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Two new flavonoids from the seeds of *Zanthoxylum alatum* Roxb.

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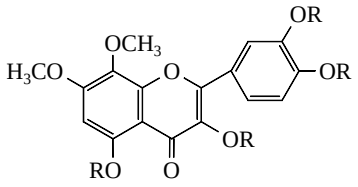
From the seeds of *Zanthoxylum alatum* Roxb. (Rutaceae) two new flavonoids, named zanthoxyl flavone and geranioloxy-alatum flavone, have been isolated. Their structures were established as 3,5,3′,4′-tetrahydroxy-7,8-dimethoxy flavone and 3,5,3′-trihydroxy-6,7-dimethoxy-4′-(7″-hydroxygeranyl-1″-ether) flavone, respectively, on the basis of structural data analysis and chemical means.

1. Introduction

Among the genus Rutaceae are aromatic, prickly, dioecious or rarely monoecious, mainly pantropical trees or shrubs. About 13 species are recorded in India. *Zanthoxylum alatum* Roxb. (Syn. *Z. armatam* DC: *Z. planispinum* Sieb & Zucc.) is an evergreen shrub with dense foliage, found in the hot valleys of the Himalayas at altidues of 1000–2100 m, in Khasi hills at 600–1800 m and in the eastern ghats of Orissa and Andhra Pradesh at 1200 m. Its seeds are employed as an aromatic tonic in fever and dyspepsia. Due to their deodorant, disinfectant and antiseptic properties, the seeds are used in dental disorders and in lotions against scabies as well as to ward off house-flies [1, 2]. Volatile oil constituents [3], fatty acids [4–7] and a flavonoid (3,5-dihydroxy-7,8,4′-trihydroxy flavone) [8] have been reported from the seeds of *Z. alatum*. We describe in this communication the isolation and structure elucidation of two new flavones from the seeds of the plant.



1. R = H
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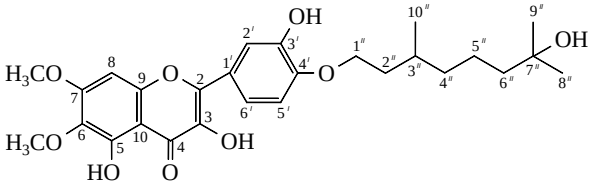

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2. Investigations, results and discussion

The finely powdered seeds of *Z. alatum* were extracted successively with petroleum ether and ethanol (95%). The ethanol extract on subjection to silica gel column chromatography furnished zanthoxyl flavone (**1**) and gerianoloxyalatum flavone (**3**).

The new compound **1**, obtained as golden yellow crystalline product, was shown to be a flavonoid from its colour reactions and UV absorption. Its molecular formula was determined as $C_{17}H_{14}O_8$ by high resolution MS spectrometry and ^{13}C NMR spectra. The UV absorption spectra on addition of NaOMe, $AlCl_3$, $AlCl_3-HCl$, NaOAc and $NaOAc-H_3BO_3$ indicated the presence of C-3 and C-5 hydroxyl groups, B-ring O-dihydroxy and 7-hydroxy substituted group of flavonol. Its IR spectrum exhibited absorption bands at 3412 (free OH) and 1675 (conjugated CO) cm^{-1}. The down field signal in the ^{1}H NMR at δ 11.85, and three other signals at δ 8.84, 8.65 and 8.27, exchangeable in D_2O, supported the presence of four free hydroxyl groups. Acetylation of **1** with acetic anhydride-pyridine yielded a tetraacetate **2** ($C_{25}H_{22}O_{12}$, M^+ m/z 514). The ^{1}H NMR spectrum of **1** showed the presence of two methoxyl groups (δ 3.95 and 3.20 ppm) and four aromatic protons. One meta, ortho-coupled aromatic signal at δ 7.89 (J = 9.5, 2.5 Hz) was assigned to C-6′ protons. A meta-coupled signal at δ 7.76 (J = 2.5 Hz) ppm, was due the C-2′ proton. One ortho-coupled signal was seen at δ 6.97. This was substantiated by the EIMS data in which, besides the molecular ion at m/z 346, an RDA ion peak was observed at m/z 151 resulted from the B-ring having two hydroxyl groups. The other ion peaks appeared at m/z 331 $[M\text{-}Me]^+$, 315 $[M\text{-}OMe]^+$, and 134 $[151\text{-}OH]^+$ supporting the substitution pattern of the molecule provided by the ^{13}C NMR spectrum. The existence of carbon signals at δ 148.88 (C-2), 135.76 (C-3) and 175.90 (C-4) supported a C-3 free hydroxy group of the flavone. The observance of a signal near 95.00 indicated a substituted C-8 position. The ^{13}C NMR values were compared with 7,8,3′,4′-tetrahydroxy flavonol and wightin [9, 10]. The configuration of each carbon was determined by DEPT experiments. Based on these data, the structure of **1** was determined as 3,5,3′,4′-tetrahydroxy-7,8-dimethoxy flavone. This is a new flavone derivative isolated from a plant source.

