

# Scutellarein-7-*O*- $\beta$ -D-Glucopyranosyl(1 $\rightarrow$ 4)-*O*- $\alpha$ -L-rhamnopyranoside from the Stems of *Thymus serpyllum* Linn.

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Phytochemical examination of the stems of *Thymus serpyllum* resulted in the isolation and identification of a glycoside, scutellarein-7-*O*- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 4)-*O*- $\alpha$ -L-rhamnopyranoside

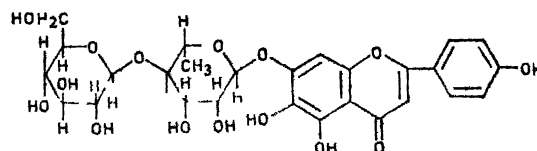
**THYMUS** *serpyllum* (N. O. Labiatae) is reputed for medicinal importance<sup>1,2</sup>. Since no work has been done on its stem, hence the chemical examination of the plant was undertaken.

## Results and Discussion

The water soluble fraction of the ethanolic extract was successively extracted with different organic solvents. The methanol soluble fraction afforded the above reported glycoside. It was purified as cream-coloured compound (1.6 g). Acid hydrolysis (7% ethanolic H<sub>2</sub>SO<sub>4</sub>) yielded an aglycone C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>, m.p. 348°, and two sugars which were characterised as rhamnose and glucose by co-chromatography with authentic samples. The aglycone was identified as scutellarein by its specific colour reactions, m.p., spectral analysis, degradation studies and co-chromatography with an authentic sample.

Periodate oxidation of the glycoside consumed 3.2 moles of periodate with the production of 1.3 moles of formic acid per mole of the glycoside, suggesting the presence of a disaccharide having both the units in pyranose form. The position of the sugar linkage in the glycoside was determined by comparing the properties of the glycoside with those of the aglycone. The aglycone gave red colour when spotted on filter paper sprayed with *p*-toluene sulphonic acid and subsequent heating, a reaction specific for C<sub>7</sub>-OH group, whereas the glycoside did not. Thereby confirming the position of glycosylation at C-7. A bathochromic shift of 7 nm in band II with sodium acetate i.r., uv spectrum further confirmed the attachment of sugar at C<sub>7</sub> position<sup>3,4</sup>.

The aqueous hydrolysate obtained by partial hydrolysis of the glycoside with 2% H<sub>2</sub>SO<sub>4</sub> showed first the appearance of glucose, suggesting it to occupy the terminal position in the sugar moiety and rhamnose is linked glycosidically with the aglycone at position-7.



Thus keeping all the facts together the structure to the glycoside has been assigned as scutellarein-7-*O*- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 4)-*O*- $\alpha$ -L-rhamnopyranoside.

## Experimental

The air dried stems (3 kg) of *Thymus serpyllum* Linn. obtained from Himalayan Range Drug Field (Simla), were extracted with ethanol and the extract concentrated under reduced pressure and the concentrated extract was poured into excess of distilled water with continuous stirring. The water soluble part was concentrated to a dry viscous mass and extracted with ethyl acetate, followed by methanol. The methanol soluble part gave positive test for flavonoidal glycoside<sup>5,6</sup>, and tlc examination showed two spots, indicating the presence of two compounds, which were separated by column chromatography over silica gel and eluted with ethyl acetate-methanol in the ratios of 1:1, 1:2 and 1:3. The eluates from methyl acetate-methanol (1:2) were of same R<sub>f</sub> values and so were combined, and evaporation of the solvent yielded a cream coloured compound (420 mg), crystallised from methanol. m.p. 238-39° (Found: C, 54.53; H, 5.4; C<sub>27</sub>H<sub>30</sub>O<sub>15</sub> calcd. C, 54.54; H, 5.5%), M<sup>+</sup> 594;  $\lambda_{max}$  (MeOH) 277 and 333, NaOEt  $\Delta$ +54, AlCl<sub>3</sub>  $\Delta$ +26 nm, no shift with NaOAc and AlCl<sub>3</sub>;  $\nu_{max}$  (KBr) 3400, 1680, 1640, 1560, 1500, 1280 and 821 cm<sup>-1</sup>.

