

**STUDIES ON THE CONSTITUENTS OF *SIEGESBECKIA*  
*ORIENTALIS* L**

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**ABSTRACT**

Investigation of Chinese drug from *Siegesbeckia orientalis* led to the isolation of four crystalline substances. A flavanoid, mp 247-249°,  $[\alpha]_D^{25} +62^\circ$ , was assigned as 3,7-dimethylquercetin(I) on the basis of its spectroscopic properties and in chemical correlation with 3,3',4',7-tetramethylquercetin(III). This is the first time a flavonoid had been isolated from *Siegesbeckia orientalis*. Aside from the considerable amount of inorganic salts, potassium nitrate, and two unknown terpenoids, mp 241-242° and mp 115-120° were also isolated.

*Siegesbeckia orientalis* L. is an annual herb of Compositae and widely distributed all over Taiwan island. According to the Pentsao<sup>1)</sup>, it is a well-known Chinese folk medicine as tonic antidote and analgesics and used for treating gout, rheumatism, anemic numbness of extremities, weakness of knee and lumbar, deviation of the mouth angle and eye balls or hemiplegia, and it also has blood-pressure-reducing activity. Besides, both *Siegesbeckia orientalis* and its related plant, *Siegesbeckia pubescens*, exhibit antitumor activity as reported lately by Hartwell<sup>2)</sup>. As to the constituents, A. Diara<sup>3)</sup> et al. have isolated new compounds of diterpenol and its glucoside, darutin and darutigenol, from this plant. In addition, K.-D. Han<sup>4)</sup> reported that its related plant, *Siegesbeckia pubescens*, contained pimarane and kaurane type diterpenes and steroids. In view of the importance of this plant as in medicinal use and because that the chemical constituents of such plant raised in Taiwan had never been investigated yet, we were prompted to search for the active constituents of *Siegesbeckia orientalis*.

The whole plant, air dried, was coarsely cut into small section. Then followed the procedures described in the experimental part four crystalline compounds A, B, C, D were obtained.

Compound A: Compound A was identified as potassium nitrate on the basis of the analytical evidences described in the experimental part.

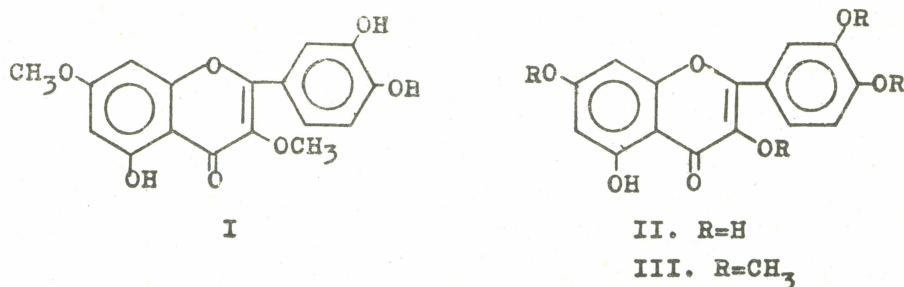
Compound B: The  $\text{CHCl}_3$  insoluble portion was chromatographed over silica gel. Then the eluate of fraction 23-24 was concentrated to afford yellow crystals with mp 248-249°,  $[\alpha]_D^{25} +62^\circ$  ( $c=0.21$ , EtOH), positive in  $\text{FeCl}_3$  test, also in Mg-HCL test. These suggested compound B to be a phenolic flavonoid. It remained unchanged by 5%  $\text{H}_2\text{SO}_4$  hydrolysis indicated that compound B was not a glycoside. The IR spectrum of compound B had an absorption peak of OH group at  $3380\text{ cm}^{-1}$ , of conjugated carbonyl group at  $1660$  and  $1640\text{ cm}^{-1}$  and of aromatic ring at  $1600$ ,  $1555$ ,  $1510$  and  $1500\text{ cm}^{-1}$ . The UV spectrum had max absorption at 257, 268(sh), 300(pl) and 360nm indicating compound B was a flavanol, the UV pattern of which was similar to that of rutin. The NMR spectrum (in  $\text{CDCl}_3 + \text{C}_5\text{D}_5\text{N}$ ) showed peaks at 3.69 and 3.78 $\delta$  indicating two methoxyl groups, the two-proton singlet at 6.43 $\delta$  corresponding to 6, 8-protons of A-ring, 7.15 $\delta$  corresponding to 2'-proton and the doublet centered at 7.64, 7.78 and 8.02 $\delta$  ( $J=3\text{HZ}$ ) corresponding to 6', 5'-protons of B-ring. The NMR spectrum revealed that no sugar was contained, just as the result from hydrolysis test mentioned above. In order to understand the more detailed oxidation pattern of flavonoid, after adding different detecting reagents, the bathochromic or hypsochromic shifts in the UV spectrum of compound B are shown in the following table:

