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Coumarin Glycoside from the Stembark of *Fraxinus sieboldiana* var. *angustata*

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「좀물푸레나무」 수피의 쿠마린 배당체

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한국에 자생하는 「좀물푸레나무」의 수피에서 fraxin, fraxetin-8-glucoside (mp. 204~5°)를 달리 확인 하였다.

Fraxinus sieboldiana var. *augustata* Nakai, belonging to *Oleaceae* Family, is one of the native plants of *Fraxinus sp.* in Korea. At present, there are 6 species and 7 varieties of *Franxinus sp.* plants in Korea. The barks of this plant have been used as folk medicine under the name of "*Gin-Pin*", Ashbark or *Fraxini Cortex* in Korea, China, Japan and America.

In recent years, it has been applied in the pharmaceutical industry as therapeutics for gout. Plants of genus *Fraxinus* are characterized by hydroxy coumarins, esculetin [1~4,6] frxetin[4], fraxinol[4~6], esculin [4,7,8], fraxin[8~10] isofraxetin[6]. From the bark of *F. sieboldiana* var. *augustata* Nakai, Shimada[3] reported the presence of esculetin.

In addition, we are pleased to report our research findings of another coumarin glycoside. From the methanol extract of the bark of *F. sieboldiana* var. *augustata* Nakai, we isolated a coumarin glycoside through the chromatographic procedure. The coumarin glycoside had the composition of $C_{15}H_{10}O_9$, mp 204~205°. The further physicochemical studies revealed that it corresponded to fraxin.

Experiment

Extraction: Finely cut fresh bark of *F. sieboldiana* var. *angustata* Nakai, collected in July 1981 at Mt. Chii, was extracted with MeoH exhausively. The extract was evaporated under the reduced pressure and suspended with the two volume of water and fractionated into tther, and EtOAC successively. EtOAC soluble fraction, the richest in glycoside, was concentred under the reduced pressure to near dryness.

The resultant residue was passed through the neutral alumina column (5×20cm) and eluted with the mixture of EtOAc and MeoH(1 : 1 by vol.). The eluates were gathered and concentrated under the reduced pressure. From the EtOAC extract we observed 8 spots of fluorescence by TLC (in long wave of UV-light).

Chromatography and Characterization: The EtOAc fraction was deposited on a chromatographic column filled with Wako-gel (h=80, d=5) and was eluted with $CHCl_3$-MeOH, the discrete method. The fine yellowish needle crystalline substance was obtained with mp 204~

205° (Compound I) from $CHCl_3$:MeOH (9:2 by vol) eluate.

This compound showed a blue-green fluorescence under the long-wave UV-light in TLC.

TLC Rf: 0.49 (BuOH=HAc:H_2O=4:1:5;a)
0.05 (Toluene: formic acid: ethylformate=5:1:4;b)

Color reaction: Yellow(Diazotized sulphanilic acid; DSA) Yellow-green ($FeCl_3$).

Anal. calcd: $C_{16}H_{15}O_{10}$, C, 51.80; H, 4.85.
Found: C,50.01; H, 5.30.

IR (KBr): 3410(-OH), 2938 (CH), 2866 (OMe), 1696(C=O), 1507(Arom.)

H-NMR (in D_2O, 90MHZ, external TMS standard): =3.75 (S, 3H, Ph-OMe), 6.15 (d, lH, C_3-H), 6.75 (S, lH, C_5-H).

Acetylation of Compound I: The glycoside (30mg) was acetylated thru the usual method and crystallized in 50% EtOH to obtain needle crystals of mp 193°.

H-NMR δ (in $CDCl_3$, 90MHZ, internal TMS standard): =2.1 (12H $4CH_3-C=O-O-$), 3.84 (3H Ph-OMe) 6.39 (lH C_3—H), 6.78 (s lH C_5—H), 7.64 (lH C_4—H)

Acid hydrolysis of Compound I: Glycoside (500mg) was hydrolyzed with 300ml of 5% sulfuric acid for one hour at 100°. After the hydrolysis process, the reaction mixture was extracted with ether. The ether soluble part was evaporated and the residue was crystallized by 50% EtOH into yellow crystals with mp 220~225°. The aglycone exhibited a dark green fluorescence under UV-light (long wave).

