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FLAVONE *O*- AND *C*-GLYCOSIDES OF *RHYNCHOSIA BEDDOMEI*

DAMA ADINARAYANA*, DUVVURU GUNASEKAR*, OTTO SELIGMANN† and HILDEBERT WAGNER†

* Department of Chemistry, Sri Venkateswara University, Tirupati-517502, India;
† Institut für pharmazeutische Arzneimittellehre der Universität München, Karlstraße 29, D-8000 München 2, W. Germany

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Key Word Index—*Rhynchosia beddomei;* Leguminosae; 3′,4′-di-*O*-methylluteolin-7-*O*-glucuronide; vitexin; isovitexin; orientin; isoorientin; vicenin-2; lucenin-2; rutin; naringenin; D-inositol.

Abstract—A new flavone-*O*-glycoside isolated from the leaves of *Rhynchosia beddomei* has been characterized as 3′,4′-di-*O*-methylluteolin-7-*O*-glucuronide.

INTRODUCTION

Plants belonging to the genus *Rhynchosia* (tribe Phaseoleae, subfamily Papilionoideae) have not been thoroughly examined for their flavonoid constituents. Besson *et al.* [1] reported the presence of four *C*-glycosylflavones in the leaves of *R.minima*. Based on the absence of flavonoid aglycones before or after acid hydrolysis, they concluded that only *C*-glycosides are present in this plant. In our chemical examination of the leaves of *R.beddomei* (Bak.) we have isolated six flavone-*C*-glycosides, a flavonol-*O*-glycoside, a new flavone-*O*-glycoside and a flavanone.

RESULTS AND DISCUSSION

Acetone extract of the leaves afforded, after repeated column chromatography employing silica gel, PC and TLC (cellulose and silica gel), four mono-*C*-glycosides (vitexin, isovitexin, orientin and isoorientin) and two di-*C*-glycosides established as vicenin-2 and lucenin-2 by mass spectral study of their permethylated derivatives. Methanol extract of the leaves yielded a cyclitol (identified as D-inositol), a flavanone (established as naringenin) and two *O*-glycosides. One of them was obtained as yellow needles (yield 0.002%) melting at 188–90° and showed identity with rutin. The second compound was obtained as pale yellow crystals (yield 0.004%) $[\alpha]_D^{31} - 160.71°$ (*c* 0.56, Py–H_2O, 1:1 v/v) which did not melt below 320°. It gave a brown ferric colour, a positive Molisch's test and pale pink colour with Mg–HCl. It also effervesced with $NaHCO_3$.

A strong IR absorption at 1585 cm^{-1} is characteristic for carboxylate. The flame test gives evidence for the presence of a potassium salt. According to the UV spectra, the flavone nucleus must bear protected OH groups in 7,3′,4′-positions and a free 5-OH group. The aglycone peak at *m/e* 314 with the fragment ions at *m/e* 153 and 162 are in congruence with a 3′,4′-di-*O*-methylluteolin structure. This was confirmed by hydrolysis and comparison of the aglycone with an authentic sample obtained by dehydrogenation of synthetic 3′,4′-di-*O*-methyleriodictyol-7-*O*-neohesperidoside followed by hydrolysis [2]. The per-deuteromethylated glycoside showed a M^+ of *m/e* 575. The fragmentation sequence *m/e* 244, 210, 175, 147, 122 and 107 is typical for PDM-hexuronides [3]. All these data are in agreement with the structure of a 3′,4′-di-*O*-methylluteolin-7-*O*-β-D-glucopyranuronide.

EXPERIMENTAL

Shade-dried leaves of *R.beddomei* (3.2 kg) were extracted successively with petrol (bp 60–80°), C_6H_6, Me_2CO and

MeOH. The Me_2CO extract on repeated chromatography (PC and TLC) yielded six flavone-*C*-glycosides, which were identified as orientin, isoorientin, vitexin, isovitexin, lucenin-2 and vicenin-2 by chromatographic and mass spectral studies as well as by comparison with authentic samples. In addition, a new flavonol was isolated [4]. The methanolic extract on concn to *ca* 250 ml gave a brown solid (11.4 g). It was Soxhleted with MeOH and from the MeOH-soluble part initially a solid separated which on repeated crystallization with absolute ethanol containing a few drops of HOAc gave a crystalline solid (250 mg), mp 226–27° (inositol). Further concn of the MeOH-soluble part deposited a yellow–green solid which on recrystallization from MeOH yielded yellow needles (70 mg), mp 188–90° (rutin). After separation of the brown solid the methanolic extract was concentrated under red. pres. and the viscous residue treated with cold H_2O to remove H_2O-soluble sugars (identified as glucose and fructose). The H_2O-insoluble material was then solvent fractionated with Et_2O, $CHCl_3$, Me_2CO, EtOAc and MeOH. The Et_2O-soluble part yielded a solid which separated from MeOH as a crystalline solid (42 mg), mp 248–49° (naringenin). The Me_2CO-soluble part was chromatographically separated to yield three *C*-glycosides identified as isoorientin, lucenin-2 and vicenin-2. The $CHCl_3$ and EtOAc extractions did not yield any crystalline principles.

