생 약 학 회 지
Kor. J. Pharmacog.
7(3):195~198 (1976)

# Coumarin glycoside of *Fraxinus chiisanensis*

Dug Ryong Hahn, Chang Jong Kim, Jung Hee Kim, and Soon Kak Hong

College of Pharmacy, Chung Ang University, Seoul, Korea

## 지리산물푸레나무의 coumarin 配糖體

韓德龍・金昌種・金貞姬・洪淳玼

中央大學校 藥學大學

지리산 물푸레나무의 수피의 메탄올추출물에서 m.p.206°, $C_{15}H_{16}O_9 \cdot \frac{1}{2}H_2O$의 조성을 갖는 coumarin glycoside (수득율 0.09%)를 분리하여 물리화학적인 분석에 의하여 esculin임을 동정하였다.

## Introduction

The bark of *Fraxinus* sp. plants has been used as folk medicine and Chinese drug from the ancient in China, Japan, Europe and America under the name of *Fraxini* cortex and ash bark.

Except Korea, the bark of *Fraxinus* sp. plant has been used for medicinal purpose in many countries of the world. So for the identified constituents in *Fraxinus* sp. plants were coumarin derivatives.

The Drug names and their botanical origin of *Fraxini* sp. are as follows:

| Region being used | Common drug name | Scientific name | Botanical origin |
|---|---|---|---|
| China | Sinpi (榊皮 秦皮) | *Fraxini* cortex | *Fraxinus rhychophylla*<br>*F. bungeana* [1-a)] |
| Japan | Ginpi (秦皮) | *Fraxini* cortex | *F. japonica*<br>*F. sieboldiana* |
| Europe and America | Ash bark | | *F. excelsior* |
| Korea and partially in China | Ginpi (秦皮) | | *Juglans manshurica* |

The plants containing coumarin derivatives and related compounds are as follows:

| Botanical origin | Chemical constituents |
|---|---|
| *Fraxinus excelsior* | planteose[1)], fraxin[15)], isofraxidin[13)], carbohydrates[5,6)] |
| *F. oxycarpa var. mediterrane* | esculin, fraxin[2)] |
| *F. oxycarpa var. pannonica* | esculin, fraxin[2)] |
| *F. potamophila* | isofraxetin[3)], fraxin, fraxinoside[8)], esculetin, fraxinol[12)] |
| *F. manshurica* | isoquercetin,[4)] esculetin, fraxinol, isofraxetin[12)] |
| *F. ornus* | carbohydrate,[5,6)] esculin, fraxin, esculetin, fraxetin, cichoriin[10)] |
| *F. americana* | fraxin, fraxetin[7)] |
| *F. japonica* | oleuropein (secoiridoid glycoside), esculin[9)] |
| *F. densata* | mannitol, esculin[11)] |

| | |
|---|---|
| *F. oxyphylla* | fraxin[17] |
| *F. rynchophylla* | esculin[20] |
| *Eleutherococcus senticosus* | isofraxidin[13-a] |
| *Aesculus hipocastanum* | esculin[16] |
| *Artemisia capillaris* | esculetin dimethylether[18] |
| *A. messer-schmidtiana* BESSER *var. viridis* BESSER f. *typica* NAKAI, | esculetin-6-methyl ether, esculetin-7-methyl ether[19] |

In general, coumarin derivatives have vitamin-p-like activity[21], adaptogenic activity[22], and choleretic activity[23]. *Fraxini* cortex is known to show astringent, antiinflammatory and antipyretic effects as well as an opthalmic remedy.

On the other hand, it has been found that the alkaloid sinine isolated from *Fraxinus sinica* showed quinine-like action[24] and the rhizomes of *Fraxinus malacophylla* had the antimalarial action[25].

Based on the fact that *Fraxinus chiisanensis* is an indigenous plant of Korea, the present study was carried out for the isolation and identification of esculin from the bark of this plant.

