HERQULINE, A NEW ALKALOID PRODUCED BY *PENICILLIUM HERQUEI*

FERMENTATION, ISOLATION AND PROPERTIES

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A new alkaloid named herquline has been isolated from culture broth of *Penicillium herquei* Fg-372 by solvent extraction and silica gel chromatography. The molecular formula of herquline has been determined as $C_{19}H_{26}N_2O_2$ on the basis of elemental analysis and its high resolution mass spectrometry. The compound does not possess antimicrobial activities, but weakly inhibits blood platelet aggregation induced by adenosine diphosphate.

In the continuing search for new alkaloids from microorganisms, a fungal strain, Fg-372 was found to produce a new nitrogen-containing basic substance named herquline. The strain was obtained from a soil sample collected at Saitama Prefecture, Japan, and classified as *Penicillium herquei*. The compound was isolated from a fermentation broth of the strain by solvent extraction and silica gel chromatography.

The present paper deals with taxonomy of the producing strain, fermentation, isolation and physicochemical properties of herquline.

Taxonomy of the Producing Strain

For the identification of the fungus, CZAPEK's agar, malt extract agar and potato dextrose agar were prepared. A stock culture of the isolate was inoculated onto these media and incubated at 27°C and 37°C, and the culture was observed for about 10 days. Cultural characteristics and morphology of the strain Fg-372 are summarized in Tables 1 and 2, respectively.

The producing organism, strain Fg-372, was identified as *Penicillium herquei* by comparison of the cultural characteristics and morphology with those given by RAPER and THOM[2] for the type species and with *P. herquei* BAINIER and SARTORY (IFO 4674 and 7904) from Institute for fermentation, Osaka, Japan.

Fermentation

The culture of *Penicillium herquei* Fg-372 was maintained on potato dextrose agar or as freeze-dried stock. The stock culture was inoculated in a 500-ml ROUX flask containing 100 ml of potato dextrose agar, and incubated for 7 days at 27°C. Preparation of the spore suspension was performed by addition of 100 ml of sterilized distilled water to the 500-ml ROUX flask followed by vigorous agitation of the water over the agar surface with a sterile loop. Two ml of the spore suspension were used for the inoculation of a 5-liter conical flask containing 1 liter of the following medium; glucose 1.0%, sucrose 2.0%, $NaNO_3$ 0.2%, K_2HPO_4 0.1%, KCl 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $FeSO_4 \cdot 7H_2O$ 0.001%, corn steep liquor 1.0% (pH adjusted to 6.0 prior to sterilization). The inoculated fermentation medium was incubated at 27°C for 10 days for the production of herquline. Herquline was not produced by

Table 1. Cultural characteristics of strain Fg-372.

	Growth (colony size) in 7 days at 27°C	Color[1]
Malt extract agar	Good, 26~27 mm Deeply velvety	Sage green (24 ig) - dusty green (24 ge) Reverse: antique gold (1½ ne) Soluble pigment: dusty yellow (1½ gc)
Potato dextrose agar	Good, 17~19 mm Deeply velvety	Sage green (24 ig) Reverse: gold (1½ nc) Soluble pigment: citron yellow (1 lc)
CZAPEK's agar	Poor-moderate, 8~10 mm Velvety	Pea green (24 ie) Reverse: dusty yellow (1½ gc) Soluble pigment: dusty yellow (1½ gc)

Growth at 37°C was very poor in these media.

Table 2. Morphology of strain Fg-372.

Penicilli	Biverticillate and usually symmetrical
Conidiophores	Coarse and long, 360~480 μ in length by 3~4 μ in diameter
Sterigmata	Tapered, 9~10 $\mu \times 2.5 \sim 3.5$ μ
Conidia	elliptical, smooth, 3.4~4.0 $\mu \times 2.0 \sim 3.0$ μ

submerged culture. The potency of the alkaloid accumulated in culture broth was determined by DRAGENDORFF's method described in our previous article[3].

