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ON THE CONSTITUENTS OF THE BARK OF *VIBURNUM AWABUKI* K. KOCH

By

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From the bark of Viburnum awabuki K. Koch ψ-taraxasterol acetate, β-sitosterol, succinic acid, β-methylmalic acid and a mixture of catechine and epicatechine were isolated. The mixture showed antimicrobial activity against *Escherichia coli*.

Introduction and Results

In the course of a study on the screening the plants which contain the antimicrobial components, the antimicrobial activities of the plants which had been used for poular remedy in the Satsunan Islands have been examined[1].

One of these plants, *V. awabuki* K. Koch (Sangoju in Japanese) is reputed to be a fish-poisonous plant in Satsunan Islands. Recently, a piscicidal substance and a plant growth inhibitor were characterized by Kawazu, et al[2].

Since the methanolic extract from the bark of *V. awabuki* K. Koch exhibited antimicrobial activity, we studied the chemical constituents of the same plant.

According to the procedure of isolation shown in Fig. 1, five crystalline compounds, **A**, **B**, **C**, **D** and **E** were isolated.

A was crystallized as colorless needles, mp 220–221°, from methanol-chloroform. The molecular formula, $C_{32}H_{52}O_2$, was assigned on the basis of the elementary analysis and the mass spectrum. The IR spectrum showed absorption bands of an ester group and an olefinic group at 1740 and 1240, and 1640 cm^{-1}, respectively. The NMR spectrum included the signals for an acetoxyl group at δ 2.03 (3H, s), and eight methyl groups at δ1.67 (3H, s), 1.43 (3H, bd), 1.27 (3H, s), 1.03 (3H, s), 0.93 (3H, s), 0.83 (6H, s) and 0.78 (3H, s). These data and a positve Liebermann-Burchard's reaction suggested **A** was a pentacyclic phytosterol. The mass spectrum was identical with that of ψ-taraxasterol acetate reported by C. Djerassi, et al[3].

B was crystallized needles, mp 139–140°, from methanol-chloroform. It seemed to be a phytosterol by positive Liebermann-Burchard's reaction. IR spectrum had a band at 3400 cm^{-1} assigned to a hydroxyl group and was very similar to that β-sitosterol. Teatment of **B** with acetic anhydride and pyridine gave a monoacetate, mp 128°. Both the IR spectrum and the melting point of the acetate were in good

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agreement with those of β-sitosterol acetate.

C, colorless needles, mp 79–80.5°, analysed for the molecular formula $C_5H_8O_5$ $1/5H_2O$. The IR spectrum showed bands at 3470 cm^{-1} due to a hydroxyl group, at 2700–2600 and 1660 cm^{-1} due to a carboxyl group with intramolecular hydrogen bonding, and at 1740 cm^{-1} due to an ester group. Signals due to a $-CH_A-CH_BH_C-$ group in the NMR spectrum of **C** were very similar to those of potassium malate which indicated at δ4.83 (1H, dd, J_{AB}=3.5, J_{BC}=8.5 Hz), 2.78 (1H, dd, J_{AB}=3.5, J_{BC}=16.8 Hz) and 2.70 (1H, dd, J_{AC}=8.5, J_{AB}=16.8 Hz)[4]. From these data, **C** appeared to be β-methylmalic acid. Its identity as β-methylmalic acid was established by comparison of the IR spectrum with that of an authentic sample.

D was crystallized colorless prisms from ethyl acetate, mp 168–169°, molecular formula, $C_4H_6O_4$ established by both of elementary and mass analyses. The compound was identified as succinic acid by comparing its IR spectrum with that of an authentic sample.

E which is colorless prisms, mp 240° (decompn.), has the molecular formula $C_{15}H_{14}O_6$ based on the mass spectrum (M^+ 290) and the molecular formulas of its derivatives which will be described below. The compound had hydroxyl and aromatic group bands at 3200, and 1629, 1612 and 1519 cm^{-1}, respectively.

