Communications to the editor

PHENOPICOLINIC ACID, A NEW MICROBIAL PRODUCT INHIBITING DOPAMINE β-HYDROXYLASE

Sir:

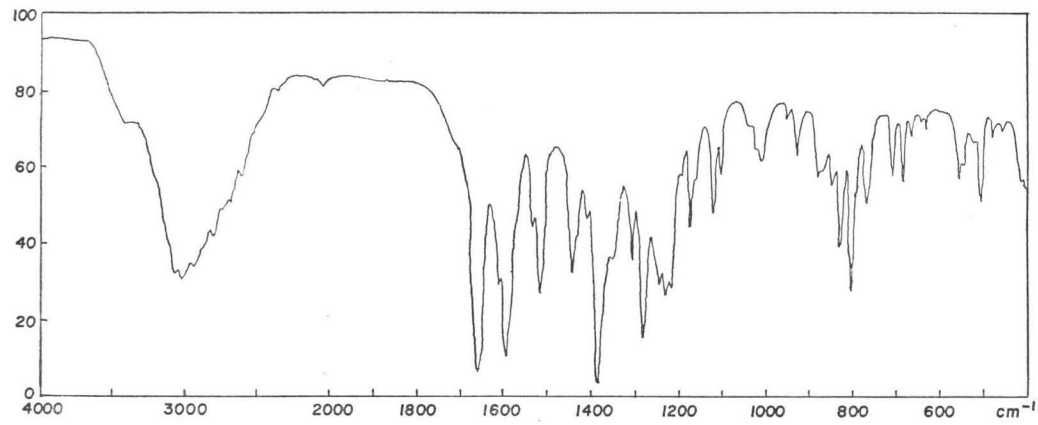
A new potent inhibitor of dopamine β-hydroxylase, named phenopicolinic acid, was found in culture filtrates of a strain of a fungus, *Paecilomyces* sp. AF2562. In the present communication we describe the isolation, structural elucidation and biological properties of phenopicolinic acid.

Paecilomyces sp. AF2562, which was isolated from soil, was shake-cultured at 30°C in a medium containing 3.0% glucose, 0.5% Polypepton and 0.1% yeast extract. After 48 hours 200 ml of the shake-culture was transferred into 30-liter jar fermentor containing 20 liters of the same medium. The fermentation was carried out at 30°C for 66 hours under aeration and stirring. The inhibitory activity on dopamine β-hydroxylase of beef adrenal medulla was assayed by the method of NAGATSU *et al.*[1] The combined cultural filtrate (34 liters), which contained about 500 mg of phenopicolinic acid, was applied to a column of Dowex 50×8 H^+ type (1 liter) and washed with 5 liters of water. The active principle was eluted with 2.6 liters of 1 N NH_4OH. The eluate was adjusted to pH 2.0 with 3 N HCl and extracted three times with an equal volume of ethyl acetate. The solvent layers were combined and concentrated *in vacuo* yielding 20.5 g of brown residue, which was applied to an alumina column (Neutral, E. Merck, W. Germany) and was developed with methanol - 4 N NH_4OH (4 : 1, v/v). The active fraction was concentrated *in vacuo* and was further purified by silicic acid column chromatography with chloroform - methanol (19 : 1, v/v). The active fraction was concentrated *in vacuo* and 60 mg of phenopicolinic acid was crystallized from hot methanol.

Phenopicolinic acid had the following properties: colorless needles; m. p. 222~226°C; soluble in aqueous alkali, pyridine and dimethyl sulfoxide; slightly soluble in methanol; sparingly soluble in water and ethyl acetate; insoluble in benzene and *n*-hexane. The molecular formula $C_{13}H_{11}NO_3$ of phenopicolinic acid was calculated from the analytical result (calcd.: C 68.11, H 4.84, N 6.11, found: C 68.06, H 5.13, N 6.22). It was also deduced from the mass spectral analysis of the methyl ester (M^+, *m/e* 243), which was prepared by methylation of phenopicolinic acid with diazomethane.

