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A NEW BIFLAVONE GLYCOSIDE FROM FLOWERS OF *Asystasia gangetica*

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The yellow parts of the flowers of Asystasia gangetica *were extracted with 85% methanol under reflux. The alcoholic extract was concentrated* in vacuo *and the aqueous extract was fractionated with solvents of increasing polarity. The ethyl acetate fraction yielded a yellow solid, which was characterized as apigenin 7-*O*-glucosyl(3′→6″)luteolin 7″-*O*-glucoside using UV,* ^{1}*H,* ^{1}*H–*^{1}*H COSY,* ^{13}*C NMR, and mass spectral studies.*

Keywords: *Asystasia gangetica*, Acanthaceae, biflavone glycoside.

Asystasia gangetica Linn. of the Acanthaceae family is an erect, procumbent herb found in peninsular India. It is cultivated in the garden as an ornamental plant. The leaves are good sources of thiamine [1]. The juice of the plant is used as an anthelmintic. It is also given in swellings and rheumatism [2, 3]. The flowers are white or yellow in color. The yellow flowers have violet or pink coloration at the center of the flower. Previous phytochemical investigations of this plant showed the presence of flavonoids [4–6]. With the view to locate the flavonoids of yellow flowers of this plant, yellow parts of the flowers alone were chosen, and the results are presented hereunder. They were found to contain a biflavone glycoside.

The yellow flowers of *Asystasia gangetica* were extracted and fractionated, and the ethyl acetate extract yielded a yellow solid **1**. The UV spectrum of the glycoside had λ_{max} at 340 nm (band I) and 265 nm (band II), suggesting the presence of a flavone skeleton. In the NaOMe spectrum, a bathochromic shift of 45 nm was noticed. This indicates the presence of a 4′-OH group. No bathochromic shift was noticed in the NaOAc spectrum of the glycoside, whereas a bathochromic shift of 11 nm was noticed in the case of the aglycone. This shows the absence of free OH at C-7 in the glycoside and the presence of the same in the aglycone. Spectral changes with other shift reagents could not be identified because of the superimposition of bands. The presence of free OH at C-5 was evidenced by the positive Wilson's boric acid test.

In the ^{1}H NMR spectrum (DMSO-d_6, TMS) of the glycoside there are two signals, one at δ 12.98 and another at δ 12.95, indicating the presence of two chelated hydroxyl groups. These signals are assigned to OH groups present at C-5 and C-5″, respectively. The C-6′ proton appears as a doublet at δ 7.9 due to *ortho* coupling with H-5′. The C-5′ proton appears as a doublet at δ 6.9 due to *ortho* coupling with H-6′. The C-2′ proton that is *ortho* to the interflavonoid linkage experiences an upfield shift and appears at δ 7.62. The C-6 proton appears at δ 6.44 ppm, and the C-8 proton appears as a doublet at δ 6.86 due to *meta* coupling with H-6. The C-8″ and C-3″ protons appear as singlets at δ 6.78 and δ 6.74, respectively. The C-2‴ and C-6‴ protons appear at δ 7.45 and δ 7.42, respectively. The C-5‴ proton appears as a doublet at δ 6.94 due to *ortho* coupling with H-6‴ (7.45) as observed in the ^{1}H–^{1}H COSY spectrum. The two doublets due to anomeric protons of the glucose units attached to the C-7 and C-7″ carbons merge at δ 5.1.

^{13}C NMR (DMSO-d_6, TMS) spectral data provide supporting evidence for the structure of the biflavone glycoside. The signal positions and their complete assignments to difference carbon atoms are given. Due to glycosylation, the signals of C-7 and C-7″ shifted upfield and appear at δ 162.9. The *ortho* carbon atoms C-6, C-8, and C-8″ experience a downfield shift, confirming the glycosylation at C-7 and C-7″. Due to interflavonoid biphenyl linkage, the C-3′ carbon experiences a downfield shift of 4.3 ppm, and the C-6″ carbon experiences a downfield shift of 4.2 ppm. These carbon atoms appear at δ 120.1 and δ 104.0, respectively.

