

Note

A new flavone glycoside from *Zanthoxylum acanthopodium* DC

B Ravindra Babu, Shilpi Khurana, Rajeev Sakhuja, Amit K
Srivastava & Subhash C Jain*

Department of Chemistry, University of Delhi, Delhi 110 007,
India

E-mail: jainsc48@hotmail.com

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Examination of the dry fruits of *Zanthoxylum acanthopodium* has led to the isolation of a new flavone glycoside along with herbacetin-8,4'-dimethyl ether. The new flavone glycoside is characterized as 7-O- α -D-glucosyl-3,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-1-benzopyran-4-one on the basis of its spectral studies and that of its aglycone. This is the first report of the isolation of a 5-O-substituted flavone from the genus *Zanthoxylum*.

Keywords: *Zanthoxylum acanthopodium* DC, fruits, rutaceae, flavone glycoside, 7-O- α -D-glucosyl-3,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-1-benzopyran-4-one, herbacetin-8,4'-dimethyl ether

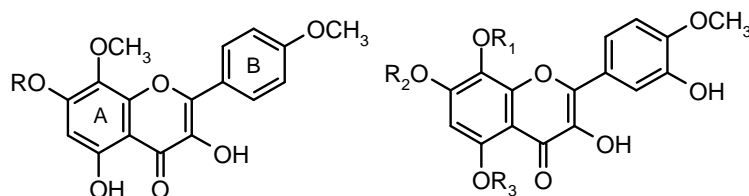
IPC: Int.Cl.⁸ C07D

The fruits of *Z. acanthopodium* (Rutaceae) are commonly known in India as Tambul. They possess the peculiar flavour of coriander and have long been used as a spice. The fruits are extensively used in the Ayurvedic and Unani systems of medicine¹. Previous phytochemical investigations of the fruits and seeds had led to the isolation of herbacetin-7,8,4'-trimethyl ether²⁻⁵ (tambulin, **1**) and 8-O-glucosyl-gossypetin-7,4'-dimethyl ether^{6,7} (tambuletin, **2**). The fruits have now been re-examined and herein are reported two flavonols **3** and **4**. Compound **3** is identified as herbacetin-8,4'-dimethyl ether^{8,9} while **4** is a new flavone glycoside. A number of flavone glycosides have also been reported earlier from various natural sources¹⁰.

Compound **4** gave positive Molisch's and Shinoda's tests, indicating it to be a flavone glycoside. Its IR spectrum had strong absorption bands at 3370 and 1658 cm⁻¹ showing the presence of free hydroxyl(s) and a flavonoid carbonyl respectively in the molecule. The presence of the flavonoid skeleton was also supported by its UV-Vis spectrum¹¹ which exhibited

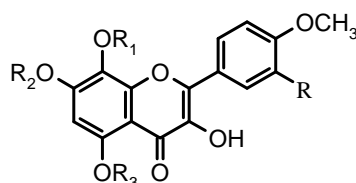
absorption maxima at 258 and 376 nm. Further, a bathochromic shift of 62 nm in band-I observed in the presence of AlCl₃ was typical of a flavonoid containing 3-hydroxyl group¹¹. The spectrum remained unaffected in the presence of sodium acetate suggesting thereby that either C-7 is unsubstituted or C-7 hydroxyl is blocked; the former possibility however looked rather remote and unlikely on biogenetic grounds. Careful interpretation of the ¹H NMR spectrum corroborated the previous findings and also revealed the presence of two methoxys and a sugar unit. The glycoside **4** was hydrolysed and the sugar moiety obtained was identified as D-glucose by its comparison with the authentic sample on paper chromatography in *n*-BuOH:pyridine:H₂O (6:4:3). The GLC chromatogram of its TMS derivative was also comparable with that of the standard sample.