Table, The UV spectral data of compound B ( $\lambda_{\text{max}}$  nm)

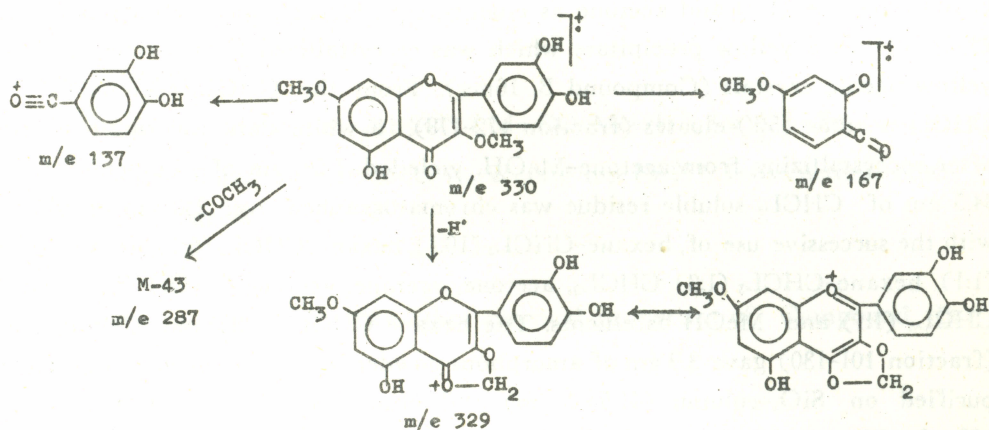
$\lambda_{\text{max}}$ EtOH	257, 268(sh), 300(pl), 360
$\lambda_{\text{max}}$ $\text{ALCl}_3$	276, 300(inf), 420
$\lambda_{\text{max}}$ $\text{ALCl}_3/\text{HCL}$	268, 300(inf), 363 403
$\lambda_{\text{max}}$ AcONa	262, 295(inf), 375(sh), 415
$\lambda_{\text{max}}$ AcONa/ $\text{H}_3\text{BO}_3$	262, 295(inf), 383
$\lambda_{\text{max}}$ EtONa	267, 398

As shown in the table, the low wavelength band II shifted only 5 nm in the presence of AcONa thereby showing that the hydroxyl at position 7 was methylated. Upon addition of a mixture of boric acid and sodium acetate the bathochromic shift in band I shifted 23 nm, and the hypsochromic shift of 17 nm in band I in presence of a mixture of  $\text{ALCl}_3$  and HCL compared to that of band I in  $\text{ALCl}_3$  both indicated the compound contain 3', 4'-dihydroxy group. After adding  $\text{ALCl}_3$  and HCL, the bathochromic shift in band I shifted 43 nm, that indicating presence of 5-hydroxyl-3-substituted group. All these experimental data suggested<sup>5)</sup> that

compound B was a 3-substituted flavanol with 3',4', 5-trihydroxy and 7-methoxy, of which the structure was shown to be the following formula:



Methylation of compound B with diazomethane gave its O-methyl derivative (III), mp 153-155°, which was identified as 3,3',4',7-tetramethylquercetin by mixed melting point and tlc comparison with that of the authentic sample which was synthesized from quercetin. The mass spectrum of I showed the molecular ion peak at m/e 330 corresponding to molecular formula C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>, and the other significant ions at m/e 329 (M-1, base peak), 287 (M-43), 167 (A+1) and 137 (aroyl cation). The principal mass fragmentation pattern is shown as follows; it is this evidential fact which strongly supported<sup>6)</sup> the formula I for compound B.