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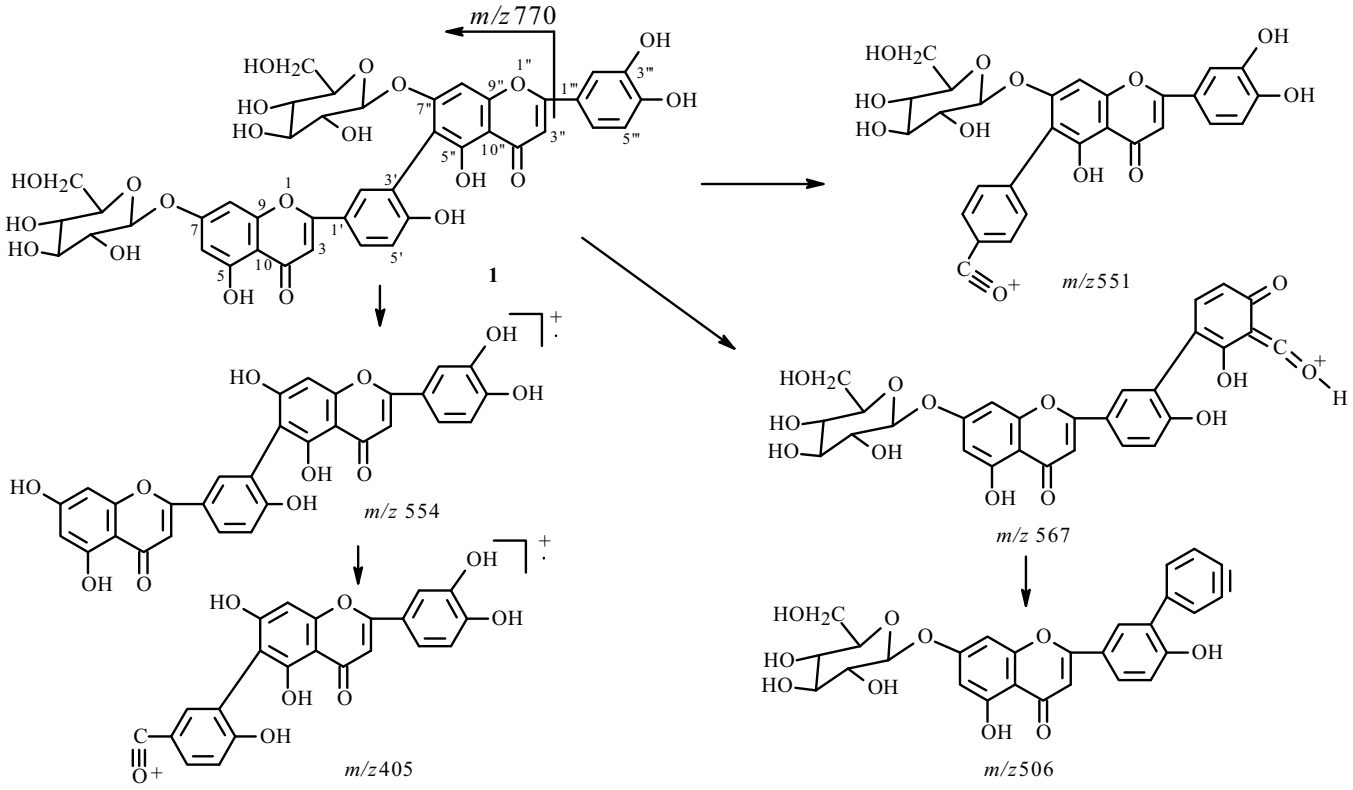

Fig. 1. Mass fragmentation pattern of **1**.

The ^1H–^1H homonuclear shift correlated 2D spectrum contains information that completely establishes the coupling relationship among all the protons except OH protons.

