various physiological activities can be obtained as microbial metabolites.

The insecticidal activities of physostigmine (**1**) and N^8-norphysostigmine (**2**) toward silkworms are shown in Table II. Both **1** and **2** caused a 100% mortality rate at a concentration of 100 ppm. At a concentration of 10 ppm, **1** showed strong insecticidal activity but **2** showed only weak activity, suggesting that the methyl at *N*-8 plays an important role in the activity.

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