Compound C: Compound C was obtained from the eluate of fraction 172-178 after compound B was eluted. It was a white needle crystal, mp 241-242°,  $[\alpha]_D^{25} +55^\circ$  (c=0.8, EtOH), positive in Liebermann Burchard test, purple spot in tlc with cerium sulfate, suggesting that compound C was a triterpenoid. Acetylation with acetic anhydride in pyridine yielded micro-needle crystals with mp 89-91° and florid spot in tlc with cerium sulfate, which was different from that of compound C. The chemical structure of compound C is now under investigation.

Compound D: The CHCl<sub>3</sub> soluble portion was chromatographed over silica

gel to afford a small amount of white scale crystals, mp 115-122°. The other physical properties of this compound are now under investigation.

This is the first instance that 3,7-dimethylquercetin was found in *Siegesbeckia orientalis* L.

## EXPERIMENTAL

### Extraction and isolation:

The whole herb (10.7 Kg) of *Siegesbeckia orientalis* L., collected in Taipei in April, 1974, was successively extracted with hot EtOH. This gave, after evaporating off the solvent in vacuo, a white ppt. Which was collected by suction and recrystallized from hot water to afford 37 gm of colorless prismatic crystals with mp above 300° (Compound A). The filtrate was concentrated to dryness to give a dark green residue (900 gm) which was made slurred with MeOH-H<sub>2</sub>O (3:1) and then shaken with n-hexane. The aqueous layer was washed well with hexane and the hexane layer was extracted with MeOH-H<sub>2</sub>O (3:1). The combined aqueous extracts, after distilling off MeOH, was added with 500ml of water and extracted with CHCl<sub>3</sub>. The CHCl<sub>3</sub> extract was concentrated to obtain 45.5 gm of greenish tar and the CHCl<sub>3</sub> insoluble portion yielded 86 gm of dark brown residue. This residue, 20 gm, was chromatographed over 190 gm of silica gel (Wako gel C-200) with the successive use of CHCl<sub>3</sub>, CHCl<sub>3</sub>-acetone (10:1), CHCl<sub>3</sub>-acetone (5:1), CHCl<sub>3</sub>-acetone (1:1) and acetone as eluents. the CHCl<sub>3</sub>-acetone eluates (fraction 23-24) afford a yellow precipitate which was recrystallized from EtOH to afford yellow needle crystals (Compound B, 16mg). From the CHCl<sub>3</sub>-acetone(10:1) and CHCl<sub>3</sub>-acetone (5:1) eluates (fraction 172-178), a white solid was obtained, which after recrystallizing from acetone-MeOH, yielded 1.56 gm of compound C. The 34.5 gm of CHCl<sub>3</sub> soluble residue was chromatographed over 230 gm of silica gel, with the successive use of, hexane-CHCl<sub>3</sub> (10:1), hexane-CHCl<sub>3</sub> (5:1), hexane-CHCl<sub>3</sub> (1:1), hexane-CHCl<sub>3</sub> (1:3), CHCl<sub>3</sub>, acetone, acetone, acetone-MeOH (7:1), MeOH-CHCl<sub>3</sub> (1:1) and MeOH as eluents. The hexane-CHCl<sub>3</sub> (1:3) and CHCl<sub>3</sub> eluates (fraction 101-130) gave 3.9 gm of amorphous powder which was chromatographically purified on SiO<sub>2</sub>-column (41.5×3 cm) and eluted with hexane-CHCl<sub>3</sub> (2:5) to afford white scales with mp 115-120° (Compound D)

### Potassium nitrate (Compound A):

Colorless prismatic crystals, mp above 300°, easily soluble in water but slightly soluble in hot EtOH, tasted cool. The aqueous solution of residue after ignition exhibited basic property in phenolphthalein test suggesting that Compound A was an inorganic compound of alkali or alkaline earth metal. Its aqueous solution had white crystalline ppt. after adding HClO<sub>4</sub> and yellow needle crystals after adding sodium picrate solution. Besides, its aqueous solution exhibited positive ring test, but its HNO<sub>3</sub> solution did not produce white ppt. after adding AgNO<sub>3</sub> solution. From all above, the crystal proved to be potassium nitrate.

