

Methylapigenin 7-*O*- β -D-Glucuronate—A New Flavone Glycoside from *Acanthus ilicifolius*

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Methylapigenin 7-*O*- β -D-glucopyranuronate, a new flavone glycoside, has been isolated along with apigenin 7-*O*-glucuronide from leaves of *Acanthus ilicifolius*. The structure of the new glycoside established by chemical and uv, ir, ^1H nmr, ^{13}C nmr and CI mass spectral data, has been confirmed by partial synthesis.

Acanthus ilicifolius Linn.^{1,2} (Family : Acanthaceae) is an evergreen spinous herb, found in the mangroves of Indian peninsula. The leaves are recorded to be used for fomentation in rheumatism and neuralgia. Two sterols and five triterpenoids have been earlier isolated from *A. ilicifolius*³; there being no record of any work on flavonoid. Leaves of *A. spinosus*⁴ and *A. mollis*⁴ have been reported to contain luteolin glycosides.

In continuation of our studies on the flavonoids of Acanthaceae⁵⁻⁸, we have examined the fresh leaves of *A. ilicifolius* collected from Pondicherry, and the isolation and characterisation of two flavone glycosides including the new methylapigenin 7-*O*- β -D-glucuronate are presented here.

Results and Discussion

Two flavone glycosides were isolated from the EtOAc soluble fraction of the aqueous alcoholic concentrate of the leaves of *A. ilicifolius*. They were separated by preparative tlc (silica gel; CHCl_3 - MeOH, 3 : 1) into more polar (lower R_f) and less polar (higher R_f) components and individually purified by recrystallisation from methanol. The more polar compound, $\text{C}_{21}\text{H}_{18}\text{O}_{11}$ had m.p. 321–22°, $[\alpha]_D^{25}$ -86.4°. It yielded, on acid as well as enzyme (β -glucuronidase) hydrolysis apigenin and D-glucuronic acid. It was identified as apigenin 7-*O*- β -D-glucopyranuronide by uv, ^1H and ^{13}C nmr spectra and the identity confirmed by super-imposable ir spectra of the sample and authentic compound from *Ruellia tuberosa*⁵.

The less polar compound, $\text{C}_{23}\text{H}_{20}\text{O}_{11}$, m.p. 235–37°, $[\alpha]_D^{25}$ -84.2° had almost identical uv fluorescence and λ_{max} as those of apigenin 7-glucuronide⁹. On acid hydrolysis, it yielded apigenin and D-glucuronic acid. It had R_f values different from apigenin 7-glucuronide; also there was no significant lowering of R_f in 5% HOAc compared to water, pointing to the absence of free COOH group. The ir spectra exhibited prominent ester absorption at 1750 cm^{-1} . The ^1H nmr spectra

provided characteristic signals of apigenin 7-*O*-glucuronide along with a three-proton signal at δ 3.40 indicating an OMe group. The presence of a COOMe group was also revealed by the signal at δ 51.92 in the ^{13}C nmr spectra which provided further evidence for the apigenin 7-*O*- β -D-glucuronide structure (see Experimental). Chemical ionisation mass spectra (CIMS) using NH_3 as well as Cl showed signals characteristic of apigenin as well as methyl glucuronate parts (see Experimental). Mild alkaline hydrolysis followed by acidification and usual work up gave apigenin 7-*O*- β -D-glucuronide. Based on these data, the compound was identified as methylapigenin 7-*O*- β -D-glucopyranuronate and the identity confirmed by direct comparison (including super-imposable ir spectra) of the natural sample with a synthetic sample prepared by the estrification of authentic apigenin 7-*O*- β -D-glucopyranuronide with MeOH. That methyl apigenin 7-*O*-glucuronate was a natural product and not an artefact, was concluded from the absence of its detection in plant sources^{9,10} richer in apigenin 7-*O*-glucuronide.

This is the first record of the occurrence of methylapigenin 7-glucuronate. Isolation of ethylapigenin 7-glucuronate from *Archillea cartilaginosa*⁹ and *Centaurea aspera*¹⁰ (Compositae) and methylacacetin 7-glucuronate from *Clerodendron infortunatum*¹¹ (Verbenaceae) was reported earlier.

The present isolation of flavone glucuronides from *A. ilicifolius* reveals its resemblance to *Ruellia*^{5,6} and *Thunbergia*⁶ species and difference from *Echolium*⁸ and *Siphonogloba*¹² species containing flavone C-glycosides in the Acanthaceae.