It gave +ve colour reactions for flavonoidal glycoside.  $\nu_{max}$  at 3400 indicated the presence of OH groups in it. Acetylation of the glycoside (50 mg) with Ac<sub>2</sub>O/pyridine yielded nono-*O*-acetyl derivative, m.p. 164-66° (Found: C, 55.54; H,

4.8.  $C_{45}H_{48}O_{24}$  calcd. C, 55.55; H, 4.9%,  $M^+$  927, thereby confirming the presence of nine hydroxy groups. Hydrolysis of the glycoside with 7% ethanolic  $H_2SO_4$ , yielded an aglycone, m.p. 348°, (Found: C, 61.96; H, 4.2.  $C_{15}H_{10}O_6$  calcd. C, 62.94; H, 4.2%),  $M^+$  286. The aqueous hydrolysate was found to contain two sugars, glucose rhamnose (paper chromatography,  $R_f$ ).

The aglycone showed bands:  $\lambda_{max}$  277 and 333; +NaOEt  $\Delta$  54 nm, +  $AlCl_3$   $\Delta$  + 26, NaOAc  $\Delta$  + 35 nm;  $\nu_{max}$  (KBr) 3400, 2940, 1725, 1640, 1560, 1500, 1280 and 821  $cm^{-1}$ . A peak at  $\nu_{max}$  3400, indicates the presence of OH groups in it. On alkaline fusion, the aglycone (50 mg) yielded *p*-hydroxybenzoic acid and 1,2,3,5-tetrahydroxybenzene, m.p. 165°, confirmed by m.m.p., co-pc and co-tlc. Thus indicating the aglycone as 4,5,6,7-tetrahydroxyflavone which was further confirmed by its  $^1H$  nmr and mass spectral studies. The important peaks obtained in the spectrum of the tetra-*O*-acetyl derivative of the aglycone:  $\delta$  6.51 (s,  $C_8$ -H), 6.73 (s,  $C_8$ -H); 7.56 (d,  $J$  8.4 Hz,  $C_2$ -H and  $C_6$ -H), 6.89 (d,  $J$  8, 2Hz,  $C_8$ -H and  $C_6$ -H), 2.45 (s,  $C_4$ -OAc), 2.58 (s,  $C_5$ -OAc), 2.52 (s,  $C_6$ -OAc) and 2.54 (s,  $C_7$ -OAc);  $m/z$  286  $m^+$ , 258, 257, 169, 158, 140 and 118.

The glycoside (50 mg) was hydrolysed by refluxing with 2% sulphuric acid. Aliquots at different interval were examined by paper chromatography. Glucose showed its appearance first, followed by rhamnose. The glycoside on permethylation<sup>7</sup> followed by hydrolysis, yielded 2:3 di-*O*-methyl-L-rhamnose and 2,3,4,6-tetra-*O*-methyl-D-glucose (confirmed by co-pc and co-tlc) clearly indicating the linkage between the two sugars through 1 $\rightarrow$ 4. The glycoside (20 mg) was dissolved in 25 ml of ethanol and was treated with 0.1*N* solution (25 ml) of sodium metaperiodate. The periodate consumed 3.2 moles and formic acid and liberated 1.3 moles were titrated by the trimetric method<sup>8</sup>. Enzymatic

hydrolysis<sup>9</sup> of the glycoside indicated  $\beta$ -linkage between the sugars and  $\alpha$ -linkage between L-rhamnose and the aglycone.

Based on the above facts when kept together, the glycoside was assigned the structure as scutellarein-7-*O*- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 4)-*O*- $\alpha$ -L-rhamnopyranoside, which was finally confirmed by  $^1H$  nmr spectrum<sup>10,11</sup> of the nona-*O*-acetyl derivative:  $\delta$  6.51 (H, s,  $C_8$ -H), 6.73 (1H, s,  $C_8$ -H), 7.56 (d,  $J$  8.4 Hz,  $C_2$ -H,  $C_6$ -H), 6.89 (2H, d,  $J$  8 2Hz,  $C_8$ -H,  $C_6$ -H), 2.45 (s,  $C_4$ -OAc); 2.58 (3H, s,  $C_5$ -OAc), 2.52 (3H, s,  $C_6$ -OAc), 2.08 (3H, s,  $C_7$ -OAc); 2.12 (3H, s,  $C_8$ -OAc), 2.05 (3H, s,  $C_2$ -OAc), 2.04 (3H, s,  $C_6$ -OAc), 2.02 (3H, s,  $C_4$ -OAc), 2.0 (3H, s,  $C_6$ -OAc), 4.75 (1H, d,  $J$  7.8 Hz,  $C_1$ -H), 5.47 (1H, d,  $J$  2Hz,  $C_1$ -H), 3.75-4.30 (m, 6 glucosyl protons) and 4.66-4.82 (4H, m, rhamnosyl protons).

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