Isolation

Cultured broth (30 liters) of *Penicillium herquei* Fg-372 obtained by incubation in 30 conical flasks (5-liter capacity) was used as starting material for the isolation of alkaloid herquline. The broth containing mycelia was adjusted to pH 10 with aqueous ammonia. The alkaloid produced was extracted with 12 liters *n*-butyl acetate and then transferred into 3 liters 0.1 N hydrochloric acid. The water layer was subsequently adjusted to pH 10 with aqueous ammonia and extracted twice with 1 liter ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo* to yield pale yellowish crystalline material. After recrystallization from *n*-hexane, colorless plates of herquline (260 mg) were obtained. The hydrochloride of the compound for biological tests was prepared as following. After the crystals were dissolved in anhydrous benzene, hydrogen chloride gas was introduced into the solution until no more precipitate formed. The hydrochloride of herquline was thus obtained.

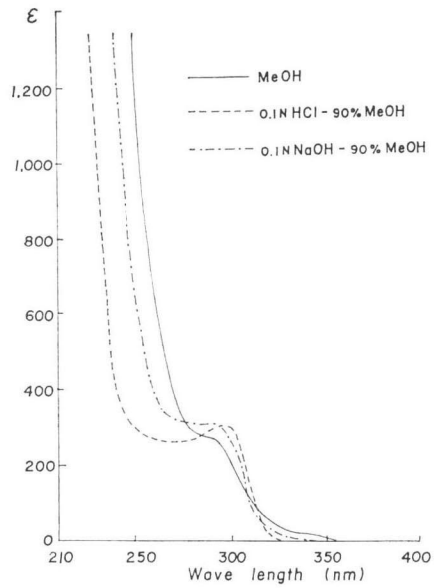
Physical and Chemical Properties

Herquline can be obtained as basic and lipophilic crystals. Its physical and chemical properties are summarized as follows.

1) Melting point: 171~174°C (dec.)
2) Optical rotation: $[\alpha]_D^{27}$ −388° (*c* 1, MeOH)
3) Elemental analysis: Found, C 72.75%; H 8.33%; N 8.87%
   Calculated for $C_{19}H_{26}N_2O_2$, C 72.58%; H 8.34%; N 8.91%
4) Molecular weight (by mass spectrometry): 314

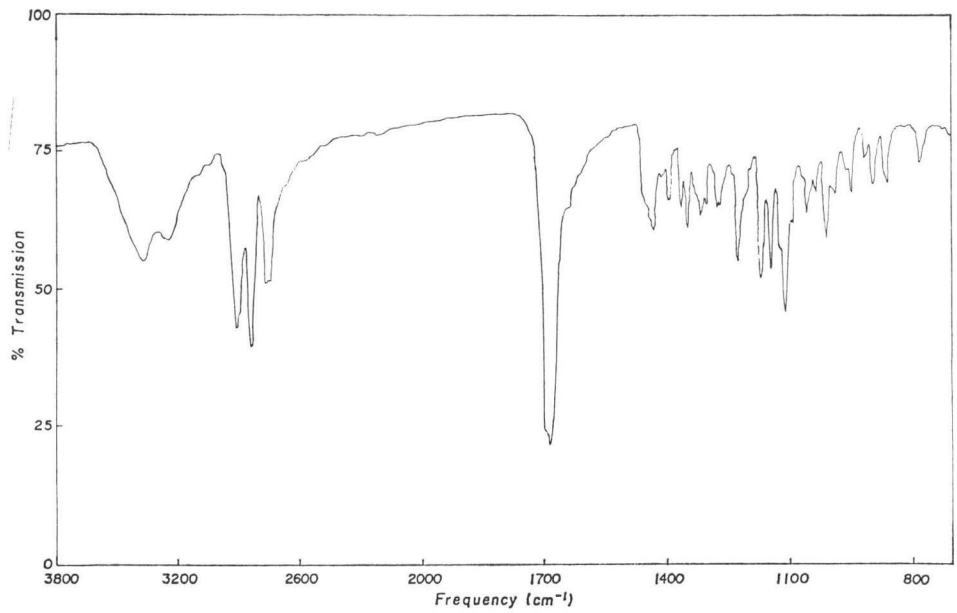
5) Molecular formula: $C_{19}H_{26}N_2O_2$
6) UV absorption: 288 nm (ϵ283 sh.) in methanol, 291 nm (ϵ 331) in 0.1 N HCl - 90% methanol, 287 nm (ϵ317 sh.) in 0.1 N NaOH - 90% methanol (Fig. 1).
7) Color reaction: Positive: DRAGENDORFF, iodine. Negative: BEILSTEIN, ninhydrin, aniline phthalate, ferric chloride, RYDON-SMITH.
8) Solubility: Soluble in chloroform, benzene, methanol and ethyl acetate. Slightly soluble in *n*-hexane.
9) Rf values on silica gel TLC (Merck, Kieselgel G): Chloroform - methanol (10: 1), 0.39; benzene - acetone (7: 3), 0.13; *n*-butanol - acetic acid - water (4: 1: 2), 0.35.