The structure of phenopicolinic acid was assigned mainly from NMR spectral data together with IR and UV spectral data and some color reactions. The presence of a phenolic hydroxyl group was shown by a positive MILLON's reaction. The positive reaction with ferrous sulfate suggeted the presence of α-carboxy pyridine ring. UV absorption peaks were at 271 nm (ε 11,100) and 225 nm (shoulder, ε 11,900) in 1 N HCl and 300 nm (shoulder, ε 3,700), 277 nm (shoulder, ε 7,300)

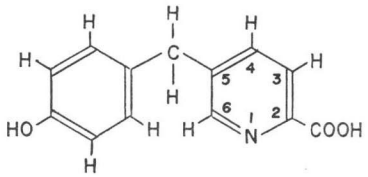
Fig. 1. IR spectrum of phenopicolinic acid (KBr)



and 271 nm (shoulder, ε 8,000) in 1 N NaOH. The IR spectrum of phenopicolinic acid is shown in Fig. 1. NMR spectrum was measured in d_6-DMSO using tetramethylsilane as an internal standard. A signal at δ 3.96 ppm (2H, s) was assigned to methylene protons. Aromatic protons were assigned to signals at 6.90 (4H, q) which seemed to be based on a typical absorption of *p*-substituted benzene. The signals at 7.75 (1H, dd, J=2, 8Hz), 8.02 (1H, d, J=8 Hz) and 8.60 (1H, d, J=2 Hz) were assignable to C-4, C-3 and C-6 protons of a 5-substituted picolinic acid nucleus, respectively. Consequently, the structure of phenopicolinic acid was represented by 5-(4-hydroxybenzyl) picolinic acid (Fig. 2). The conclusion was confirmed by the chemical synthesis of phenopicolinic acid.[2]

Fusaric acid (5-butylpicolinic acid) was reported to be a potent inhibitor of dopamine β-hydroxylase, produced by fungi.[3] The inhibitory activity of phenopicolinic acid was about twice as much as that of fusaric acid. The concentration of phenopicolinic acid required for 50% inhibition was 3.9×10^{-8} M. Studies on the kinetics of dopamine β-hydoxylase inhibition revealed that phenopicolinic acid was uncompetitive with tyramine and competitive with ascorbic acid, and that the Ki value was 5×10^{-9} M. A potent hypotensive effect of phenopicolinic acid was observed in preliminary experiments on spontaneously hypertensive rats. When 50 mg/kg of phenopicolinic acid was administered orally, the decrease in blood pressure was 21, 16 and 23% in 1, 3 and 5 hours, respectively, after administration. The LD_{50} of phenopicolinic acid for mice was about 350 mg/kg by intraperitoneal injection.

Fig. 2. Structure of phenopicolinic acid.



TERUYA NAKAMURA
HIDEYUKI YASUDA
AKIRA OBAYASHI
OSAMU TANABE

Central Research Laboratories,
Takara Shuzo Co., Ltd.,
Otsu-shi, Shiga-ken, Japan

SHINGO MATSUMURA
FUSAO UEDA
KATSUYA OHATA

Research Laboratories,
Nippon Shinyaku Co., Ltd.,
Nishioji, Minami-ku,
Kyoto, Japan

(Received March 14, 1975)

References

1) SUDA, H; T. TAKEUCHI, T. NAGATSU, M. MATSUZAKI, T. MATSUMOTO & H. UMEZAWA: Inhibition of dopamine β-hydroxylase by 5-alkylpicolinic acid and their hypotensive effects. Chem. Pharm. Bull. (Tokyo) 17: 2377~2380, 1969

2) MURAI, H; T. OKUBO, S. TADA, H. WATANABE, S. MATSUMURA, T. NAKAMURA & H. YASUDA: Synthesis of a new metabolite, phenopicolinic acid. (in preparation)

3) HIDAKA, H; T. NAGATSU, K. TAKAYA, T. TAKEUCHI, H. SUDA, K. KOJIRI, M. MATSUZAKI & H. UMEZAWA: Fusaric acid, a hypotensive agent produced by fungi. J. Antibiotics 22: 228~230, 1969