Compound **3**, obtained as light yellow crystalline mass, also responded positively to the flavonoid tests. Its molecular formula was established as $C_{27}H_{34}O_9$ for flavonol molecule with an oxygenated monoterpenic moiety by high resolution MS and ^{13}C NMR spectra. Its UV absorption indicated a flavonol skeleton with a free 3-hydroxyl group. The UV absorption spectra on addition of various shift reagent supported free hydroxyl groups at the 3 and 5 positions, and bounded 7 and 4′-hydroxy groups. Its IR spectrum exhibited absorptions at 3422 (free OH), 3250 (chelated OH) and 1652 cm^{-1} (conjugated CO). The ^{1}H and ^{13}C NMR spectra of **3** displayed signals both in the upfield and downfield regions. The D_2O exchangeable signals in the ^{1}H NMR at δ 11.71, 7.36 and 6.72, supported the presence of hydroxy groups. The ^{1}H NMR of **3** closely resembled that of **1** except for the appearance of

monoterpenic moiety signals at δ 1.25 (Me-8″, Me-9″), 0.85 (d, J = 6.5 Hz, Me-10″), 4.15 (d, J = 10.5 Hz, CH_2-1″a), 4.07 (d, J = 10.5 Hz, CH_2-1″b) and for remaining methine and methylene protons in between δ 2.82–1.30. The EIMS of **3** showed the RDA fragment ion peaks at m/z 151 and other ion peaks at m/z 329 $[M\text{-}C_{10}H_{21}O_2]^+$, 345 $[M\text{-}C_{10}H_{21}O]^+$, 173 $[C_{10}H_{21}O_2]^+$, 157 $[C_{10}H_{21}O_2]^+$, 143 $[C_9H_{19}O]^+$, 129 $[C_8H_{12}O]^+$, 101 $[C_6H_{13}O]^+$, 87 $[C_5H_{11}O]^+$, 73 $[C_4H_9O]^+$, and 59 $[C_3H_7O]^+$. The presence of the C_{10} unit at 4′ position was deduced from the ^{13}C NMR spectral data in which value of C-4′ shifted to δ 156.34 in comparison to that of **1**. The usual carbon at δ 149.50 (C-2) 135.61 (C-3) and 175.62 (C-4) supported free C-3 hydroxyl group in the flavone: A signal at δ 95.22 and C-9 (156.39) and C-10 (103.36) signal disclosed the unsubstituted nature of C-8. The ^{13}C NMR values were compared with the related flavones patuletin, 6-hydroxy-luteolin derivatives, salvigenin and pectolinarigenin [9, 10]. The signals at δ 62.13 and 68.96 were assigned to the C-1″ and the C-7″ carbinol carbon, respectively. The multiplicities of each carbon was determined by DEPT experiments. Based on the above evidences the structure of **3** was established as 3,5,3′-trihydroxy-6,7-dimethoxy-4′-(7″-hydroxy geranyl-1″-ether) flavone. It was named geranioloxyalatum flavone.

3. Experimental

All melting points were determined on a Perift apparatus in one end open capillaries and are uncorrected. UV and IR spectra were recorded on Beckmann DU-64 and Perkin-Elmer-882 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were screened in $CDCl_3$ on a Brucker AC 300 F spectrometer at 300 MHz and 75 MHz, respectively, with TMS as internal standard. The chemical shifts are recorded in δ (ppm) values. EIMS spectra were obtained on a Jeol-JMS D 300/JMA-2000 mass spectrometer operating at 70 eV.

3.1. Plant material

Seeds of *Z. alatum* were purchased from the local market Khari Baowli, Delhi and identified by Dr. M. P. Sharma, Deptt. of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen of the seeds is preserved in the Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard.

3.2. Extraction, isolation and purification of the compounds

The dried, powdered and defatted seeds (1.5 kg) were Soxhlet extracted exhaustively with EtOH (95%). The concentrated extract (300 g) was dissolved in minimum amount of MeOH and adsorbed on silica gel (60–120 mesh) to form a slurry. The air-dried slurry was chromatographed over a silica gel column prepared in petroleum ether (b.p. 60–80 °C). The column was eluted successively with petroleum ether, petroleum ether/$CHCl_3$ (9:1, 7:3, 1:1, 3:7, 1:4), $CHCl_3$, $CHCl_3$/MeOH (9.5:0,5, 9:1, 4:1, 3:1, 1:1, 1:3) and MeOH, to isolate the following compounds.

