

## NOTE

**A New Flavonol Diglycoside from *Solenostemma arghel* Leaves**

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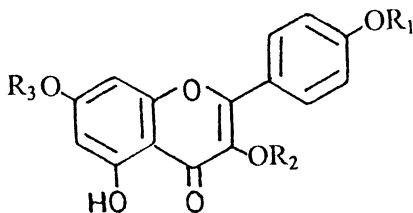
Chemical investigations on the leaves of *Solenostemma arghel* [Del] Hayne resulted in the isolation and identification of the new natural flavonol glycoside: kaempferol 3,4'-di-O- $\beta$ -D-glucoside beside its 3-glucuronide, 3-rutinoside, 7,4'-diglucoside and the aglycone kaempferol were characterized. Their structure was established on the basis of chromatographic analysis, chemical degradation and UV spectroscopy and confirmed by  $^1\text{H-NMR}$  analysis.

*Solenostemma arghel* [Del] Hayne (Asclepiadaceae) is used in various traditional medicines<sup>1</sup> such as an effective remedy for cough; infusion of its leaves is used for gastro-intestinal cramps, colds and urinary tracts. Investigation of its alcoholic extract for antimicrobial properties against 4 bacteria and 5 fungi proved that it had a high antimicrobial activity<sup>2</sup>. The only report concerning its chemical constituents was the isolation and identification of kaempferol and steroid glycosides<sup>3</sup>. The present manuscript deals with the isolation and identification of one new flavonol glycoside which is reported for the first time from a natural source, beside 4 known flavonol glycosides and one aglycone and that all are derivatives of the flavonol kaempferol. It is noteworthy that it was reported<sup>4</sup> that the distribution of flavonoid glycosides in south Indian plants belonging to the family Asclepiadaceae was studied and found to be significantly in favour of quercetin, kaempferol was present in traces only and flavone was absent from all plants.

The new naturally obtained flavonol glycoside, kaempferol 3,4'-di-O- $\beta$ -D-glucoside was isolated from the fraction eluted by 40% ethanol from the polyamide column of the methanolic extract of *S. arghel* leaves. It appeared as a brown spot on paper chromatograms under UV light unchanged on exposure to ammonia vapours. UV spectral data in MeOH and in the presence of diagnostic reagents are in accordance with those of 2,4'-disubstituted kaempferol<sup>5,6</sup> where a bathochromic shift (55 nm) in band I was produced upon the addition of NaOMe with a decrease in intensity indicating that position 4' was occupied. The addition of NaOAc led to a 5 nm bathochromic shift in band II indicating a free 7-OH group which was confirmed by the appearing of a shoulder at 325 nm on the addition of NaOAc. No shift was produced in band I upon the addition of NaOAc/H<sub>3</sub>BO<sub>3</sub>, *i.e.*, the absence of 3,4'-dihydroxyl group in ring (B). The

(47 nm) bathochromic shift produced in band I after the addition of  $\text{AlCl}_3/\text{HCl}$  indicated the presence of free 5-OH group and a substituted one at position 3. Complete acid hydrolysis yielded kaempferol as the aglycone moiety and the sugar glucose which were identified by co-paper chromatography with authentic markers. Partial acid hydrolysis yielded an intermediate which was identified as kaempferol-3-O- $\beta$ -D-glucoside through  $R_f$ -values, colour reactions and UV spectral data<sup>7</sup>.

Enzymatic hydrolysis using  $\beta$ -glucosidase yielded kaempferol. The  $^1\text{H-NMR}$  spectrum of this compound was also in accordance with the proposed structure, where the doublet signals at 8.06 and 6.93 ppm of H-2',6' and H-3',5', respectively were shifted downfield than those of kaempferol ( $\delta$  7.88 and 6.77 ppm for H-2',6' and H-3',5') indicating glycosylation in position 4'. The two glucose anomeric protons gave rise to two doublet signals, the positions of which ( $\delta$  5.45 and 5.3 ppm) indicated the attachment of the anomeric carbons of each glucose moiety to the kaempferol hydroxyls<sup>5</sup> at positions 3 and 4'.



