the C-7 (8) double bond in the parent oligoglycoside (**14** or **19**) is presumed to be shifted to C-8 (9) in the hydrolysates during the acidic hydrolysis.[1)]

9) Based on this finding, another possible sugar sequence (3-Me-glu^{3}—xyl^{4}—3-Me-glu^{3}—glu^{4}—xyl^{3}(—glu^{2})—aglycone)

for B_1 has been ruled out. If this alternate sequence is correct, glycerol should be obtained instead of erythritol.

Faculty of Pharmaceutical Sciences,
Osaka University,
1-6, Yamada-oka, Suita,
Osaka 565, Japan

Isao Kitagawa*
Motomasa Kobayashi
Tatsuya Inamoto
Tohru Yasuzawa

Institute for Protein Research,
Osaka University,
3-2, Yamada-oka, Suita,
Osaka 565, Japan

Yoshimasa Kyogoku

Received June 22, 1981

[Chem. Pharm. Bull. 29(8)2391—2393(1981)]

Anti-Platelet Aggregation Principles from the Bark of *Fraxinus japonica* Blume[1)]

The methanol extract of the bark of *Fraxinus japonica* Blume was fractionated with the guidance of inhibitory activity on rabbit platelet aggregation induced by arachidonic acid. The following active principles were identified: 3-methoxy-4-hydroxyphenylethanol (I) (which also exists in the animal body as a dopamine metabolite), *p*-hydroxyphenylethanol (II), 2,6-dimethoxy-*p*-benzoquinone (III), compounds (IV), (V), and esculetin.

Keywords——the bark of *Fraxinus japonica* Blume; anti-aggregatory activity on platelet; 3-methoxy-4-hydroxyphenylethanol; *p*-hydroxyphenylethanol; 2,6-dimethoxy-*p*-benzoquinone; esculetin; 3,4-dihydroxyphenylethanol; dopamine metabolites

The bark of *Fraxinus japonica* Blume (the Oriental medicine "Shinpi"), which is widely distributed in Japan, has been used as a home remedy diuretic, antifebrile analgesic and so on.

The methanol extract of the bark is reported to have an anti-inflammatory action and to promote the excretion of uric acid.[2,3)] Esculin, a coumarin glycoside which is a main constituent of the extract, is an active principle having these physiological activities.[4)] Until now no other biologically active compound has been found in the bark.

The methanol extract of the bark was bioassayed for inhibitory activity on rabbit platelet aggregation induced by arachidonic acid (AA) and found to be active. We report here the isolation of the active principles guided by this bioassay[5)] and their potencies.

The methanol extract of the bark was partitioned between ethyl acetate and water. The acidic portion, which was fractionated from the ethyl acetate layer in the usual manner and had a significant inhibitory activity, was separated into eight fractions by Sephadex-LH 20 column chromatography. Two of the fractions, which showed a potent inhibitory activity, were further purified by the combination of high performance liquid chromatography (μBondapak C_{18}) and preparative thin-layer chromatography (silica gel). Finally, the compounds I, II, III, IV, V, and esculetin were isolated as the active principles. All except esculetin gave very low yields.

Compound I, colorless oil; $C_9H_{12}O_3$; MS *m/e*: 168 (M^+); IR ν_{max}^{KBr} cm^{-1}: 1601; UV λ_{max}^{MeOH} nm: 228, 281, 287sh. The IR and UV spectra suggested that I is an aromatic type compound. The proton nuclear magnetic resonance (^1H NMR) spectrum ($CDCl_3$) of I showed signals due to a Phe–$C\underline{H}_2$–$C\underline{H}_2$–O– group at δ 2.80 and and 3.83 (2H, triplet, J=7 Hz, respectively), an aromatic methoxy group at δ 3.89 (3H, singlet), and 1,2,4-trisubstituted benznee ring system at δ 6.70 (1H, double doublet, J=2, and J=8 Hz), 6.73 (1H, doublet, J=2 Hz) and 6.87 (1H, doublet, J=8 Hz). The above data indicated I to be 3-methoxy-4-hydroxyphenylethanol which is an alkaline hydrogenation product of wood[6] and a dopamine metabolite.[7] This was comfirmed by direct comparison with an authentic specimen using IR, MS, ^1H NMR spectrometry.

