METHYLSPINAZARIN AND DIHYDROMETHYLSPINAZARIN, CATECHOL-O-METHYL TRANSFERASE INHIBITORS PRODUCED BY *STREPTOMYCES*

Sir:

Two inhibitors of catechol-O-methyl transferase (COMT) were found in a cultured broth of a *Streptomyces* by our screening studies of enzyme inhibitors. The active substances were crystallized. By the structural studies, the one was identified to be methylspinazarin and the other dihydromethylspinazarin. In this communication, isolation, chemical and physical properties, identification, and biological activities of these inhibitors are reported.

In the course of screening for enzyme inhibitors, a cultured broth of a strain of a *Streptomyces* exhibited strong inhibition against COMT. The strain designated as MD 157-A9 was isolated from a soil collected at Nara City of Japan, and was classified as *Streptomyces filipinensis*. The activity in inhibiting COMT was assayed by a modified method described by AXELROD *et al.*[1]

The strain MD 157-A9 was shake-cultured at 27°C in a medium containing 2.0% potato starch, 2.0% glucose, 2.0% soybean meal, 0.5% yeast extract, 0.25% NaCl, 0.35% $CaCO_3$, 0.0005% $CuSO_4 \cdot 5H_2O$, 0.0005% $MnCl_2 \cdot 4H_2O$, 0.005% $ZnSO_4 \cdot 7H_2O$ (pH 6.6~6.8), and 0.01% silicone oil. The maximum production was obtained at 50~60 hours.

The cultured broth was filtered, and the filtrate was extracted with butanol at pH 2.0. The mycelium part was also extracted with methanol. The methanol extracts were combined and concentrated to remove methanol. The concentrate was extracted with butanol at pH 2.0. The butanol extract was evaporated under reduced pressure, yielding a dark reddish violet syrup. After being washed with *n*-hexane, the residue was dissolved in acetone. The acetone-soluble fraction was dried to a reddish syrup. It was further purified by silica gel column chromatography developed with benzene-acetone (10:1) and benzene-acetone (5:1) successively. The active material was separated into two fractions. One, a fast eluted compound (I), was crystallized from benzene-acetone to give reddish violet needles, m.p. 236°C. The second compound (II) was crystallized from benzene-acetone to yellowish orange needles, m.p. 198°C.

Compound I has a molecular formula $C_{11}H_8O_6$ (MW 236). Found: C 56.10, H 3.32, O 39.75. Calcd.: C 55.94, H 3.41, O 40.65. The molecular formula was confirmed by mass spectrometry: M^+ of dimethyl ether, *m/e* 264 ($C_{13}H_{12}O_6$). It has no optical activity. ν_{KBr}: 3400 cm^{-1} (OH), 1670, 1630 cm^{-1} (peri-hydroxy quinone). λ_{max}^{MeOH} (log ε): 253 nm (4.25), 295 nm (3.90), 490 nm (3.88). It gives positive ferric chloride (violet) and magnesium acetate (violet) tests for peri-hydroxy quinone. The NMR spectrum (internal reference TMS) in DMSO-d_6 solution showed methyl signal at δ 2.18 and methine signal at δ 7.07 which coupled with each other (J=*ca.* 1 Hz). Two broad OH proton signals were present centered at δ 10.4 and 12.1. The nuclear OVERHAUSER effect between methyl and methine signals was observed, +27% CH{CH_3}.

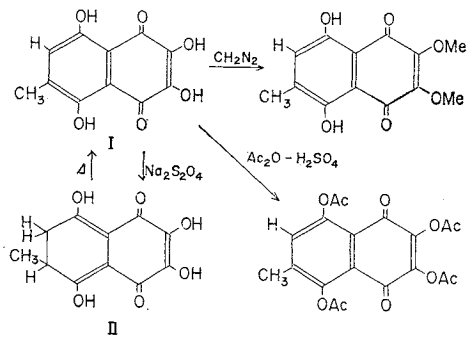
Compound I gave the dimethyl ether by treatment with diazomethane, m.p. 116~118°C. The NMR spectrum in $CDCl_3$ solution showed two methoxy signals at δ 4.10 and 4.13 and two hydrogen bonded OH protons at δ 12.77 and 12.36.