The aglycone **5** gave a molecular ion peak at *m/z* 346 corresponding to the molecular formulae C₁₇H₁₄O₈. The substitution pattern in ring **A** and **B** was deduced by considering its mass fragmentation pattern along with the UV-Vis and ¹H NMR data. A fragment ion at *m/z* 151 (due to retro Diels-Alder cleavage) indicated ring **B** to contain a hydroxy and a methoxy group. The presence of a 3',4'-dioxygenation pattern was indicated by its ¹H NMR spectrum as it showed peaks characteristic of 1,2,4-trisubstituted aromatic moiety at δ 7.10 (d), 7.77 (d) and 7.84 (dd). The hydroxy group was placed at C-3' and the methoxy at C-4' on the basis of its UV-Vis data. A bathochromic shift of 39 nm in band-I with a decreased intensity on addition of sodium methoxide (characteristic of a 3-hydroxy flavone lacking 4'-OH) indicated that the C-4' hydroxyl is substituted and hence the methoxy was placed at C-4'. Further EIMS data showed another important ion at *m/z* 181 (for A⁺-H) thus indicating the presence of two hydroxy and a methoxy in ring **A**. The two hydroxy were present as *ortho*-dihydroxy and placed at C-7 and C-8 based on its UV-Vis spectrum. The presence of C-7 hydroxyl was indicated by a bathochromic shift of 9 nm in band-II on addition of fused NaOAc. This maxima further shifted by 11 nm on addition of H₃BO₃ (typical for *o*-dihydroxy groups) which supported the presence of C-8 hydroxy. The absence of any peak in the region δ 94.0-100.0 in its ¹³C NMR spectrum



- 1 R = CH₃
3 R = H

- 2 R₁ = glu, R₂ = CH₃, R₃ = H
4 R₁ = H, R₂ = glu, R₃ = CH₃
5 R₁ = R₂ = H, R₃ = CH₃



- 1 R = OH, R₁ = H, R₂ = glu, R₃ = CH₃
2 R = R₂ = R₃ = H, R₁ = CH₃

further suggested that C-8 is not unsubstituted¹² but oxygenated and showed its signal at δ 125.89. Moreover, a chemical shift¹³ of δ 103.28 was attributed to unsubstituted C-6 which was confirmed by its ¹H NMR that showed a singlet at δ 6.56. The UV-Vis spectrum also confirmed the unsubstituted C-6 position⁹ as it showed a bathochromic shift of 48 nm in AlCl₃-HCl vs. MeOH. Since ¹H NMR revealed the presence of two methoxy and one of them was already placed at C-4', the other may be present at the only other available position, C-5. This assignment was indirectly confirmed as no signal in the region δ 11-14 for chelated-OH was observed in its ¹H NMR spectrum. Based on the above spectral characteristics, the aglycone was assigned the structure 3,7,8-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4*H*-1-benzopyran-4-one.

The sugar moiety was placed at C-7 on the basis of UV-Vis spectrum of **4**, as it remained unaffected in the presence of NaOAc. A downfield shift of the C-7 signal by 3.92 ppm in its ¹³C NMR spectrum, as compared with that of aglycone **5**, confirmed the presence of sugar at C-7. The sugar which was identified as a glucopyranose also got support from its ¹³C NMR spectrum. The anomeric configuration was assigned to be an α -linkage because C-1'' resonated at δ 95.21. Moreover, in its ¹H NMR spectrum, H-1'' appeared as a doublet at δ 5.23 with J = 4.5 Hz, which further supported the assignment of α -linkage.

All the above data suggested **4** to be 7-O- α -D-glucosyl-3,8-dihydroxy-2-(3-hydroxy-4-methoxyph-

nyl)-5-methoxy-4*H*-1-benzopyran-4-one. This compound has not been reported earlier from any other natural source. This is the first report of the isolation of 5-methoxylated flavone from the genus *Zanthoxylum*.

Experimental Section

The melting points were obtained in open glass capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model BX-II IR spectrometer in KBr pellets and ¹H and ¹³C NMR spectra were recorded on Bruker 300 MHz and 75.47 MHz instruments in DMSO-*d*₆. All chemical shifts were reported in δ (ppm) downfield from tetramethylsilane. UV-Vis spectra were recorded on Perkin-Elmer Lambda spectrophotometer. Mass spectra were recorded on a Jeol-DX 303 instrument by employing electron ionization at 70 eV and only major peaks are quoted. TLC spots were visualized under UV (254 and 366 nm) and by heating after spraying with 5% H₂SO₄.

Plant Material

The fruits of *Z. acanthopodium* were collected from the Botanical Survey of India (BSI) Experimental Garden, Barapani, Shillong, and identified by Dr. T.M. Hynniewta.

Extraction and Isolation

Dried fruits of *Z. acanthopodium* (1.35 Kg) were crushed and extracted with petroleum ether and

MeOH. The solvent free MeOH extract was treated with acetone. The acetone-soluble fraction was subjected to column chromatography and eluted with Hexane, Hexane-EtOAc, EtOAc, EtOAc-MeOH, MeOH gradient in order of increasing polarity. Elutions with Hexane-EtOAc (8:2) gave fractions that contained compound **3** along with minor impurities while EtOAc-MeOH (9:1 and 8:2) eluted a brown residue that contained mainly compound **4**. MeOH was added to the brown residue. The yellow solid that separated was filtered and purified by recrystallization from hot MeOH.

