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Ethoxy-hydroxy-benzoic Acid; A Platelet Antiaggregating Substance from *Acanthopanacis Cortex*

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오가피로 부터 혈소판 응집억제작용 물질 Ethoxy-hydroxy-benzoic Acid의 분리

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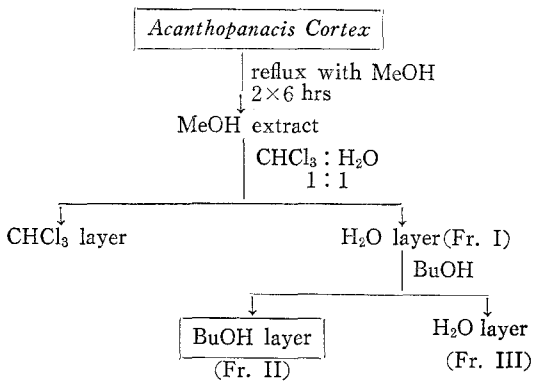
The BuOH fraction prepared from the methanol extract of *Acanthopanacis Cortex* showed inhibitory activity against ADP-induced platelet aggregation. The inhibitory activity remained in ether layer when the BuOH fraction was refluxed with 5% aq. HCl-EtOH (1 : 1 mixture) and extracted with ether. From the ether layer, ethoxy-hydroxy-benzoic acid (m.p. 128~130°C), a platelet antiaggregating substance, was isolated.

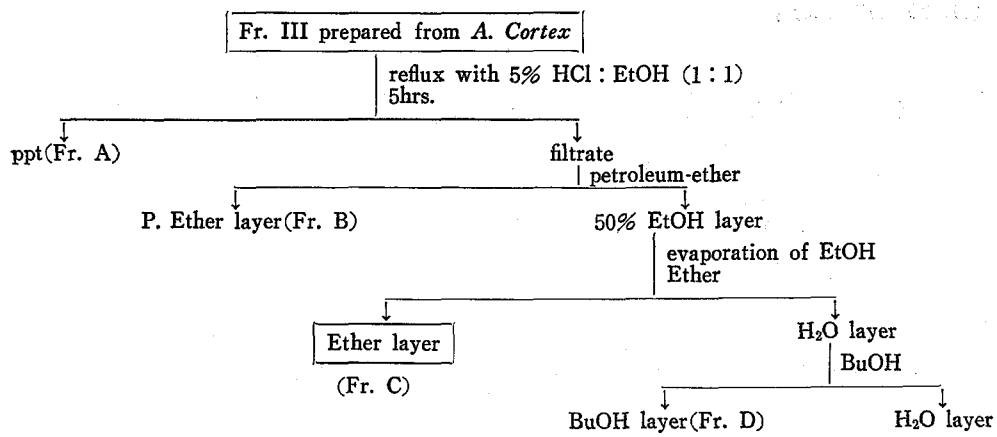
It was reported that the H_2O fraction prepared from the methanol extract of *Acanthopanacis Cortex* showed inhibitory activity against ADP-induced platelet aggregation.[1)]

As in Scheme 1, the H_2O fraction (Fr. I) was fractionated again into butanol soluble (Fr. II) and water soluble (Fr. III) fractions. Fr. II was inhibitive against platelet aggregation at the concentration of 2.5 mg/ml while Fr. III showed no effect at 5 mg/ml. The active fraction (Fr. II) was treated with 5% aq. HCl-EtOH (1 : 1 mixture) and fractionated as shown in Scheme 2 and each fraction (Fr. A~D) was subjected to test for the aformentioned activity. The activity was concentrated in Fr. C which exhibited inhibition of platelet aggregation at the concentration of 1 mg/1ml. On silica gel chromatography of Fr. C eluting with $CHCl_3$: acetone (20 : 1~7 : 1), an antiplatelet aggregating substance of m.p. 128~130°C (Comp. A) was isolated.

It's UV spectrum (λ max 251 and 286.5 nm, shifted to 313.5 nm in alkali) indicated its phenolic nature. The NMR spectrum showed a triplet at δ 1.37 (J=7) and a quartet at δ 4.32 (J=7) suggesting the presence of $-OCH_2CH_3$ function. In the aromatic region there were three protons. Two D_2O exchangable protons at δ 5.84 and δ 1.64 disappeared when Comp. A was methylated and a signal for two methyl groups appreared at δ 3.92 as a singlet. In the



Scheme 1. Extraction and fractionation of *Acantho panacis Cortex*.