3.2.1. Zanthoxyl flavone (**1**)

Elution of the column with $CHCl_3$/MeOH (3:1) afforded golden crystals of 1, recrystallized from MeOH, 75 mg, positive to $FeCl_3$ and Mg/HCl m.p. 156–157 °C, UV (λ_{max}, MeOH) 271, 350 nm (log Σ 4.2, 3.8, 3.9), λ_{max} (MeOH + NaOMe) 255, 273, 397 nm, ($AlCl_3$) 270, 395, (MeOH + $AlCl_3$ + HCl) 272, 285 nm, λ_{max} (MeOH/NaOAc, and NaOAc/H_3BO_3) 271, 365, nm. IR (KBr, ν, cm^{-1}) 3412, 1675, 1611, 1576, 1508, 1469, 1443, 1336, 1259, 1196, 1159, 1115, 1005, 806. ^1H NMR δ 7.89 (1 H, dd, J = 9.5, 92.5 Hz, H-6′) 7.76 (1 H, d, J = 2.5 Hz, H-2′), 6.97 (1 H, d, J = 9.5 Hz, H-5′), 6.42 (1 H, brs, H-6), 3.95 (3 H, brs, OMe), 3.20 (3 H, brs, OMe), 11.85 (1 H, brs, D_2O exchangeable), 8.84 (1 H, brs, D_2O exchangeable, OH), 8.65 (1 H, brs, D_2O exchangeable, OH), 8.27 (1 H, brs, D_2O exchangeable, OH). ^{13}C NMR δ 148.88 (C-2), 135.76 (C-3), 175.90 (C-4), 152.84 (C-5), 94.08 (C-6), 125.84 (C-7), 125.82 (C-8), 143.81 (C-9), 103.24 (C-10), 123.61 (C-1′), 110.54 (C-2′), 145.72 (C-3′), 146.07 (C-4′), 120.23 (C-5′), 114.51 (C-6′), 55.86 (OMe), 55.28 (OMe) HR EIMS m/z 346.2905 ($C_{17}H_{14}O_8$ requires 346.2896). EIMS m/z (rel. int.) 346 $[M]^+$ (99.8), 331 (100), 315 (12.6), 194 (3.0), 163 (5.1), 151 (26.3), 134 (16.3). Acetylation of compund **1**: Compound **1** (10 mg) was acetylated with Ac_2O/pyridine to get the tetra acetylated derivative **2**, m.p. 133–134 °C, EIMS m/z 514 $[M]^+$ $[C_{25}H_{22}O_{11}]$ (11.2).

3.2.2. Geranioloxyalatum flavone (**3**)

Further elution of the column with $CHCl_3$/MeOH (1:1) furnished light yellow coloured crystals of **3**, recrystallised from MeOH, 50 mg m.p. 190–191 °C. UV (CH_3OH, λ_{max}) 205, 220, 272, 330 nm (log Σ 6.5, 4.6, 5.1, 5.5), λ_{max} (MeOH/$AlCl_3$) 205, 222, 270, 370 nm, λ_{max} (MeOH + $AlCl_3$ + HCl) 205, 221, 273, 350 nm, λ_{max} (MeOH/NaOMe) 205, 220, 275, 380 nm. λ_{max} (MeOH + NaOAc; MeOH + NaOAc/H_3BO_3) 205, 222, 272, 331 nm. IR (KBr, ν_{max}) 3422, 3250, 2928, 2855, 1652,, 1482, 1317, 1259, 1175, 1032, 922, 836, 795, 742 cm^{-1}. ^1H NMR δ 7.89 (1 H, dd, J = 9.5, 2.5 Hz, H-6′), 7.18 (1 H, d, J = 2.5 Hz, H-2′), 6.97 (1 H, d, J = 9.5 Hz, H-5′), 6.39 (1 H, brs, H-8′), 4.15 (1 H, d, J = 10.5 Hz, H_2-1″b), 4.07 (1 H, d, J = 10.5 Hz, H2-1″b), 3.96 (3 H, brs, OMe), 3.90 (3 H, brs, OMe), 2.82 (1 H, m, H-3′), 2.39 (2 H, m, H_2-6″), 2.01 (2 H, m, H_2-2″), 1.61 (2 H, m, H_2-5″), 1.30 (2 H, m, H_2-4″), 1.25 (6 H, brs, Me-8″, Me-9″), 0.85 (3 H, d, J = 6.5 Hz, Me-10″), 11.71 (1 H, brs, D_2O exchangeable, OH), 7.36 (1 H, brs, D_2O exchangeable, OH), 6.72 (1 H, brs, D_2O exchangeable, OH). ^{13}C NMR ($CDCl_3$): δ 149.50 (C-2), 135.61 (C-3), 175.62 (C-4), 161.30 (C-5), 130.09 (C-6), 159.50 (C-7), 95.22 (C-8), 156.39 (C-9), 103.36 (C-10), 123.1 (C-1′), 114.26 (C-2′), 145.50 (C-3′), 156.36 (C-4′), 114.26 (C-5′), 129.6 (C-6′), 62.13 (C-1″), 32.00 (C-2″), 34.12 (C-3″), 22.68 (C-4″), 14.20 (C-5″), 29.67 (C-6″), 68.96 (C-7″), 29.78 (C-8″), 29.78 (C-9″), 14.20 (C-10″), 56.48 (OMe), 55.49 (OMe). HR EIMS m/z: 502.5505 ($C_{27}H_{34}O_9$ requires 502.5566), EIMS m/z (rel. int.) 502 $[M]^+$ (3.1), 345 (100), 329 (98.9), 315 (12.6), 173 (6.3), 157 (40.1), 152 (26.3), 143 (32.7), 139 (14.1), 129 (6.1), 111 (5.2), 101 (3.1), 87 (3.4), 73 (3.6), 69 (18.3), 59 (10.2), 55 (4.5).

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