Compound I afforded the tetraacetyl derivative by acetylation with acetic anhydride and sulfuric acid, m.p. 186~187°C, 4 acetyl signals (in $CDCl_3$) at δ 2.42, 2.36 and 2.30 (6H).

The carbon skeleton of compound I was confirmed to be naphthalene by zinc dust distillation.

From the above data, compound I was deduced to be methylspinazarin (2, 3, 5, 8-tetrahydroxy-6-methyl-1,4-naphthoquinone). Methylspinazarin was synthesized by KURODA *et al.*[2] in their studies on pigments of sea urchins. The identity was confirmed by direct comparison of UV and IR spectra with an authentic sample.

Compound II has molecular formula $C_{11}H_{10}O_6$ (MW 238). Found: C 55.97, H 4.23, O 39.80. Calcd.: C 55.46, H 4.23, O 40.30. M^+, *m/e* 238. ν_{KBr}: 3350 cm^{-1} (OH), 1630 cm^{-1} (hydrogen bonded quinone). λ_{max}^{MeOH} (log ε):



265 nm (4.24), 345 nm (3.82), 385 nm (3.89). The NMR spectrum in DMSO-d_6 solution showed 1H at δ 3.20, 2H at 2.95, 3H at 1.25 and OH protons at 11.3 (broad). The double resonance experiment revealed the presence of the following partial structure: CH_3-CH-CH_2-. Dimethyl ether (m.p. 128°C) and tetraacetate of **II** were prepared by the same methods applied to the compound **I**. These results suggested that compound **II** should be 6,7-dihydromethylspinazarin.

After heating up to about 200°C, compound **II** was converted partly to methylspinazarin. By reduction with sodium dithionate, methylspinazarin afforded compound **II**. Thus, compound **II** was determined to be 6,7-dihydromethylspinazarin.

It has been reported that COMT is involved in inactivation of circulating or exogenously supplied catechol amines.[3] The following compounds have been reported to inhibit COMT: polyphenols,[4] dihydroxyphenyl acetamides,[5] 3-hydroxyestradiol,[6] 3,5-dihydroxy-4-methoxybenzoic acid,[7] tropolones,[8] pyridoxal 5'-phosphate,[9] flavonoid[5] and 8-hydroxyquinoline.[5] As reported in this paper, methylspinazarin and dihydromethylspinazarin also inhibited COMT strongly *in vitro*. ID_{50} of methylspinazarin and dihydromethylspinazarin were 0.80 μg/ml (3.3×10^{-6} M) and 0.78 μg/ml (3.2×10^{-6} M) respectively. Their dimethoxy derivatives did not show any inhibition at 100 μg/ml.

The effect of methylspinazarin and dihydromethylspinazarin on tyrosine hydroxylase (TH)[10], dopa decarboxylase (DDC)[11] and dopamine-β-hydroxylase (DβH)[12] were also studied. DβH was inhibited by 6.6 μg/ml (2.7×10^{-5} M) of dihydromethylspinazarin. They did not inhibit the other enzymes at 100 μg/ml.

Fig. 1. Effect of methylspinazarin on blood pressure of SH-rats.

Mean depression percentage (5 rats) of each dose.
Bars show the standard error.