### 3,7-dimethylquercetin (Compound B):

Yellow needles from EtOH, mp 248-249°,  $[\alpha]_D^{31} + 62^\circ$  (c=0.21, EtOH). It gave positive Mg-ribbon test (pink) and FeCl<sub>3</sub>-test (dark green). IR  $\overset{\text{KBr}}{\text{max}} \text{ cm}^{-1}$ : 3380 (broad, OH), 1660 and 1650 (conjugated carbonyl), 1600, 1555, 1510 and 1500 (aromatic ring). UV  $\lambda_{\text{max}}^{\text{EtOH}}$  nm: 257, 268 (sh), 300(pl) and 360;  $\lambda_{\text{max}}^{\text{EtOH}+\text{ALCL}_3}$  nm: 276, 300 (inf) and 420;  $\lambda_{\text{max}}^{\text{EtOH}+\text{ALCL}_3+\text{HCL}}$  nm: 268, 300 (inf), 363, 403;  $\lambda_{\text{max}}^{\text{EtOH}+\text{AcONa}}$  nm: 262, 295 (inf), 375 (sh), 415;  $\lambda_{\text{max}}^{\text{EtOH}+\text{EtONa}}$  nm: 267, 398;  $\lambda_{\text{max}}^{\text{EtOH}+\text{AcONa}+\text{H}_3\text{BO}_3}$  nm: 262, 295 (inf), 383. NMR $\delta$  (CDCl<sub>3</sub>+C<sub>5</sub>D<sub>5</sub>N): 3.69 3.78 (each 3H, s., two OCH<sub>3</sub>), 6.43 (2H, s., C<sub>6,8</sub>-Hs), 7.15 (1H, d., C<sub>2'</sub>-H), 7.64, 7.78 and 8.02 (2H, each d, J=3HZ, C<sub>6',5'</sub>-Hs). Mass spectrum m/e: 330 (M<sup>+</sup>, C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>), 329 (M-1, base peak), 287 (M-43), 167, 137.

Methylation of quercetin (II) and compound B (I): 3,3',4',7-tetramethylquercetin (III).

A solution of quercetin (30mg) in methanol-ether (15ml) was treated with ethereal solution of diazomethane (prepared by 1.5 g of nitrosomethylurea and 5 ml of 40% KOH in 15 ml of ether solution); the solution was allowed to stand for 10 hours at room temperature. The solvent was evaporated and the residue was recrystallized from methanol-chloroform to yield 3,3',4',7-tetramethylquercetin(III) as yellow needles with mp 153-155°. Compound B (7.4 mg) was treated by using the same method as described above to yield an O-methyl derivative of Compound B. It was to be identical with 3,3',4',7-tetramethylquercetin (III) by comparison of the tlc (SiO<sub>2</sub>/Me<sub>2</sub>CO/I<sub>2</sub>) and mixed melting point.

### Triterpenoid (Compound C):

Colorless needle crystals, mp 241-242° (Me<sub>2</sub>CO-MeOH),  $[\alpha]_D^{31} - 55^\circ$  (c=0.8, MeOH), Liebermann Burchard test: pink, its acetate mp 89-91°. Its structural elucidation is now in progress.

### Diterpenoid (Compound D):

Colorless scales, mp 115-120°. The other physical properties of Compound D are now under investigation.

## ACKNOWLEDGEMENT

The author expresses due thanks to Mr. C. P. Wang of Chemical Research Center, National Taiwan University, for the NMR data and Mr. J. H. Lin, National Research Institute of Chinese Medicine for the UV data. This work was supported in part by the National Science Council of the Republic of China.

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## 豨薟之成分研究

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### 摘 要

爲要探究中藥豨薟 (*Siegesbeckia orientalis* L.) 有效成分，從本植物分離得一種類黃素 mp. 247~249,  $[\alpha]_D^{25} + 62^\circ$  (EtOH, C=0.213)。此等物理化學諸恆數及光譜儀等分析結果及自quercetin(II)經 methylation 所得之3, 3', 4', 7-tetramethylquercetin (III) mp 153~155° 比較推定爲 3, 7-dimethylquercetin (I)。豨薟中單離得flavonoid 尚屬首次。另外得無機成分硝酸鉀及二種未知構造 terpenoids 結晶，融點各爲241~242° 及 115~120°，其物理化學特性繼續研究中。