The C-6‴ proton signal is found at δ 7.45. This is coupled with the C-5‴ proton, which appears at δ 6.94. This coupling is again shown by cross peaks. The C-6 proton, which appears at δ 6.44, and the C-8 proton, which appears at δ 6.88, are coupled with each other (*meta* coupling). Similarly, coupling between C-2′ and C-6′ is shown by the cross peaks appearing between δ 7.62 and δ 7.9. The cross peaks seen between δ 7.45 and δ 7.42 are due to the *meta* coupling of C-2″ and C-6″ protons.

The structure of the biflavone glycoside has been confirmed by mass spectral data. The fragmentation pattern (MS-FAB$^+$) (Fig. 1) of the biflavonoids follows by the corresponding monoflavonoids. Some A and B-ring fragments are exactly the same as those observed for monoflavonioids, except that they have an intact flavonoid skeleton attached to them [4]. The compound had peaks at *m/z* 770, 567, 554, 522, 405, 389, 362, 270, 229, 137, and 121. The fragmentation pattern agrees with the identity of the compound. Based on the above evidence, the glycoside has been characterized as apigenin 7-*O*-glucosyl(3′→6″)luteolin 7″-*O*-glucoside.

EXPERIMENTAL

Plant Material. **Extraction and Isolation.** The yellow parts of the flowers of *Asystasia gangetica* collected at Kumbokonam of Tanjore District, India during November were extracted with 85% methanol under reflux. The alcoholic extract was concentrated *in vacuo*, and the aqueous extract was fractionated with petroleum ether (60–80°C) (5 × 500 mL), peroxide-free ether (3 × 500 mL), and ethyl acetate (5 × 500 mL). The EtOAc fraction alone was taken for study. EtOAc fraction: biflavone glycoside, apigenin 7-*O*-glucosyl(3′→6″)luteolin 7″-*O*-glucoside. The EtOAc fraction was concentrated *in vacuo* and left in an ice chest for a few days. The yellow solid that separated was filtered and subjected to silica gel column chromatography and eluted with solvents of increasing polarity, 50% EtOAc in $CHCl_3$, yielding the glycoside, which was recrystallized from methanol and studied. It was soluble in MeOH but insoluble in water. It gave a yellow color with NH_3 and a red color with Mg-HCl. It responded to Wilson′s boric acid test and Molisch′s test. It did not respond to the Horhammer-Hansel test. It had λ_{max}, MeOH: 265, 340; +NaOMe: 270, 385; +NaOAc: 267, 342 nm. ^1H–^1H COSY and mass spectrum were recorded and interpreted.

Hydrolysis of Glycoside 1. The glycoside (50 mg) was dissolved in hot aqueous MeOH, and an equal volume of H_2SO_4 was added to it. This mixture was refluxed at 100°C for 2 h. The excess alcohol was distilled off and the resulting solution was extracted with Et_2O.

The residue obtained was studied for the aglycone. It responded to Wilson′s boric acid test. It did not respond to the Horhammer-Hansel test and Molisch′s test. It had λ_{max}, MeOH: 253, 267, 291 sh, 335; +NaOMe: 266, 329 sh, 390; + NaOAc: 278, 329 sh, 335 nm. Spectral changes with other shift reagents could not be identified due to overlapping of bands.

The filtrate was neutralized with $BaCO_3$ and concentrated. The concentrated filtrate was examined by paper chromatography. The R_f values agreed with those of glucose. The identity of the sugar moiety was further confirmed by co-chromatography with an authentic sample of glucose.

Spectroscopic Methods. NMR spectra were recorded on a Bruker AMX400 spectrometer at 400.13 MHz for ^1H NMR and 100.61 MHz for ^{13}C using standard Bruker pulse sequences. TMS was used as an internal standard. Mass spectra were recorded in Auto spec FAB+ Magnet Bpm: 55 BPI 446544. UV spectra were obtained from a Perkin–Elmer 301 UV/Vis spectrometer.

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