3′,4′-Di-O-methylluteolin 7-O-glucuronide. The MeOH-soluble part on concn yielded a yellow solid (120 mg) which did not melt below 320°. With alc. $FeCl_3$ it gave an initial pale green colour changing to brown. A pale pink colour was formed with Mg–HCl, Molisch's test was positive and it also gave effervescence with $NaHCO_3$ soln. $[\alpha]_D^{31} - 160.71$; TLC (Cellulose); BAW (4:1:5) R_f 0.57, 15% aq. HOAc, R_f 0.34; The salt-free glucuronide (mp 189–190°) was prepared according to the method of ref. [5]. $C_{23}H_{22}O_{12}$ (508.42). (Found: C, 53.98; H, 5.04. Calc.: C, 54.33; H 4.76%). UV λ_{max}^{MeOH} nm: 250 (ε 134 300), 270 (ε 119 700) and (ε 154 700); $\lambda_{max}^{AlCl_3}$: 263, 275, 290, 355 and 382; $\lambda_{max}^{AlCl_3-HCl}$: 262, 275, 295, 362 and 385 (sh); λ_{max}^{NaOMe}: 280, 305 (sh), 395 (broad); λ_{max}^{NaOAc}: 250, 270 and 340. IR (KBr) $\bar{\nu}$ cm^{-1}: 3400 (OH), 1730 (C=O carboxyl), 1660 (C=O Flavone), 1610 (Ar), 1495, 1250, 1165, 1025, 825. MS: *m/e* (rel. int. %). (a) *Glycoside*: 314 (A+H, 100), 162 ($B_1^{+\cdot}$, 13), 153 [$(A_1+H)^+$, 44.4], 152 ($A_1^{+\cdot}$, 3.7) (b) *Perdeuteromethyl ether of the glycoside*: 575 (M^+, 11.0), 540 (M-35, 3.0), 331 (M-244, 42.0), 244 (M-331, 23), 210 (Glur-34, 100), 175 (36), 147 (53), 122 (40), 107 (80), 88 (35), 81 (61), 72 (34), 43 (85); ^1H NMR: (DMSO-d_6+TFA-*d*, TMS int.): δ 3.21–3.60 ppm (*m*, 3H, H-2″, 3″, 4″), 3.82, 3.85 (*s*, 6H, OMe-3′, 4′), 4.05 (*m*, 1H, H-5″), 5.18 (*br*, 1H, H-1″), 6.40 (*d*, $J=2$ Hz, 1H, H-6), 6.76 (*d*, $J=2$ Hz, 1H, H-8), 6.80 (*s*, 1H, H-3), 6.98 (*d*, $J=8$, 5 Hz, 1H, H-5′), 7.32–7.67 (*m*, 2H, H-2′, 6′).

(a) *Acid hydrolysis of 3′,4′-di-O-methylluteolin-7-O-glucuronide.* Acid hydrolysis of the glycoside (10 mg) in MeOH (2 ml) with 5% aq. HCl (8 ml) by heating at 100° for 5 hr gave a mixture of aglycone and the unchanged compound which were separated by PLC (microcrystalline cellulose, 15% aq. HOAc). The aglycone showed the same UV characteristics as earlier recorded for luteolin 3′,4′-di-*O*-methyl ether [6] and gave the same mp as the synthetic compound [2] (mp 279–280°). The sugar was characterized as glucuronic acid by paper chromatography [7].

(b) *Acetylation of 3′,4′-di-O-methylluteolin-7-O-glucuronide.* A mixture of the glycoside (25 mg) in 1 ml Py and 5 ml Ac_2O was kept at room temp. for 56 hr, poured into crushed ice (50 g). The resulting solid gave an amorphous substance (7 mg) from dry $CHCl_3$–petrol, mp 132–144°.

(c) *Hydriodic acid treatment of 3′,4′-di-O-methylluteolin-7-O-glucuronide.* Gentle reflux of the glycoside (8 mg) in a mixture of HI (0.5 ml) and phenol (0.1 ml) and usual work-up yielded a green–yellow solid, separating from MeOH as a yellow crystalline solid and identified as luteolin by direct comparison with an authentic sample.

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