Agr. Biol. Chem., **39** (3), 739~740, 1975

Isolation and Identification of Nigragillin as a Insecticidal Metabolite Produced by a *Aspergillus niger*

Akira ISOGAI, Tetsuo HORII, Akinori SUZUKI, Shigeo MURAKOSHI,* Katsutoshi IKEDA,** Shouhachi SATO** and Saburo TAMURA

Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo
**Kanagawa Prefectural Institute of Sericultural Science, Ebina-shi, Kanagawa-ken **Kanagawa Prefectural Livestock Experiment Station, Ebina-shi, Kanagawa-ken*

Received October 17, 1974

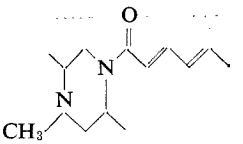
It is well known that some kinds of moulds growing on feeds produce toxins which cause poisoning, occasionally leading to death, in livestock such as chickens and cattles. To elucidate the chemical nature of the causative principles, we isolated various fungi from imported feed and examined their toxicity at first. In the experiment we orally administered culture filtrates or their extracts to silkworm larvae (*Bombyx mori* L.), because the insect has been proved to be very sensitive to the toxicity of mycotoxins.[1] During the process we found that a culture broth from a strain of *Aspergillus niger* revealed a strong insecticidal effect on the worm. Then, we attempted the isolation of the toxin and finally identified it as nigragillin, which has been obtained in 1969 by Caesar *et al.*[2] as a fungal alkaloid from the *Asp. niger* group though they did not refered to its biological activity. In this paper we wish to report the isolation, identification and insecticidal activity of the compound.

Our fungus strain was cultured stationarily in the Czapek-Dox medium containing 2.0% yeast extract at 25°C for 1 week. The cultured broth was autoclaved and filtered, and the filtrate was extracted with ether at pH 10. The extract was shaken with 5% hydrochloric acid, and the aqueous layer was adjusted to pH 10 with 20% sodium hydroxide and reextracted with ether. Evaporation of the extract yielded a basic fraction as a yellowish oil, which was successively applied to preparative alumina thin-layer chromatography (TLC) (benzene-ethyl acetate, 6: 1) and preparative silicic acid TLC (ethyl acetate-methanol, 2: 1). The active zone on the chromatogram was extracted with ethyl acetate and methanol, respectively, and the extract was converted into picrate in ethanol. The picrate thus obtained was applied to a basic alumina column, and the column was eluted with ethyl acetate. After evaporation of the solvent, a toxic substance was obtained as an oil in a yield of 15 mg from 1 liter of culture filtrate.

The molecular formula of the toxin (I) was found to be $C_{13}H_{22}N_2O$ with high resolution mass spectrometry (M^+ *m/e*: 222.1764; calcd. 222.1731). $[\alpha]_D^{26}+101°$ ($c=1.0$, $CHCl_3$). λ_{max}^{MeOH} nm (ε): 262 (26200).* The IR spectrum of I is shown in Figure. The picrate of I melted at 180~181°C. I is positive to the Dragendorff reagent. It is also detected under UV lamp on thin-layer chromatograms.

The NMR (100 MHz) of I in $CDCl_3$ showed signals at δ 0.93 (3H, d, $J=6.7$ Hz), 1.31 (3H, d, $J=7.0$ Hz), 1.84 (3H, d, $J=5.0$ Hz), 2.27 (3H, s), 2.2~2.4 (1H, m), 2.72 (1H, dd, $J=5.0$, 12.3 Hz), 2.92 (1H, m), 3.38 (1H, dd, $J=3.2$, 13.6 Hz), 3.93 (1H, br.d, 13.6 Hz), 4.48 (1H, m), 5.8~6.4 (3H, m) and 7.2~7.5 (1H, m).

On catalitic hydrogenation over platinum oxide in acetic acid, I consumed two molar equivalents of hydrogen to yield tetrahydro-derivative (II), which showed no UV absorption maximum between 230 and 280 nm and no NMR signal on the field lower than δ 5.0 ppm. These data as well as the mass spectra of I and II were found to be identical with those of nigragillin (N-methyl-*trans*-2,5-dimethyl-N′-sorbyl-pyperazine) and its tetrahydro compound.[2]



I

To test the insecticidal activities, I and II were mixed with artificial diets and applied orally to silkworm larvae. As shown in the Table, I produced immediate effect on the insect through oral administration. For the topical application each sample was dissolved in a definite amount of ethyl acetate. I revealed a strong contact toxicity to silkworm larvae. Even a dose of 5 μg/g, I caused immediate posioning and knockdown, occasionally followed by death, whereas II was tenth as active as I. *dl*-Nigragillin synthesized by the method of Caesar *et al.*[2] indicated same biological activities as the natural one. The effect of I was quite similar to that of aspochracin from *Aspergillus ochraceus*[3] which also contains a conjugated triene amide moiety.

Toxicity of nigragillin to live stock will be reported elsewhere.

* According to Ref. 2), λ_{max} of nigragillin was 252 nm, but synthesized *dl*-nigragillin showed the maximum at 262 nm.

TABLE I. INSECTICIDAL ACTIVITIES OF NIGRAGILLIN (I) AND TETRAHYDRONIGRAGILLIN (II) TO SILKWORM LARVAE THROUGH ORAL ADMINISTRATION

Each samle was mixed into a definite amount of artificial diet. Ten larvae just after third molting were used for each diet.

Compound	Dosage (ppm)	Symptoms[a] in larvae after treatment		Lethality (%) after treatment		
		1 hr	24 hr	24 hr	48 hr	72 hr
I	5	−	−	0	0	0
	10	−	+	0	0	0
	20	+	++	0	0	0
	40	++	+++	0	40	70
	80	+++	+++	0	100	—
II	220	+	++	0	0	30
Control	—	−	−	0	0	0

[a] Poisonous symptoms such as swooning, convulsing and vomitting were observed immediately after feeding. +++, strong; ++, moderate; +, weak; −, no effect.

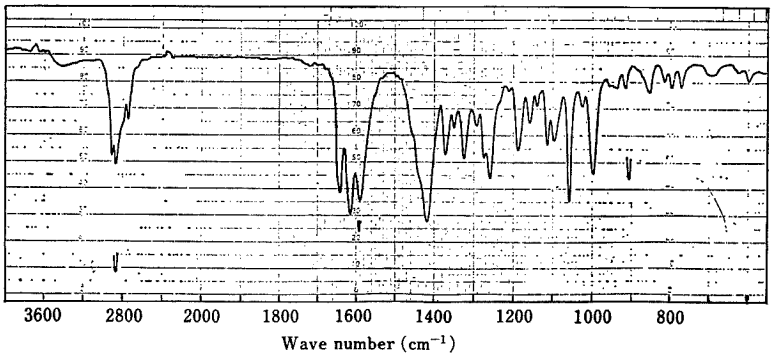


FIG. IR Spectrum of Nigragillin (I) (Film).

Acknowledgement. We thank to Dr. K. Aizawa for the measurement of high resolution mass spectra, and to Mr. K. Morii for his encouragement through this work.

REFERENCES

1) S. Murakoshi, T. Ohtomo and H. Kurata, *J. Food Hyg. Soc. Japan*, **14**, 65 (1973).
2) F. Caesar, K. Jansson und E. Mutschler, *Pharm. Acta Helv.*, **44**, 676 (1969).
3) R. Myokei, A. Sakurai, C. F. Chang, Y. Kodaira, N. Takahashi and S. Tamura, *Agr. Biol. Chem.*, **33**, 1491 (1969).