Fig. 1. UV spectra of herquline.



The molecular formula of herquline, $C_{19}H_{26}N_2O_2$, was determined on the basis of elemental analysis and its high resolution mass spectrometry (found: *m/e* 314.199, calculated for $C_{19}H_{26}N_2O_2$: *m/e* 314.199). The IR spectrum (Fig. 2) shows absorptions at 3200~3500 cm^{-1} (amine and/or hydroxyl groups), at 2750~2950 cm^{-1} (methyl and methylene groups), and at 1694 and 1705 cm^{-1} (carbonyl group). The proton NMR spectrum (Fig. 3) measured in $CDCl_3$ shows characteristic signals at δ 5.59, 3.93, 3.40 and 1.7~3.1 ppm.

Fig. 2. IR spectrum of herquline (KBr).

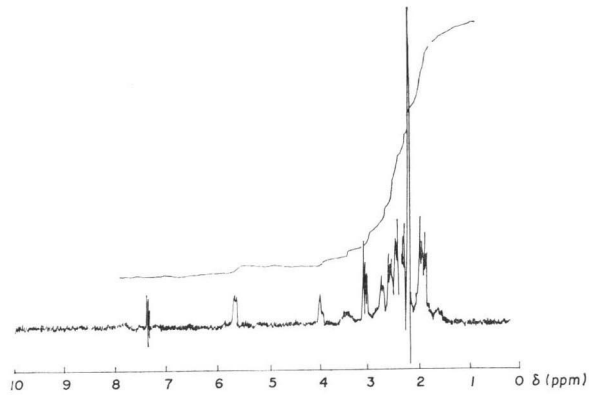


Biological Properties

Herquline inhibited weakly blood platelet aggregation induced by adenosine diphosphate in primary test (100 μg/ml *in vitro*, rabbit PRP). Further investigation on its pharmacological activities is now in progress.

The acute toxicity (LD_{50}) of the compound by intraperitoneal administration in mice was greater than 100 mg/kg.

Fig. 3. NMR spectrum of herquline (100 MHz, $CDCl_3$).



Antimicrobial activities of herquline was assayed by paper disc method, but the compound was inactive at 1 mg/ml against the following test organisms: *Staphylococcus aureus* FDA 209P, *Bacillus subtilis* PCI 219, *Sarcina lutea* PCI 1001, *Pseudomonas aeruginosa* P-3, *Xanthomonas oryzae, Escherichia coli* NIHJ, *Candida albicans, Saccharomyces sake, Aspergillus niger, Aspergillus brevipus* and *Piricularia oryzae*.

Discussion

We have previously reported on the isolation of new alkaloid neoxaline[4] from *Aspergillus japonicus* Fg-551. In the continuing search for new alkaloids from fungi, we found a novel alkaloid, herquline to be produced by *Penicillium herquei* Fg-372, a soil isolate.

It is known that some alkaloids, such as oxaline[5], diketopiperazines[6], tremorgins[7], cyclopiazonic acid[8], rugulovasines[9], verruculogen[10] and roquefortines[11] are produced by *Penicillium*, and many other basic metabolites, which contain a nitrogen atom in the molecule, have been isolated from fungi. However, herquline can be distinguished from those known fungal metabolites by its UV spectrum, IR spectrum and molecular formula. Herquline is therefore, considered to be a novel alkaloid of fungal origin. Its chemical structure is now under investigation.

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