7-O-METHYLSPINOCHROME B AND ITS 6-(3-HYDROXY-*N*-BUTYL)-DERIVATIVE, CATECHOL-O-METHYL TRANSFERASE INHIBITORS, PRODUCED BY *FUNGI IMPERFECTI*

Sir:

In a previous paper[1] we reported isolation of two catechol-O-methyl transferase (COMT) inhibitors produced by a *Streptmyces* and identified as methylspinazarin (2, 3, 5, 8-tetrahydroxy-6-methyl-1, 4-naphthoquinone) and 6, 7-dihydromethylspinazarin. In this paper we report the isolation of other inhibitors which are produced by *Fungi imperfecti*. Their structures were determined to be 7-O-methylspinochrome B (2, 3, 5-trihydroxy-7-methoxy-1, 4-naphthoquinone) and 6-(3-hydroxy-*n*-butyl)-7-O-methylspinochrome B, a new compound.

In the course of our screening for COMT inhibitors, cultured broth of a *Fungi imperfecti* obtained from Sanraku-Ocean Co., Ltd., Tokyo, showed strong inhibition against COMT by a modification of the assay method described by AXELROD *et al.*[2] This strain was classified by Mr. K. KOHNO of Research Institute of Sanraku-Ocean Co., Ltd., to be *Corynespora cassi cola*. Maximum production was observed after 96 hours of the submerged culture in a medium containing 1.0% potato starch, 0.5% glucose, 1.0% soybean meal, 0.25% yeast extract, 0.05% KH_2PO_4 and 0.025% $MgSO_4 \cdot 7H_2O$, at 27°C.

The cultured broth was filtered, and the filtrate was extracted with butyl acetate at pH 2.0. The separated cake was extracted with methanol. The methanol extract was concentrated and extracted with butyl acetate at pH 2.0. The combined butyl acetate extract was dried under reduced pressure, yielding a reddish gummy material. After washed with petroleum ether, the residue was dissolved in acetone. The acetone-soluble fraction was dried to a reddish powder. The active agent was further purified by silica gel column chromatography with benzene-acetone (10 : 1). The activity appeared in two fractions. The most rapidly eluted compound (**I**), was further purified by Sephadex LH-20 column chromaotgraphy with methanol and then crystallized from acetone-benzene to give reddish needles, m. p. 267°C. The second compound (**II**) was isolated by the same procedure to give reddish orange needles, m. p. 202°C.

Compound **I** has a molecular formula $C_{11}H_8O_6$ (MW 236). Found: C, 56.34; H, 3.84; O, 39.82. Calcd.: C, 55.94; H, 3.41; O, 40.65. ν_{KBr}: 3480 cm^{-1} (OH), 1660, 1620 cm^{-1} (peri-hydroxy quinone. λ_{max}^{EtOH} (log ε): 272 nm (4.33), 285 (shoulder), 322 (3.99), 385 (3.56) and 485 (3.26). It gives positive ferric chloride (violet) and magnesium acetate (violet) tests for peri-hydroxy quinone. The NMR spectrum in DMSO-d_6 solution showed a methoxy signal at δ 3.88 and meta-coupled aromatic proton signals at δ 6.50 and 6.95 (J=2.5 Hz). Broad OH proton signals were centered at *ca.* δ 10.8 and a hydrogen bonded OH signal was seen at δ 12.10. Nuclear OVERHAUSER effects were observed between the methoxy and both aromatic proton signals, +12.0% $CH_{\delta 6.50}\{OCH_3\}$, +12.0% $CH_{\delta 6.95}\{OCH_3\}$.

Compound **I** gave a dimethyl ether by treatment with diazomethane, m.p. 113°C, M^+ *m/e* 264 ($C_{13}H_{12}O_6$). The NMR spectrum in $CDCl_3$ shows three methoxy signals at δ 3.87, 4.07 and 4.11, meta-coupled aromatic proton signals at δ 6.61 and 7.17 (J=2.5 Hz) and a hydrogen bonded OH signal at δ 12.08.

From the above data, **I** was suggested to be 7-O-methylspinochrome B (2, 3, 5-trihydroxy-7-methoxy-1, 4-naphthoquinone) and its dimethyl ether to be the 2, 3-dimethyl ether of **I**.

Both compounds are known compounds derived from spinochrome B, a pigment isolated from the sea urchin. Spinochrome B trimethyl ether[3~5] and its partially demethylated product[6] were identical with the dimethyl ether of **I** and compound **I** respectively, when compared with the melting point, UV and NMR spectra described in the literature[3~6].

Compound **II** has a molecular formula of $C_{15}H_{16}O_7$ (MW 308). Found: C, 58.14; H, 5.36; O, 36.50. Calcd.: C, 58.44; H, 5.23; O, 36.33. ν_{KBr}: 3400 cm^{-1} (OH), 1650, 1610 cm^{-1} (peri hydroxy quinone). λ_{max}^{EtOH} (log ε): 273 nm (4.26), 286 (shoulder), 331 (3.87) and 400 (3.44). It gives the same color reactions as **I**.