Compound II, colorless needles; mp 92°C; $C_8H_{10}O_2$; MS *m/e*: 138 (M^+); IR ν_{max}^{KBr} cm^{-1}: 1603, 1596.; UV λ_{max}^{MeOH} nm: 224, 278, 285sh. The ^1H NMR spectrum (CD_3OD) showed the presence of a Phe–$C\underline{H}_2$–$C\underline{H}_2$–O– group at δ 2.71 and 3.68 (2H, triplet, J=7 Hz, respectively), and a *p*-substituted benzene ring system at δ 6.69 and 7.02 (2H, doublet, J=9 Hz, respectively). These data indicated II to be *p*-hydroxyphenylethanol and it was identified by direct comparison with an authentic sample of mp, IR, MS, and ^1H NMR spectra.

Compound III, yellow needles; sublimes; $C_8H_8O_4$; MS *m/e*: 168 (M^+); IR ν_{max}^{KBr} cm^{-1}: 1669, 1642, 1621, 1596.; UV λ_{max}^{MeOH} nm: 286, 294sh, 382. The ^1H NMR spectrum ($CDCl_3$) showed the presence of two equivalent methoxy groups at δ 3.82 (6H, singlet) and two equivalent olefinic protons at δ 5.85 (2H, singlet). From these data, III was assumed to be 2,6-dimethoxy-*p*-benzoquinone and this was confirmed by direct comparison of the IR and MS spectra with those of an authentic specimen.

Esculetin which is an aglycone of esculin was identified by direct comparison (mp, IR, MS, PMR) with an authentic sample.

The inhibitory potency of compounds I, II, III, and esculetin on rabbit platelet aggregation induced by AA or collagen was examined (Table I). Table I includes the results obtained by using aspirin and indomethacin as positive controls, and by using 3,4-dihydroxyphenylethanol[8] which is one of the dopamine metabolites as the same with compound I and was found to have relatively potent inhibitory effects.

None of compounds I, II, and 3,4-dihydroxyphenylethanol except compound III (ADP[9] 10 μM final concentration: IC_{50}=80—100 μM[10]) inhibit ADP-induced rabbit platelet aggrega-

Table I. Inhibitory Effects on Platelet Aggregation[a]

Inhibitors	Platelet aggregation induced by[b]	
	Arachidonic acid (100 μM)[c,d]	Collagen (15 μg/ml)[c]
3-Methoxy-4-hydroxyphenylethanol[e] (I)	13.9 (10.9— 17.7)	30.5 (23.0— 40.6)
3,4-Dihydroxyphenylethanol[e]	19.7 (13.6— 28.6)	35.4 (23.9— 52.6)
p-Hydroxyphenylethanol (II)	119.3 (83.9—169.6)	157.4 (111.8—221.6)
2,6-Dimethoxy-*p*-benzoquinone (III)	145.2 (96.7—217.8)	48.5 (40.5— 58.1)
Esculetin	62.6 (47.8— 82.0)	71.6 (57.2— 89.6)
Aspirin	32.3 (24.8— 42.0)	20.0 (14.5— 27.6)
Indomethacin	1.01 (0.80— 1.26)	0.91 (0.77— 1.09)

Aggregation experiments were performed in an aggregometer (SIENCO, dual sample aggregation meter, model DP-247E). For each assay, rabbit platelet-rich plasma (440 μl, 300000 platelets/mm^3) was preincubated with inhibitor (2 μl in ethanol or DMSO) for 3 min at 37°C before the addition of the aggregating agents (40 μl).

a) Figures represent IC_{50} (μM: concentration that inhibits 50% of the agonists' effects) evaluated from 2 to 3 different concentrations of the inhibitor on log probit paper. Each value is the mean and its 95% confidence limits for individual determinations in 5 to 8 different platelet preparations.

b) The maximum decrease in absorbance expressed as percentages of that in the controls (submaximal equipotent) is taken as the criterion for the degree of platelet aggregation.

c) Final concentration.

d) Sodium arachidonate.

e) Exists as a dopamine metabolite in an animal bodies.

tion, just as aspirin and indomethacin, anti-inflammatory drugs which are well known as inhibitors of prostaglandin biosynthesis,[11] have no effect on ADP. Thus, these compounds like aspirin and indomethacin may be expected to have some effect on prostaglandin biosynthesis and anti-inflammatory activity.