Analytical data

Compound **4**: Yellow solid (75 mg), m.p. 263–64°C; $R_f = 0.5$ in CHCl_3 -MeOH (4:1); $[\alpha]_D^{22+27.5^\circ}$ (MeOH, $c = 0.2$); UV-Vis (λ_{max} , nm): 376, 332 (sh), 258; AlCl_3 : 438, 332 (sh), 265; AlCl_3 +HCl: 430, 338, 265; NaOAc: 380, 332 (sh), 258; NaOAc+ H_3BO_3 : 378, 332 (sh), 258; NaOMe: 416, 332 (sh), 263; IR (KBr): 3420, 3370, 2380, 2370, 1658, 1605, 1560, 1520, 1435, 1340, 1275, 1220, 1200, 1180, 1125, 1040, 960, 875, 805 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$): δ 3.59 (2H, m, H-6'), 3.86 (3H, s, 4', -OMe), 3.90 (3H, s, 5-OMe), 4.25 (1H, m, H-4''), 4.81 (1H, m, H-3''), 4.94 (1H, m, H-3''), 5.07 (1H, H-5''), 5.23 (1H, d, $J = 4.5$ Hz, H-1''), 6.58 (1H, s, H-6), 7.07 (1H, d, $J = 8.6$ Hz, H-5'), 7.83 (1H, d, $J = 2.2$ Hz, H-2'), 7.88 (1H, dd, $J = 2.2$ and 8.6 Hz, H-6'), 9.18 (1H, s, 8-OH), 9.52 (1H, brs, 3-OH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 55.49 (4'-OMe), 56.59 (5-OMe), 61.17 (C-6''), 70.11 (C-4''), 74.14 (C-2''), 76.18 and 77.18 (C-3'' and C-5''), 95.21 (C-1''), 103.15 (C-6), 103.32 (C-10), 111.48 (C-2'), 115.39 (C-5'), 120.16 (C-6'), 123.55 (C-1'), 124.35 (C-8), 135.91 (C-3), 146.98 (C-2), 148.17 (C-3'), 149.34 (C-4'), 155.78 (C-5), 156.04 (C-7), 157.58 (C-9), 176.28 (C-4); EI-MS: m/z (%) 484 (M^+ , 2), 376 (3), 360 (15), 347 (24), 346 (100), 331 (25), 317 (10), 303 (10), 275 (7), 260 (5), 243 (4), 232 (4), 215 (4), 201 (4), 181 (9), 164 (7), 151 (10), 143 (8), 126 (6), 116 (3), 73 (5).

Compound **5**: Obtained as yellow solid on hydrolysis of **4** with 7% ethanolic H_2SO_4 . UV-Vis (λ_{max} , nm): 380, 335 (sh), 262; AlCl_3 : 452, 337 (sh),

267; AlCl_3 +HCl: 428, 334, 261; NaOAc: 383, 335 (sh), 271; NaOAc+ H_3BO_3 : 389, 335 (sh), 282; NaOMe: 419, 332 (sh), 263; IR (KBr): 3373, 3097, 1658, 1617, 1562, 1534, 1502, 1445, 1362, 1340, 1222, 1215, 1040, 875, 808 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$): δ 3.85 (3H, s, 4' -OMe), 3.91 (3H, s, 5-OMe), 6.56 (1H, s, H-6), 7.10 (1H, d, $J = 8.7$ Hz, H-5'), 7.77 (1H, d, $J = 2.1$ Hz, H-2'), 7.84 (1H, dd, $J = 2.1$ and 8.7 Hz, H-6'), 8.77 (1H, s, 7-OH), 9.34 (1H, s, 8-OH), 9.44 (1H, brs, 3-OH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 55.28 (5-OMe), 55.51 (4'-OMe), 103.28 (C-6), 103.32 (C-10), 111.66 (C-2'), 114.67 (C-5'), 119.93 (C-6'), 123.55 (C-1'), 125.89 (C-8), 135.81 (C-3), 146.06 (C-2), 146.54 (C-3'), 149.33 (C-4'), 152.12 (C-7), 153.47 (C-5 and C-9), 176.0 (C-4); EI-MS: m/z (%) 346 (M^+ , 100), 331 (27), 318 (5), 317 (6), 303 (10), 275 (5), 260 (4), 247 (4), 232 (3), 181 (6), 164 (5), 151 (10), 143 (9), 130 (4), 121 (3).

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