Experimental

Fresh leaves (1.5 kg) of *Acanthus ilicifolius* Linn. (Voucher specimen deposited at Jawaharlal Institute) collected from Pondicherry were extracted thrice with boiling 80% EtOH and the concentrate partitioned with C_6H_6 , Et_2O and EtOAc. The residue from EtOAc (0.5 g) was subjected to

preparative tlc (silica gel; CHCl₃—MeOH, 3 : 1). The lower band on elution with MeOH gave apigenin 7-O-glucuronide (0.25 g) and the higher band yielded methylapigenin 7-O-glucuronate (0.15 g).

Apigenin 7-O-β-D-glucopyranuronide : M.p., uv, ir and ¹H nmr data and acid and enzyme hydrolysis were reported earlier⁸. ¹³C nmr (22.5 MHz, D₂O, dioxan internal, TMS) δ 182.47 (C-4), 175.77 (C-6"), 164.72 (C-2), 162.83 (C-7), 160.36 (C-5,4'), 156.91 (C-9), 128.43 (C-2',6'), 121.15 (C-1'), 116.14 (C-3',5'), 105.99 (C-10), 102.68 (C-3), 100.08 (C-6 1"), 95.98 (C-8), 76.99 (C-5"), 76.01 (C-3"), 72.50 (C-2") and 69.19 (C-4"); positive CI(NH₃) *m/z* (rel. int.) 271 (aglycone+H, 100), 194 (glucuronide part -OH+NH₃, 36), 150 (194-CO₂, 29) and 132 (150-H₂O, 40), negative CI(Cl) *m/z* 269 (aglycone -H, 92) and 193 (glucuronide part -H, 6).

Methylapigenin 7-O-β-D-glucopyranuronate : It gave light yellow needles, m.p. 235-37°, [α]_D²⁰ -84.2 (c, 0.4, MeOH), λ_{max} (MeOH) 268, 333, (NaOMe) 268, 300sh, 385, (AlCl₃) 275, 300, 345, 385, (AlCl₃+HCl) 277, 300, 340, 380, (NaOAc) 267, 3·5, 387, (NaOAc+H₃BO₃) 268, 340 nm; ν_{max} (KBr) 3430 br, 1750, 1670, 1610, 1500, 1185, 910 and 840 cm⁻¹; ¹H nmr (90 MHz, DMSO-d₆, TMS internal) δ 7.95 (2H, d, *J* 8.6Hz, *H*-2',6'), 6.93 (2H, d, *J* 8.6Hz, *H*-3',5'), 6.86 (2H, s, *H*-8,3), 6.48 (1H, s, *H*-6), 5.60 (1H, br s, *H*-1"), 5.35 (4H, br s, CH of sugar), 3.40 (3H, s, OCH₃); ¹³C nmr (22.5 MHz, DMSO-d₆, TMS) 181.92 (C-4), 169.17 (C-6"), 164.29 (C-2), 162.34 (C-7), 161.37 (C-5), 161.17 (C-4'), 156.88 (C-9), 128.53 (C-2',6'), 120.92 (C-1'), 115.98 (C-3',5'), 105.44 (C-10), 103.04 (C-3), 99.33 (C-6), 99.13 (C-1"), 94.65 (C-8), 75.40 (C-5"), 75.20 (C-3"), 72.73 (C-2"), 71.30 (C-4") and 51.92 (OCH₃); positive CI(NH₃) *m/z* 271 (aglycone+H, 100), 208 (methyl glucuronate part, 16), 194 (glucuronide part, 100), 150 (194-CO₂, 6), 132 (150-H₂O, 24); negative CI(Cl) *m/z* 269 (aglycone-H, 100); pc (*R_f* × 100, Whatman No. 1,

ascending, 28°) 5(H₂O), 8(5% HOAc), 23(15% HOAc), 5(50% HOAc), 80(BAW), 88(phenol), 86(Forestal) and 85(t-BAW).

Methylapigenin 7-O-glucuronate : Apigenin 7-O-glucuronide (60 mg) in MeOH (20 ml) was refluxed with HCl (0.05 ml) for 2 h cooled, solvent distilled off in vacuum and the residue crystallised from dioxan; identical with natural sample by co-tlc.

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