It is also of pharmacological interest that the two dopamine metabolites (compound I and 3,4-dihydroxyphenylethanol) exhibited anti-aggregatory activity and were about two times as effective as aspirin in AA (Table I).

Studies are now in progress on the effects of the above compounds on the prostaglandin biosynthesis and anti-inflammatory activity and on the structural elucidation of compounds IV and V.

References and Notes

1) A part of this work was presented at the 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, April 1981.
2) *a*) S. Nakaya, T. Takahashi, T. Itoh, H. Miura, K. Fukushi, and T. Saitoh, *J. Iwate Med. Ass.*, **18** (3), 244 (1966); *b*) K. Saitoh, *ibid.*, **23** (2), 227 (1971); *c*) S. Nakaya, *Yakubutsuryoho*, **3**, 325 (1970).
3) *a*) G. Iida, *Tohoku J. Exp. Med.*, **25**, 454 (1935); *b*) T. Okui, *ibid.*, **30**, 534 (1937); *c*) S. Watanabe, *Folia Pharmacologica Japonica*, **43**, 18 (1947), *ibid.*, **43**, 35 (1947); *d*) S. Nokaya, T. Itoh, T. Takahashi, and I. Kohama, *Yakubutsuryoho*, **3**, 239 (1970).
4) *a*) I. Yamagami, Y. Suzuki, and K. Itoh, *Folia Pharmacologica Japonica*, **64**, 714 (1962); *b*) I. Itoh, Proc. of the 9th Symposium on Oriental Medicine, 1975, pp. 43—50.
5) The activity in each fraction was assayed by the optical density method of Born. G.V. Born, *J. Physiol.* (London)., **162**, 67 (1962). The experimental details are also shown in Table I.
6) Herbert. G. Arlt, Jr., Sonja K. Gross, and Conrad Schuerch, *Tappi*, **41**, 64 (1958).
7) M. Goldstain, A.J. Friedhoff, S. Pomerantz, and C. Simmons, *Biochem. Biophys. Acta*, **39**, 189 (1960).
8) M. Goldstain, A.J. Friedhoff, S. Pomerantz, and J.F. Contrera, *J. Biol. Chem.*, **236**, 1816 (1961).
9) Adenosine 5′-diphosphate.
10) The result was obtained by the method shown in Table I.
11) J.B. Smith and A.L. Wills, *Nature* (London) New Biology, **231**, 235 (1971).

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan

HARUYOSHI KODAIRA
MASAYUKI ISHIKAWA*
YASUO KOMODA
TERUMI NAKAJIMA

Received June 23, 1981

[Chem. Pharm. Bull. 29(8)2393—2396(1981)]

Stereoselective Synthesis and Structure Proof of a Metabolite of Vitamin D_3, (23*S*, 25*R*)-25-Hydroxyvitamin D_3 26,23-Lactone (Calcidiol Lactone)

Stereochemical configurations of biologically prepared 25-hydroxyvitamin D_3 26,23-lactone (calcidiol lactone) at C-23 and C-25 are determined to be *S* and *R*, respectively, by comparison of its high performance LC retention time with those of (23*S*,25*R*)- and (23*R*,-25*S*)-25-hydroxyvitamin D_3 26,23-lactone which have been synthesized stereoselectively starting from C-22 steroid aldehyde and (*R*)- or (*S*)-citramalic acid.

Keywords——vitamin D metabolite; calcidiol lactone; stereoselective synthesis; determination of stereochemistry; iodolactonization