

On the Active Components of the Roots of *Citrus* genus in Taiwan

IV. The Components of the roots of *Citrus limonia* Osbeck, *C. sinensis* var. *brasiliensis* Tanaka, *C. limon* Burman, *C. reticulata* Blanco and *C. kotokan* Hayata

By

Kun-ying Yen, Ling-ling Yang, Wai-sun Tam, and Duen-ren Cheng

Dept. of Pharmaceutical Phytochemistry, Taipei Medical College

(Received April 16, 1972)

Our previous investigation on the components of three *Citrus* genera namely, *Citrus medica* L. var. *sarcodactylis* Swingle¹⁾; *C. tankan* Hayata²⁾, *C. nobilis* Lour. var. *sunki* Hort.³⁾, have been reported. Lately, the authors have decided to continue to study on the following five *Citrus* species both indigenous and cultivated in Taiwan:

- 1). *Citrus limonia* Osbeck
- 2). *C. sinensis* var. *brasiliensis* Tanaka
- 3). *C. limon* Burman
- 4). *C. reticulata* Blanco
- 5). *C. kotokan* Hayata

The above materials were treated under the general procedures of extraction and concentration. As from the concentrated ethereal extract, a mixture of coumarin and sterol were isolated and separated by eluting through silica gel column. However, evidence from their identical melting point, composition and infrared spectrum analysis, suggested their common coumarin component to be xanthyletin. As to the mixed sterols, infrared spectrum analysis, gas chromatography were applied and results were submitted as follows:-

- 1). *C. limonia* contains a mixture of stigmasterol and β -sitosterol (Fig. 1)
- 2). *C. sinensis* var. *brasiliensis* contains a mixture of stigmasterol and β -sitosterol (Fig. 2)
- 3). *C. limon* contains a mixture of stigmasterol and β -sitosterol (Fig. 3)
- 4). *C. reticulata* contains scale-like crystals of mp. 111~112° which gives a positive result in Liebermann-Burchard's reaction. However, a further investigation could not carried out due to an inadequate amount of product.
- 5). *C. kotokan* contains a sterol-like substance which gives a positive result in Liebermann-Burchard's reaction.

The pericarps of these species mentioned above all contain hesperetin, while the roots contain xanthyletin and a mixture of sterols. Thus, it is an interesting fact as viewed from the point of the motaxonomy.

Shimadzu GC-3AF:

Column: 1.5% SE-30 on Chromosorb-W (60-80 mesh)
1.8m x 4.0mm glass column.

*Temperature: 270°

Gas Carrier: N₂ 0.6 kg/cm², 30ml/min
H₂ 0.4 kg/cm²
Air 0.5 kg/cm²
Sens. 10³M, Range 6.4 V.

Fig. 1. GLC of mixed sterols isolated from *C. limonia*

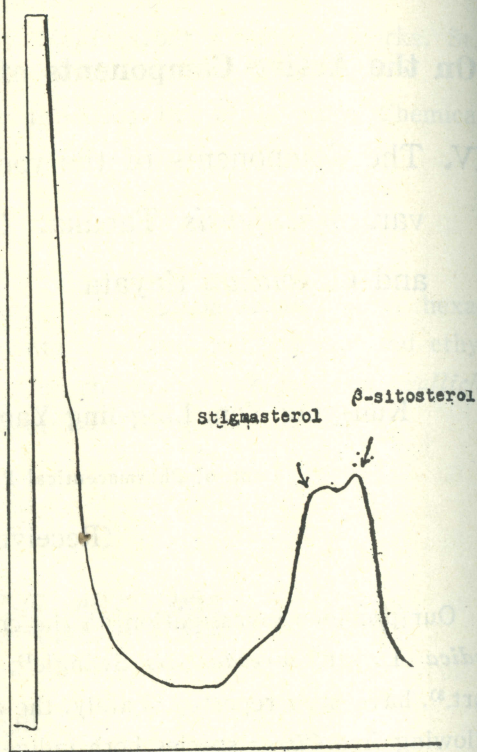


Fig. 2. GLC of mixed sterols isolated from *C. sinensis* var. *brasiliensis*

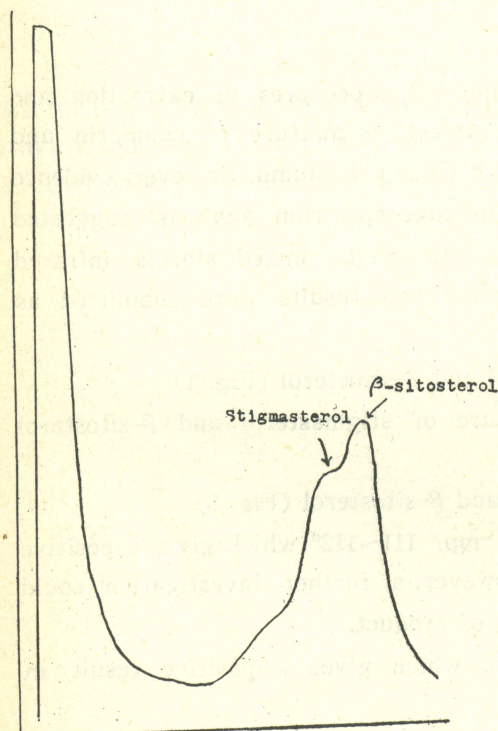
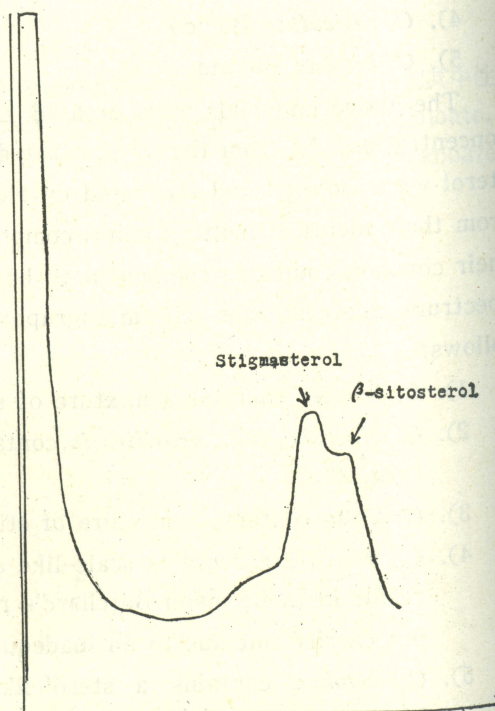