By treatment with diazomethane, **II** gave a dimethyl ether, m. p. 108~109°C which had a hydrogen bonded OH (δ 12.38, singlet, in $CDCl_3$). These data suggested that **II** and **I** have the same chromophore. The NMR studies of **II** indicated the presence of a 3-hydroxy-*n*-butyl substituent: δ 1.10 (3H, doublet, J=6 Hz),

3·50 (1H, multiplet), 1.50 (2H, multiplet) and 2.65 (2H, triplet, J=6 Hz) in DMSO-d_6. There were also one methoxy (δ 3.85), one aromatic proton (7.06, singlet), broad OH (*ca.* 11.0) and one hydrogen bonded OH (12.65, singlet) peaks. A nuclear OVERHAUSER effect was observed between the methoxy and aromatic proton signals, +18.0% CH{OCH_3}. The above results indicated that **II** is a 3-hydroxy-*n*-butyl derivative of **I**. There are two possibilities for the position of the alkyl side chain, C_6 or C_8. The chemical shift of the aromatic proton (δ 7.06) indicated that the proton should be present at C_8. Thus it showed the presence of an alkyl substituent at C_6, because the chemical shift of an aromatic proton at C_8 should be at around δ 6.5[4]. Thus, we propose 2, 3, 5-trihydroxy-6-(3-hydroxy-*n*-butyl)-7-methoxy-1,4-naphthoquinone as the structure of **II**.

Compounds **I** and **II** inhibited COMT. ID_{50} values of compounds **I** and **II** were 10 μg/ml (4.23×10^{-5}M) and 7.0 μg/ml (2.27×10^{-5}M) respectively. LINEWEAVER-BURK plots of the kinetic data on both compounds **I** and **II** showed

Fig. 1. LINEWEAVER-BURK plot of adrenaline concentration against rate of O-methylation with or without compounds **I** and **II**

Reaction mixture contained 50 μmoles of phosphate buffer, pH 8.0, various concentrations of L-adrenaline, 2.5 μmoles of magnesium chloride, 0.375 μmoles of S-adenosyl-L-methionine-methyl-H^3 (1 μCi/μmole), enzyme preparation, and distilled water to final volume of 0.5 ml

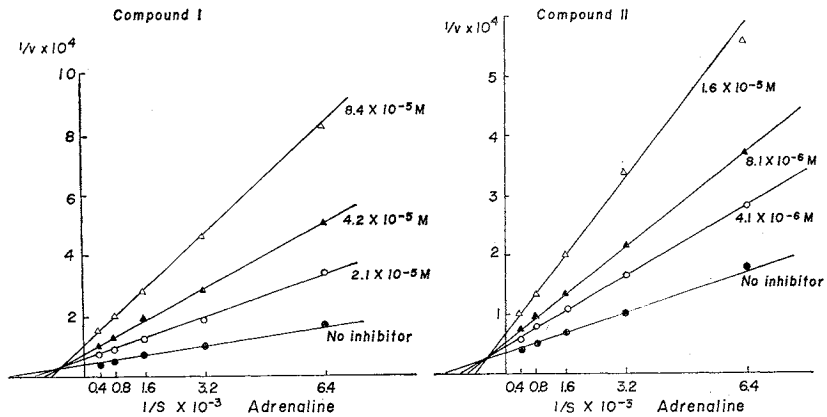
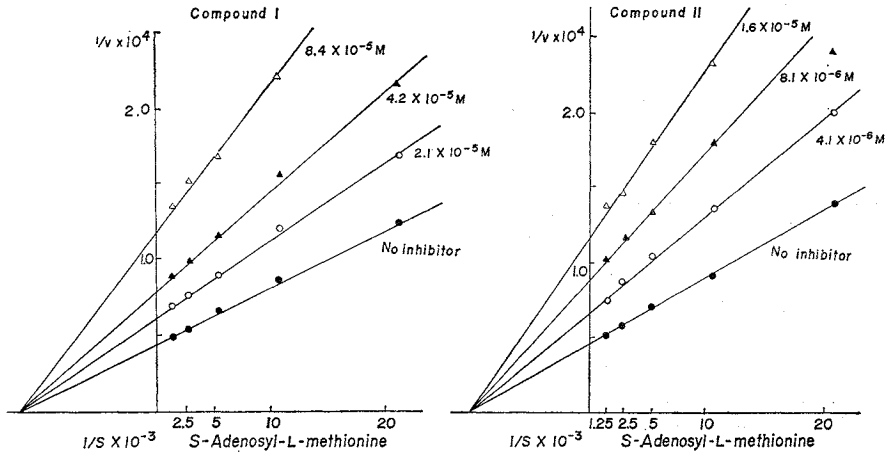


Fig. 2. LINEWEAVER-BURK plot of S-adenosyl-L-methionine concentration against rate of O-methylation with or without compounds **I** and **II**