Acetylation of **E** with acetic anhydride and pyridine yielded a pentaacetate, **F**, mp 156–157°, which had the molecular formula $C_{25}H_{24}O_{11}$. The IR spectrum of the latter compound contained no hydroxyl bands, while in the NMR spectrum the signals of newely produced five acetyl groups at δ2.31 (12H, s) and 1.93 (3H, s) were observed. The NMR spectrum also showed the signals of methylene protones ascribed to a $-CH_2CH-$ group at δ2.95 (2H, d, J=3Hz) whose environment was confirmed by spin decoupling. On irradiation at δ 2.95, two broad doublets at δ 6.60 (1H, d, J=3Hz) and 6.70 (1H, d, J=3Hz) due to aromatic meta protons sharpened, and at δ5.41 (1H, m), the doublet at δ 2.95 collapsed to a singlet. The NMR spectrum exhibited additional evidence for three aromatic protons at δ7.27–7.40 (3H, m), and a methine proton at δ5.12 (1H, m). Treatment of **E** with diazometane afforded a methylether derivative, **G**, mp 147–148°, $C_{19}H_{22}O_6$, which had IR hand due to a hydroxyl group. The hydroxyl group seemed to be secondary, since the methine proton at δ5.41 in the NMR spectrum of **F** shifted to δ4.28 ($\Delta\delta$=1.13 ppm) in the spectrum of **G**.

The above data indicated the presence of the following partial structures, which suggested that **E** was a dihydroflavanol-like compound lacking carbonyl function.

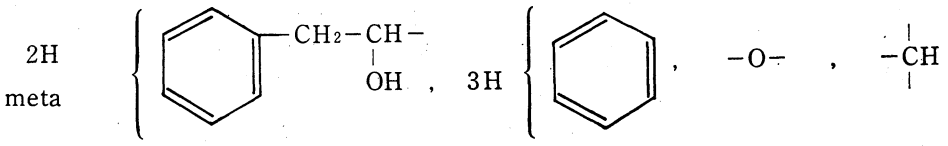

Table 1. Activity of **E**, **F** and **G** on growth inhibition of microorganisms

Compounds[a]	Microorganisms[b]			
	I	II	III	IV
E	11[c]	-[d]	-	-
F	-	-	-	-
G	6	-	-	-

a 50 mg/ml MeOH
b I, *Escherichia coli*; II, *Pseudomonas aeruginosa*; III, *Pseudomonas fluorescens*; IV, *Saccharomyces cerersial.*
c A radius of inhibition ring (mm).
d -, Non inhibition.

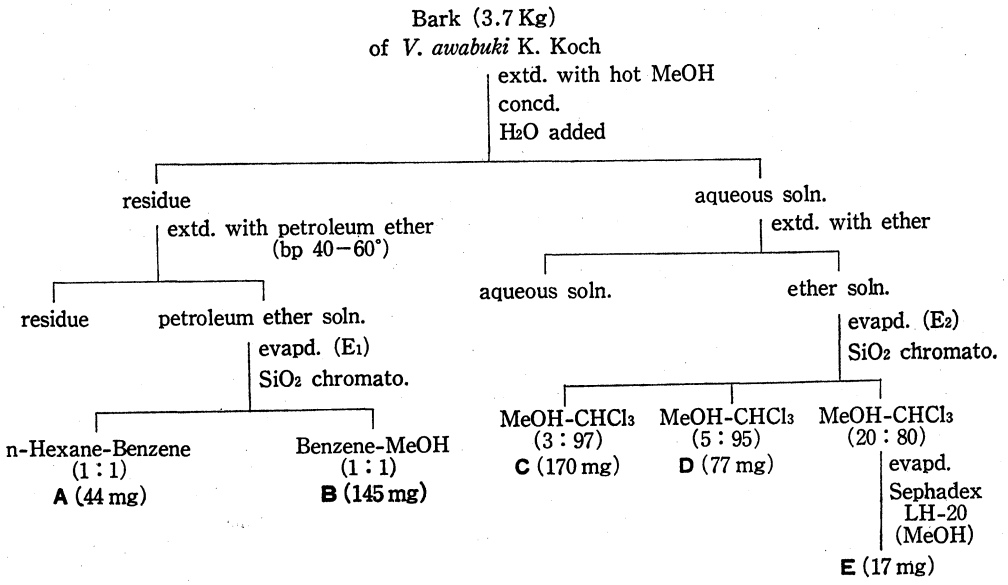

Fig. 1. Isolation of the compounds

The NMR spectrum of **G** was superimporsable on that of 3,3',4',5,7–flavanpentol (catechine or epicatechine)[5]. However, the melting points of **E**, and **F** and **G** were not in agreement with those of catechine or epicatechine, and the corresponding drivatives, respectively. Therefore, **E** should be a mixture of them.