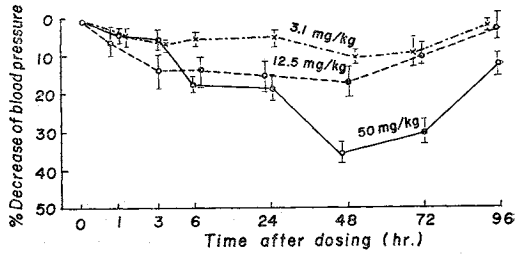
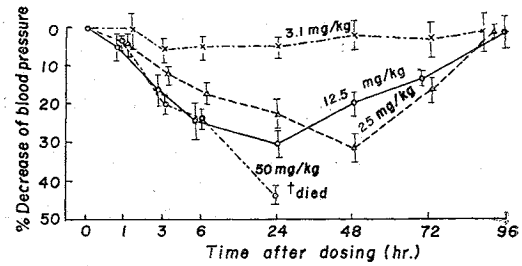


Fig. 2. Effect of dihydromethylspinazarin on blood pressure of SH-rats.

Mean depression percentage (5 rats) of each dose.
Bars show the standard error.



The hypotensive effect against spontaneously hypertensive rat (SHR) was studied. The results are shown in Figs. 1 and 2. From these results, it is clear that both methylspinazarin and 6,7-dihydromethylspinazarin have strong activity in decreasing blood pressure of SHR.

LD_{50} of methylspinazarin and dihydromethylspinazarin to mice were 50~100 mg/kg by intravenous injection. Antimicrobial activity of these compound was not found at 100 μg/ml.

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HIDEO CHIMURA
TSUTOMU SAWA
TOMOHISA TAKITA
MEIKI MATSUZAKI
TOMIO TAKEUCHI
TOSHIHARU NAGATSU*
HAMAO UMEZAWA

Institute of Microbial Chemistry,
Kamiosaki 3-14-23, Shinagawa-ku,
Tokyo, Japan
* Department of Biochemistry,
School of Dentistry,
Aichi-gakuin University,
Nagoya, Japan

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References

1) 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
2) Kuroda, C. & H. Ohshima : The pigments from the sea urchins and the syntheses of the related compounds. Proc. Imp. Acad. (Japan) 16 : 214～217, 1940
3) Axelrod, J. ; H. Weil-Malherbe & R. Tomchick : The physiological disposition of H^3-epinephrine and its metabolite metanephrine. J. Pharmacol. Exp. Ther. 127 : 251～256, 1959
4) Axelrod, J. & M. J. Laroche : Inhibitor of O-methylation of epinephrine and norepinephrine *in vitro* and *in vivo*. Science 130 : 800, 1959
5) Ross, S. B. & Ö. Haljasmaa : Catechol-O-methyl transferase inhibitors. *In vitro* inhibition of the enzyme in mouse-brain extract. Acta Pharmacol. et Toxicol. 21 : 205～214, 1964
6) Knuppen, R.; W. Lubrich, O. Haupt, U. Ammerlahn & H. Breuer : Wechselwirkungen zwischen Oestrogenen und Catechlminen. I. Beeinflussung der enzymatischen Methylierung von Catecholaminen durch Oestrogene und *vice versa*. Z. Physiol. Chem. 350 : 1067～1075, 1969
7) Nikodejevic, B.; S. Senoh, J. W. Daly & C. R. Creveling : Catechol-O-methyltransferase. II. A new class of inhibitors of catechol-O-methyltransferase ; 3, 5-dihydroxy-4-methoxybenzoic acid and related compounds. J. Pharmacol. Exp. Ther. 174 : 83～93, 1970
8) Belleau, B. & J. V. Burba : Tropolones : A unique class of potent noncompetitive inhibitors of S-adenosylmethionine-catechol methyltransferase. Biochim. Biophys. Acta 54 : 195～196, 1961
9) Black, I. B. & J. Axelrod : unpublished
10) Nagatsu, T.; M. Levitt & S. Udenfriend : Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239 : 2910～2917, 1964
11) Davis, V. E. & J. Awapara : A method for the determination of some amino acid decarboxylases. J. Biol. Chem. 235 : 124～127, 1960
12) Kuzuya, H. & T. Nagatsu : A simple assay of dopamine β-hydroxylase activity in the homogenate of the adrenal medulla. Enzymologia 36 : 31～38, 1969