## Experiment

### Isolation of glycoside

Three kg of finely cut fresh barks of *Fraxinus chiisanensis* (collected in the Chii mountain in August 1976) was extracted three times with hot methanol. The extract was evaporated to dryness under reduced pressure. The yield of the methanol extract was about 8%. The methanol extract was diluted with two volumes of water and the solution was extracted with chloroform, chloroform-alcohol mixture(2 : 1 by vol.), and water saturated N-butanol successively. The chloroform-alcohol extract, richest in glycoside, was evaporated in reduced pressure to dryness and the resulted residue (158g) was chromatographed on neutral alumina (3×60cm column) and eluted with chloroform-methanol mixture (2 : 1 by vol.).

The fractions were 250ml each; the fractions were analyzed by thin-layer chromatography on silica gel in butanol-glacial acetic acid and water (4 : 1 : 5 by vol.).

Sixteen of non-glycosidic material and 8g of a glycosidic fraction were obtained. Repeated chromatographing of the glycoside fraction on neutral alumina

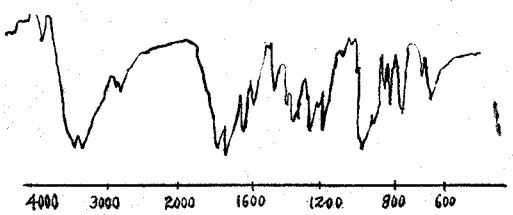


**Fig. 1.** Chromatography in a thin-bound layer of silica gel. Detection under U.V. light. Developer: N-butanol-glacial acetic acid water (4 : 1 : 5)
A. Esculin standard. B. Esculin isolated from *Fraxinus chiisanensis*. C. Esculetin standard. D. Esculetin from hydrolyzed product of Fraxinus chiisanensis esculin.

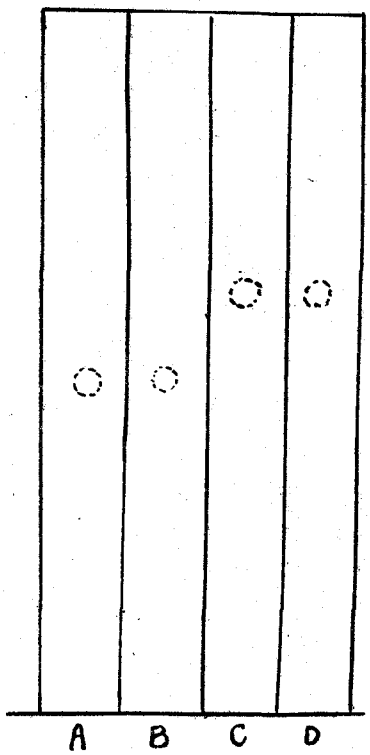


**Fig. 2.** Infrared spectra of esculetin (in KBr).

(column 4×60cm) with chloroform-alcohol mixture enables us to obtain a chromatographically homogeneous glycoside (2.8g).

The glycoside was confirmed as esculin, $C_{15}H_{16}O_9 \cdot \frac{1}{2}H_2O$ by chemo-physical-instrumental analysis, mass spectroscopy, I.R. spectrum, elemental analysis and mixed melting point with the comparison of the authentic esculin respectively (Fig.1~2).

Esculin has m.p. 206. M.W. 340.28

Found %. C, 52.75%; H, 4.8%; O, 42.25%

**Acid hydrolysis of esculin.**

Five hundred mg of esculin was hydrolyzed with 300ml of 5% sulfuric acid for 30 minutes at 100°C and deposited genin was recrystallized from hot methanol.

Leaflet silver yellowish crystals were obtained and melted at 268~270°C corresponding to the melting point of esculetin and showed no depression when mixture melting point with authentic esculetin sample. It gave blue fluorescence under U.V. light and in alkaline solution.

It was identified as esculetin by the following chemo-physical properties, mass spectroscopy, I.R. spectrum and elemental analysis respectively. Esculetin has m.p. 268~270°C. M.W. 178

I.R. spectrum. 3500(dimeric OH), 3400~3200(polymeric OH), 1760(—CO—O—C=C), 1715, 1300 (=C=O), 1620, 1570 (coumarin nucleus), 1260~1180 (arom. ether), 1200 (phenolic OH), 1075, 1020cm$^{-1}$ (=C—O—C).

Found %. C, 60.66%; H, 3.7%; O, 35.92%

Methylation of esculetin yields its dimethylether, which gave a needle crystal and melted at 146°C. Mixed melting point with authentic esculetin dimethylether did not show any depression.