Microbial growth inhibition test of **E** and its derivatives, **F** and **G** was performed with a paper disk method[6]. The results of this test are shown in Table 1. **E** and **G** exhibited antimicrobial activity against *Esherichia coli.*

Experimental

NMR spectra were determined on a JEOL MH–60 (60MHz) spectrometer in deuterochloroform solutions. IR spectra were recorded on Nujol mull with a Schimadzu IR–27 recording infrared spectrophotometer. Melting points were

measured on a Yanagimoto micromelting point determination apparatus, and uncorrected.

Extraction The bark of *V. awabuki* K. Koch (3.7 Kg) was extracted with boiling methanol (15*l*×3). The combined methanol solution was concentrated to dryness under reduced pressure and the concentrate was dissolved in water. The water-insoluble residue was extracted (Soxhlet) with petroleum ether (bp 40–60°). The extract was evaporated to give a pale-green residue (E_1), (84g), *in vacuo*. On the other hand, the aqueous solution was extracted with ether. The extract was concentrated to give a dark-brown residue (E_2), *in vacuo*.

Isolation of **A** *and* **B** *from the residue* (E_1) The residue (E_2) was chromatographed on a column of silica-gel.

Elution with 50% hexane in benzene and recrystallization from methanol-chloroform gave colorless needles (**A**, 44mg), mp 220–221°, IR 1740, 1640 cm^{-1}, NMR δ2.03 (3H, s), 1.67 (3H, s), 1.43 (3H, bd), 1.27 (3H, s), 1.03 (3H, s), 0.93 (3H, s), 0.83 (6H, s) and 0.78 (3H, s), m/e: 468 (M^+), 453, 408, 393, 386, 326, 249 and 189.

Anal. Calcd. for $C_{32}H_{52}O_2$:C, 81.99; H, 11.18. Found: C, 80,90; H, 11.22.

The elution with benzene was evaporated to give colorless needles (**B**, 145 mg), mp 139–140, IR 3400, 1640 and 1040 cm^{-1}.

Isolation of **C**, **D** *and* **E** *from the residue* (E_2)

After the residue (E_2) was dissolved in methanol, was added activated silica-gel (10g) to this solution, and the suspension was evaporated to dryness in vacuo. The silca-gel which adsorbed the residue was placed on the top of activated silica-gel (300g), and the column was eluted with solvents. Elution with 3% methanol in chlorform and recrystallization from chloroform gave colorless needles (**C**, 170 mg), mp 79–80.5°, IR 1740, and 1670 cm^{-1}, m/e: 148 (M^+).

Anal. Calcd. for $C_5H_8O_5$1/5H_2O: C, 39.58; H, 5.58. Found: C, 39.98; H, 5.58.

Elution with 5% methanol in chloroform and recrystallization from ethyl acetate gave colorless prisms (**D**, 77 mg), mp 168–169°.

Anal. Calcd. for $C_4H_6O_4$: C, 40.68; H, 5.12. Found: C, 40.75; H, 5.16.

Eluate with 20% methanol in chlorcform was evaporated under reduced pressure, and the residue which exhibited antimicrobial activity against *Escherichi coli*, was chromatographed on a column of Sephadex LH–20. Elution with methanol and recrystallization from benzene-methanol gave priams (**E**, 17 mg), mp 240° (decompn.), IR 3200, 1629, and 1620 and 1519 cm^{-1}, m/e: 290 (M^+).

Acetylation of **B**. Acetylation of **B** with acetic anhydride and pyridine at room temperature and recrystallization from ethanol gave colorless needles, mp 128°, IR 1740, 1240 cm^{-1}.

Acetylation of **E**. **E** was treated as mentioned above to give colorless needles, **F**, mp 156–157°, IR 1765, 1740, 1620, 1595, 1220 and 1200 cm^{-1}, NMR δ2.31 (12H, s) and 1.94 (3H, s), m/e: 416 (M^+–42×2).

Anal. Calcd. for $C_{25}H_{24}O_{11}$: C, 60.00; H, 4.83. Found: C, 59.78; H, 4.85.

Methylation of **E**. Treatment of **E** with diazometane in the usual manner and recrystallization from methanol gave colorless needles, **G**, mp 147–148°, IR 3500, 1620, 1590, 1510 and 1500 cm^{-1}, NMR δ1.73 (1H, s), 2.95 (2H, d, J=3Hz), 3.83 (3H, s), 3.97 (3H, s), 4.28 (1H, m), 4.96 (1H, bd), 6.12 (1H, d, J=3Hz), 6.20 (1H, d, J=3Hz) and 7.07–7.47 (3H, m), m/e: 346 (M^+).

Anal. Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.45. Found: C, 65.92; H, 6.54.

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