TLC Rf: 0.80 (a), 0.25 (b).

Color reaction: fine yellow (DSA), dark green ($FeCl_3$).

Anal. calcd: C, 57.79; H, 4.03
Found: C, 56.86; H, 4.37

IR (KBr): 3340(—OH), 2956 (CH), 2850 ($-OCH_3$), 1690 (C=O), 1600 (〉C=C〈), 1580 (Arom.)

H-HMR δ (in $CDCl_3$, 90MH_Z TMS standard): 3.87 (s 3H 1 Ph-OMe), 6.18 (d lH C_3—H), 6.55 (s lH C_5—H), 7.65 (d, lH, C_4—H)

The aguous hydrolysate was found to contain one sugar, glucose (TLC in BuOH:HAc:H_2O= 4:1:5, BuOH:HAc: Et_2O: H_2O=9:6:3:1 with the comparison of authentic sample) whens prayed by anillinediphenylamine-phosphoric acid.

Acetylation of Aglycone: The aglycone which had been acquired by dydrolysis (30mg) was acetylated through the usual method and crystalized from 30% EtOH into needle crystals with mp 193°.

NMR δ (in $CDCl_3$, 90 MH_Z, TMS standard): 2.32 (s 3H $-O-\overset{\overset{O}{\|}}{C}-CH_3$), 2.38 (s 3H $-O-\overset{\overset{O}{\|}}{C}-CH_3$), 3.86 (3H—O—$CH_3$), 6.4 (d lH C_3—H), 6.88 (s lH c_5—H), 7.62 (d lH C_4—H)

Results and Discussion

According to the present literature survey, it is reported that esculetin was isolated from the bark of *Franxinus sieboldiana* var. *angustata* Nakai.

On the basis of our research findings, however, we report another coumarin glycoside as fraxin.

Esculetin, esculin and fraxin have been isolated from *Fraxinus* species in Korea. These components were constituents of therapeutics for gout which are now manufactured by the pharmaceutical industries.

Continuous study on this medicinal plant will definitely help develop the coumarins of drug.

It is known that *Oleaceae* is divided into two classes— sect. ornus and sect. *Fraxinaster*. *F.*

sieboldiana var. *angustata* Nakai belongs to the sect. ornus. category. In sect. ornus, it has been reported that fraxetin, esculetin, esculin, fraxin are contained as components, and our research findings validate this opinion.

Summary

Fraxin, fraxetin-8-glucoside, mp 204-205° was isolated from the fresh bark of *Fraxinus sieboldiana* var. *angustata* Nakai, which is one of the indigenous plants of the Oleaceous family in Korea.

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References

1. Shimada H.: *Yakugaku Zasshi;* **58**, 636 (1938).
2. Shimada H.: *Yakugaku Zasshi;* **60**, 508 (1940).
3. Shimada, H.: *Yakugaku Zasshi;* **72**, 63 (1951), 498 (1952).
4. Terazawa, M., T. Sasaya: The Research Bulletin of College Experimental Forests, Hokkaido University **26**, 172 (1968).
5. M. Terazawa, T. Sasaya: *Journal of Japan Wood Research Society*, **17**, 167-173 (1970).
6. Artemeva, M.V., Nikonov, G.K., Karryev, M.O.: *Khim, Prir. Soedin.* **9**(4), 465 (1973).
7. Hahn, D.R.: *Kor. J. Pharmacog.*, **7**(3), 195. (1976).
8. Yook, C.S.: *Kor. J. Pharmacog.*, **12**(3), 143 (1981).
9. Wessely, F., Demmer, E.: *Chem. Ber.* **61**, 1279 (1928).
10. Wessely, F., Demmer, E.: *Chem. Ber.* **62**, 120 (1929).
11. Soren, Rosendal, Jensen, Bent, Juhl, Nielsen: *Phytochem.* **15**, 221 (1976).
12. Späth, E., Jerzmanowska, Z.: *Chem. Ber.* **70**, 1019 (1937).