Reaction mixture contained 50 μmoles of phosphate buffer, pH 8.0, 1.25 μmoles of L-adrenaline, 2.5 μmoles of magnesium chloride, various concentrations of S-adenosyl-L-methionine-methyl-H^3 (1 μCi/μmole), enzyme preparation, and distilled water to final volume of 0.5 ml



the mixed type of inhibition against adrenaline and the non-competitive type of inhibition against S-adenosyl-L-methionine (Figs. 1 and 2). The inhibition constants, *Ki*, values of compounds **I** and **II** against adrenaline were 2.1×10^{-5}M and 6.2×10^{-6}M respectively, while the MICHAELIS-MENTEN constant, *Km*, value of adrenaline was 6.3×10^{-4}M. Against S-adenosyl-L-methionine, the *Ki* values of compounds **I** and **II** were 4.2×10^{-5}M and 8.4×10^{-6}M respectively, while the *Km* value of S-adenosyl-L-methionine was 8.1×10^{-5}M. As inhibition by compounds **I** and **II** did not show any change at excess concentration of magnesium ion (2×10^{-2}M), the effect of these compounds is not due to chelation of magnesium ion.

The effect of compounds **I** and **II** on tyrosine hydroxylase[7] (TH) and dopamine-β-hydroxylase[8] (DβH) was also tested. TH was inhibited by 100 μg/ml (4.23×10^{-4}M: ID_{50}) of compound **I** and by 150 μg/ml (4.87×10^{-4}M; ID_{50}) of compound **II**. These compounds did not inhibit DβH at 100 μg/ml.

The hypotensive effect was studied in the spontaneously hypertensive rat (SHR). The results observed with the intraperitoneal injection of 12.5 mg/kg, 3.1 mg/kg and 0.8 mg/kg are shown in Figs. 3 and 4. From these results, it is clear that compound **II** markedly decreases blood pressure in the SHR.

Fig. 3. Effect of intraperitoneal injection of compound **I** on blood pressure of SHR
Mean depression percentage (2 rats) of each dose

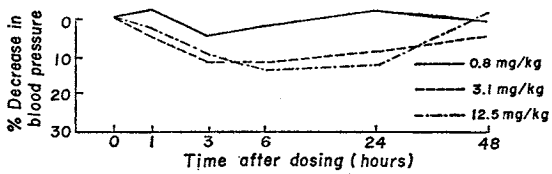
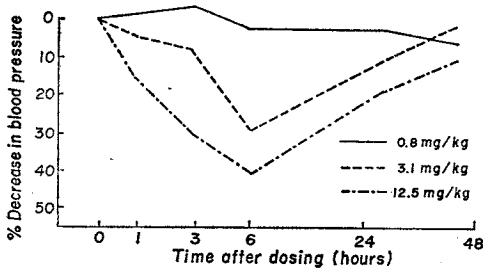


Fig. 4. Effect of intraperitoneal injection of compound **II** on blood pressure of SHR
Mean depression percentage (2 rats) of each dose



No toxicity was observed after the intraperitoneal injection of 200 mg/kg of each compound to mice. These compounds at 100 μg/ml showed no antibacterial activity.

HIDEO CHIMURA
TSUTOMU SAWA
YOSHIKI KUMADA
FUKUKO NAKAMURA
MEIKI MATSUZAKI
TOMOHISA TAKITA
TOMIO TAKEUCHI
HAMAO UMEZAWA

Institute of Microbial Chemistry
Kamiosaki, Sinagawa-ku, Tokyo, Japan

(Received July 24, 1973)

References

1) CHIMURA, H.; T. SAWA, T. TAKITA, M. MATSUZAKI, T. TAKEUCHI, T. NAGATSU & H. UMEZAWA: Methylspinazarin and dihydromethylspinazarin, catechol-O-methyltransferase inhibitors produced by streptomyces. J. Antibiotics 26; 112~114, 1973
2) AXELROD, J.; W. ALBERS & C. D. CLEMENTE: Distribution of catechol-O-methyltransferase in the nervous system and other tissues. J. Neurochem. 5 : 68~72, 1959
3) MOORE, R. E.; H. SINGH, C. W. J. CHANG & P. J. SCHEUER: Polyhydroxynaphthoquinones, preparation and hydrolysis of methoxyl derivatives. Tetrahedron 23 : 3271~3305, 1967
4) MOORE, R. E. & P. J. SCHEUER: Nuclear magnetic resonance spectra of substituted naphthoquinones. Influence of substituents on tautomerism, anisotropy, and stereochemistry in the naphthazarin system. J. Org. Chem. 31 : 3272~3283, 1966
5) GOUGH, J. H. & M. D. SUTHERLAND: The structure of spinochrome B. Terahedron Letters 1964 : 269~274, 1964
6) GOUGH, J. H. & M. D. SUTHERLAND: Pigments of marine animals. IX. A synthesis of 6-acetyl-2, 3, 5, 7-tetrahydroxy-1, 4-naphthoquinone: its status as an echinoid pigment. Aust. J. Chem. 23 : 1839~1846, 1970
7) NAGATSU, T.; M. LEVITT & S. UDENFRIEND: Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239 : 2910~2917, 1964
8) KUZUYA, H. & T. NAGATSU: A simple assay of dopamine β-hydroxylase activity in the homogenate of the adrenal medulla. Enzymologia 36 : 31~38, 1969