Scheme 2. Acid hydrolysis and fractionation of Fr. III prepared from *Acanthopanacis Cortex*.

mass spectrum, the molecular ion peak appeared at m/e 182(M^+). Other significant peaks in the mass spectrum were at m/e 154 ($M^+-CH_2CH_2$), 137(M^+-COOH) and 109 ($M^+-CH_2CH_2$, $-COOH$). The above date together with the IR spectrum (ν_{max} 3490 and 1675 cm^{-1}) suggested that Comp. A is a benzoic acid with one ethoxy and one phenolic hydroxy groups substituted; ethoxy-hydroxy-benzoic acid.

The NMR spectrum of phenyl protons of dimethylated Comp. A, a double doublet centered at δ 7.68 with J_1=8.5 and J_2=2, a doublet at δ 7.54 with J=2 and another doublet at δ 6.86 with J=8.5, suggests that three functional groups are situated at C_1, C_2 and C_4 positions. The appearance of proton signal from carboxylic acid at unusually higher field suggests the possible intramolecular hydrogen bonding between the carboxyl and phenolic hydroxyl groups. However, the determination of absolute structure should need further studies.

The comparison of TLC pattern of Fr. C prepared from *Acanthopanacis Cortex* purchased and that prepared from the cortex of *Acanthopanax senticosus* was made and the presence of Comp. A was confirmed from the latter

Experimental

Acanthopanacis Cortex was purchased from the local herb drug market. *Acanthopanax senticosus* was collected from Iksan, Chunbuk, Korea in Oct. 1982.

The following instruments were utilized for the present work: IR, Perkin-Elmer 281 B; UV, Gilford 2600; NMR, Varian FT 80 A; Mass, Hewlett Packard 5985 B; Centrifuge, Sorvall RT 6000; Microscope, AD Spencer 1051T; Thrombocounter, Coulter Electronics THC; Aggregometer, Chrono-Log 340.

Plant extraction and fractionation

Plant samples were extracted and fractionated as described in Scheme 1. Methanol extract obtained from *Acanthopanacis Cortex* was partitioned between $CHCl_3$ layer and H_2O layer (Fr. I) which was extracted wth butanol yielding Fr. II and Fr. III.

Separation of ethoxy-hydroxy-benzoic acid (Comp. A)

The butanol fraction (Fr. II) was refluxed with 5% HCl-EtOH (1 : 1 mixture) for 5 hrs. and fractionated as in Scheme 2 yielding Fr. A~D. Fr. C was applied to a silica gel column and eluted with $CHCl_3$: Acetone (20 : 1~7 : 1).

A fraction which exhibited platelet antiaggregating activity was collected and recrystallized from $CHCl_3$, m.p. 128°~130°C. IR νmax(KBr) 3490, 1675 cm^{-1} (COOH) UV λmax(MeOH) 251, 286.5nm, NMR $\delta(CDCl_3)$ 6.81~7.60 (3H, m, phenyl) 5.84 (1H, b, COOH) 4,32 (2H, q, J=7, $-OCH_2$) 1.64(1H, b, OH) 1.37 (3H, t, J=7, $-CH_3$) Mass m/e 182(M^+), 154(M^+ −28), 137(M^+ −45), 109(M^+ −73).

Methylation of Comp. A

10 mg of Comp. A in 2 ml of ethanol was treated with diazomethane for 4 hrs. And the solvent was evaporated off. IR νmax($CHCl_3$) 1705 cm^{-1} ($COOCH_3$), NMR $\delta(CDCl_3)$ 7.68 (1H, dd, J_1=8, J_2=2), 7.54(1H, d, J=2) 6.86(1H, d, J=8.5), 3.92(6H, s, OCH_3, $COOCH_3$).

Platelet-aggergation studies

Rat platelet rich plasma (PRP) was prepared and the aggregation or inhibition of aggregation was observed with either modified smear method[1] or with turbidometry method.[2,3]

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