After being separated from aglycon, the hydrolysate was neutralized with $BaCO_3$, filtered, evaporated and subjected to the ascending paper chromatography for 16 hrs by using Whatman No. 1 filter paper. Developer system was N-butanol-glacial acetic acid and water (4:1:5 by vol.) and ammoniacal silver nitrate solution was used for coloration. Paper chromatogram showed one spot of Rf 0.18 corresponding to that of glucose. The glucose osazone test was carried out

The glucosazone crystallized from diluted alcohol melted at 209~210°C. When the mixed melting point with the authentic glucosazone did not show any depresson.

## Summary

Esculin, esculetin-6-glucoside, m.p. 206°C was isolated from the fresh bark of *Fraxinus chiisanensis* Nakai, one of the indigenous plant of the oleaceous family in Korea, in yield 0.09%.

<1976. 8. 30접수>

## References

1. Lewis, D.H.: *Phytochem.*, **13**, 1519 (1974).
1-a. Hong-Yen Hsü: The studies of Chinese herb medicine, 61pp (1972).
2. Jela, V. and Salko: *Lek. Sirovine*, **6**, 25 (1968). (Through C. A. 74, 91117k 1971)
3. Artemeva, M. V., Karryev, M. O. and Nikonov, G.K.: *Izv. Akad. Nauk. Turkm. ssk, ser. Biol. Nauk.*, **1**, 63 (1973).
4. Artemeva, M. V., Nikonov, G. K. and Nezbinskaya, G.A.: *Khim. Prir. Soedin.*, **3**, 433 (1973).
5. Sabry, Z.I. and Atallah, N.A.: *Nature*, **190**, 915 (1961).
6. Paris, R.P. and Stamboul, A.: *Ann. Pharm. Franc.*, **18**, 873 (1960).
7. Edwards: *Amer. Journ. Pharm.*, **54**, 282 (1886).
8. Artemeva, M.V., Karryev, M.O. and Nikonov, G.K.: *Izv. Akad. Nauk.* Turkm. SSK, Ser. Biol. Nauk., **3**, 82 (1973).
9. Inouye, H., Nishioka, T. and Kaniwa, M.: *Phytochem.* **14**, 309 (1975).
10. Ivanov, V., Yaneva, A. and Savchev, P.: *Tr. Nauchnoizsled. Khim-Farm. Inst.*, **8**, 147 (1972). (Through C.A. 78, 156664r 1973).
11. Ryu, K.S. and Yook, C. S.: *10th. Annual meeting of Pharmaceutical Soc. of Korea* (1969).
12. Artemeva, M. V., Nikonov, C.K. and Karryev, M. O.: *Khim. Prir. Soedin* **9**, 493 (1973).
13. Späth, E. and Jerzmanowska, Z.: *Chem. Ber.*, **70**, 1019 (1937).
13-a. Ovodov, Yu. S., Frolova, G.M., Nefedova, M. Yu, and Elyakov, B.: *Khim. Prir. Soedin.*, **3**, 62

(1967).

14. Tumann: *Chem. Zentr.*, **1**, 1277 (1916).
15. Horstmar, S.: *Pogg. Ann.*, **100**, 607 (1857).
16. Reppel: *Planta med.*, **4**, 199 (1956).
17. Stambouli, P.: *Compt. Rend.*, **253**, 313 (1961).
18. Shibue, S.: *Bull. Agr. Chem. Soc. Japan*, **9**, 600 (1930).
19. Hahn, D.R.: *Journ. of Pharm. Soc. of Korea*, **10**, 20 (1966).
20. Cho, H.Y.: *Journ. of Chung-Ang Pharmcy*, **1**, 69 (1957).
21. Pollock, C.: *Arch. Biochim. Biophys.*, **49**, 1 (1954).
22. Brekhman, I.I. and Dardymov, I. V.: *Lloydia*, **32**, 46 (1969).
23. Hahn, D.R.: *J. Pharm. Soc. Korea*, **10**, 25 (1966).
24. Lü, S.K., Chang, Y.T. and Chuan, T.K.: *Natl. Med. J. China*, **27**, 327 (1941).
25. Yang, S.T.: *J. Am. Pharm. Assoc., Sci. Ed.*, **37**, 458 (1948).