K-3,4'-diglycoside	: $R_1 = R_2 = \text{glucose}, R_3 = \text{H}$ (the new compound)
K-3-glucoside	: $R_1 = R_3 = \text{H}, R_2 = \text{glucose}$ (the intermediate)
K-3-glucuronide	: $R_1 = R_3 = \text{H}, R_2 = \text{glucuronic acid}$ .
K-3-rutinoside	: $R_1 = R_3 = \text{H}, R_2 = \text{rutinose}$ .
K-7,4'-diglycoside	: $R_2 = \text{H}, R_1 = R_3 = \text{glucose}$
Kaempferol	: $R_1 = R_2 = R_3 = \text{H}$

**Plant material:** *Solenostemma arghel* [Del] Hayne leaves were collected from Aswan, Egypt. Voucher specimen is deposited at the National Research Centre Herbarium, Cairo, Egypt. It was identified by Prof. Dr. Lotfy Boulos.

**Extraction. Isolation and Identification:** Air-dried leaves of *S. arghel* (1 kg) were extracted with methanol when hot; the concentrated methanolic extract was chromatographed over polyamide (6S, Riedel-De-Haen-Ag, Seelze, Hannover) column eluted first with water and then with increasing percentage of ethanol. The new compound was isolated from the fraction of the column eluted by 40% ethanol by preparative paper chromatography using BAW (6 : 1 : 2) as the solvent system for irrigation. All the isolated compounds including the new one were purified over Sephadex LH-20 column prior to chemical and spectral analyses. Complete acid hydrolysis of the glycosides (2N HCl, 1 h at 100°C) and controlled acid hydrolysis were done using 0.1 N HCl and the mixture was examined every 5 min, yielding the sugar residues and the aglycones which were identified by co-chromatography with authentic reference markers. Enzymatic hydrolysis using  $\beta$ -glucosidase or  $\beta$ -galactosidase was achieved<sup>5</sup>. All UV spectral data were

recorded using the standard procedures<sup>5,6</sup>. <sup>1</sup>H-NMR spectra of the isolated compounds were recorded in DMSO-d<sub>6</sub>, set at 39.5 ppm which is the chemical shift in relation to  $\delta_{\text{TMS}} = 0$ .

### Kaempferol 3,4'-di-O- $\beta$ -D-glucoside

R<sub>F</sub> values  $\times 100$  on Whatman No. 1 paper chromatography. [*n*-Butanol : Acetic acid : Water (BAW, 4 : 1 : 5)] 59, [*n*-Butanol : Acetic acid : Water (BAW, 6 : 1 : 2)] 68; [15% AcOH] 57; [H<sub>2</sub>O] 19. UV spectral data:  $\lambda_{\text{max}}$  nm in (MeOH) 265, 350; (NaOMe) 280, 325\*, 405; (AlCl<sub>3</sub>) 273, 300\*, 343, 400; (AlCl<sub>3</sub>/HCl) 273, 302\*, 345, 392; (NaOAc) 270, 300\*, 360; (NaOAc/H<sub>3</sub>BO<sub>3</sub>) 265, 350 (\*shoulder). <sup>1</sup>H-NMR, aglycone moiety:  $\delta$  (ppm) at 8.06 (d, J = 8 Hz, H-2' and 6'); 6.93 (d, J = 8 Hz, H-3' and 5'); 6.52 (d, J = 2.5 Hz, H-8); 6.28 (d, J = 2.5 Hz, H-6); sugar moiety:  $\delta$  (ppm) at 5.45 (d, J = 7.5 Hz, H-1'' of glucose at position 3); 5.3 (d, J = 7.5 Hz, H-1''' of glucose at position 4'); 3.1–3.7 (m of the 12 glucose protons).

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