Fig. 3. GLC of mixed sterols isolated from *C. limon*



EXPERIMENTAL

1). *Citrus limonia*

2kg of dried roots were collected from Taichung Hsien in March 1969. They were firstly fragmented and then macerated thrice with ether. The ethereal extract was then concentrated to a dark brown oily substance (150 ml). It was then chromatographed through silica gel column using *n*-hexane as eluting solvent and different fractions were collected consecutively.

Fr. 1. An oily substance was obtained.

Fr. 2. The solvent was concentrated to give colorless scale-like crystals which gave positive result in Liebermann-Burchard's reaction (violet-red-blue). The crystals were recrystallized with ethanol giving m.p. 139~141°. However, according to gas chromatography retention time, it is suggested to be a mixture of stigmasterol and β -sitosterol.

Fr. 3. After concentration, crystals were set free and later recrystallized with ethanol. Colorless cylindrical crystals having a m. p. 128~129° was obtained which was identical with the infrared spectrum of the authentic xanthyletin. It was also verified to be xanthyletin by mixed melting point and thin layer chromatography comparison.

Anal. Calcd. for $C_{14}H_{12}O_3$ C:73.67; H:5.30, Found C:74.01; H:5.38

2). *Citrus sinensis* var. *brasiliensis*

6.8kg of dried roots were collected from Nantow Hsien in April 1969. The fragmented roots were firstly macerated with ether and the procedures were repeated as in 1). The extract was chromatographed through silica gel column with *n*-hexane as eluting solvent while each successive fraction were collected in order.

Fr. 1. An oily substance was obtained.

Fr. 2. Concentration of the fraction, crude crystals were separated out. After recrystallization with ethanol, colorless scale-like crystals of m. p. 134~136° were obtained. It was positive in Liebermann-Burchard's reaction. The color indication was from red to violet and finally turned blue. The product was determined as a mixture of stigmasterol and β -sitosterol according to its gas chromatography.

Fr. 3. Xanthyletin was obtained.

3). *Citrus limon*

3kg of dried roots were obtained from Tainan Hsien in August 1969. The roots were freely fragmented and treated as in 1).

Fr. 1. Colorless scales of m. p. 147~149° which gave positive result in Liebermann-Burchard's reaction (red-violet-blue). According to gas chromatography results, the product was determined to be a mixture of stigmasterol and β -sitosterol.

Fr. 2. Xanthyletin was obtained.

4). *Citrus reticulata*

1kg of dried roots were collected from Taichung Hsien in August 1969 and were treated as in 1).

Fr. 1. A product of m. p. 111~112° was obtained which gave positive in Liebermann-Burchard's reaction. The color indication was from red to violet and finally turned blue. However, owing to an inadequate amount obtained, further identifications were not possible.

Fr. 2. Crystalline platelets were obtained and was found identical to xanthyletin.

5). *Citrus kotokan*

2kg of dried roots were collected from Hsinchu Hsien in April 1969 and were treated as in 1).

Fr. 1. An oily substance was obtained.

Fr. 2. Colorless crystalline prisms were obtained (xanthyletin).

ACKNOWLEDGEMENT

The authors wish to express his gratitude to Dr. C. T. Hsü, Dean of the College for encouragement throughout the course of this work.

The authors also thank Dr. I. Nishioka, Professor of Pharmaceutical Sciences, Kyushu University (Japan) for gas liquid chromatography analysis and other valuable suggestions.

The authors are also indebted to the National Council for Science Development for research grant.

REFERENCES

- 1). K. Y. Yen: Journ. Taiwan Pharm. Assoc. **22**, 21 (1970)
- 2). K. Y. Yen, L. L. Yang: Journ. Taiwan Pharm. Assoc., **22**, 26 (1970)
- 3). K. Y. Yen, L. L. Yang, T. Y. Huang: Journ. Taiwan Pharm. Assoc., **22**, 29 (1970)

中 文 摘 要

臺灣產柑類根部之活性成分研究

第四報 廣東檸檬、柳橙、檸檬、椪柑

虎頭柑根之成分

顏焜熒、楊玲玲、譚維信、鄭敦仁

臺北醫學院 生藥化學科

臺灣省栽培及野生柑類五種 1) 廣東檸檬 (*Citrus limonia*), 2) 柳橙 (*C. sinensis* Osb. var. *brasiliensis*), 3) 檸檬 (*C. limon*), 4) 椪柑 (*C. reticulata*), 5) 虎頭柑 (*C. kotokan*), 作其根部之活性成分探討, 得如下的結果:

此五種均共含有香豆素化合物 (coumarin compound)——xanthyletin。同時1), 2), 3) 三種尚含有混合固醇類 (phytosterol)——stigmasterol 及 β -sitosterol。此於化學分類學 (chemotaxonomy) 探討之領域觀之, 爲一有趣之事。