New biologically active limonoids and flavonoids from *Aphanamixis polystachya*

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Evidence is presented for the structures of three new compounds: amoorinin-3-O-a-L-rhamnopyranosyl-(1\(\rightarrow\)6)-a-D-glucopyranoside, 1; 8-methyl-7, 2', 4'-tri-O-methylflavanone-5-O-a-L-rhamnopyranosyl-(1\(\rightarrow\)4)-a-D-glucopyranoside, 2 and 8-C-methyl-5, 7, 3', 4'-tetrahydroxyflavone-3-O-a-L-arabinopyranoside, 3 which occurs together with known substance aphanamixinin from the roots of *A. polystachya*.

Biological screening of the compounds reveal antifeedant and antifungal activities.

*Aphanamixis polystachya* (Meliaceae) is an Indian medicinal plant used in our indigenous system of medicine as an astringent and to cure spleen and liver disorders, tumors, abdominal diseases and rheumatism. Earlier studies on this plant had disclosed the presence of limonoids, sterols, saponin and polyphenolic compounds. The present communication deals with the isolation and characterization of three new compounds limonoid and flavonoids 1-3 along with aphanamixinin from its roots, the structures of which were confirmed by its spectral and chemical methods. In the preliminary investigation, the glycosides displayed antifeedant and antifungal activities.

Compound 1 gave positive Molisch’s test for a glycoside and belongs to the limonoid class, based on the Ehrlich test. Accordingly, on acid hydrolysis it yielded amoorinin (spectral data, chemical reactions, mp, mmp and co-TLC). The sugars were identified as glucose and rhamnose (co-PC). Permethylation followed by acid hydrolysis (5% HCl-MeOH) afforded 2,3,4-tri-O-methyl derivatives of D-glucose and L-rhamnose and amoorinin. Partial acid hydrolysis of compound 1 yielded L-rhamnose followed by D-glucose indicating the presence of L-rhamnose as the terminal sugar. Takadiastase hydrolysis of 1 afforded L-rhamnose and a prolimonoid glycoside. The latter on almond enzymatic hydrolysis gave D-glucose. The intersugar configurations were deduced from \(^1\)H- and \(^13\)C NMR which indicated the \(\beta\)-D-pyranosyl configuration for glucose and the \(\alpha\)-L-pyranosyl configuration for rhamnose. Thus 1 is amoorinin-3-O-a-L-rhamnopyranosyl-(1\(\rightarrow\)6)-\(\beta\)-D-glucopyranoside.

Compound 2 gave positive tests for a flavanone glycoside. On acid hydrolysis compound 2 yielded an aglycone, glucose and rhamnose. The \(^1\)H NMR spectrum of the aglycone displayed signals for three methoxyls, a methyl and an OH. The aglycone did not give a bathochromic shift of longest wavelength with NaOAc but the same was observed with AICI\(_3\) indicating the presence of methoxyl at C-7 and OH group at C-5 positions respectively. KOH degradation of the aglycone afforded 6-methyl-5-O-methylphloroglucinol and 2,4-di-O-methylbenzoic acid. On demethylation (48% HBr-AcOH) the aglycone afforded 8-methyl-5,7,2',4'-tetrahydroxyflavone (mmp and co-TLC). Thus the aglycone was 8-methyl-7,2',4'-tri-O-methyl-5-hydroxyflavone. Permethylation of 2 followed by treatment with 5% HCl-MeOH afforded the above reported aglycone, 2,3,4-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose. Partial acid hydrolysis of 2 yielded L-rhamnose first followed by D-glucose indicating L-rhamnose was the terminal sugar.
configurations were deduced from $^1$H- and $^{13}$C NMR spectra which confirmed the β-D-pyranosyl configuration for glucose and the α-L-pyranosyl configuration for rhamnose. Thus 2 is 8-methyl-7,2',4'-tri-O-methylflavanone-5-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Compound 3 responded positively test for a flavone glycoside. Acid hydrolysis of the compound 3 yielded 8-C-methylquercetin (mp, spectral data, mmp and co-TLC) and L-arabinose (co-PC). Methylation followed by acid hydrolysis of the compound 3 afforded 8-C-methylquercetin-5,7,3',4'-tetramethylether (mp, spectral data, mmp and co-TLC) and 2,3,4-tri-O-methyl-L-arabinose. The sugar configuration was deduced from $^1$H- and $^{13}$C NMR spectra which confirmed the α-L-pyranosyl arabinose. Thus 3 is 8-C-methyl-5,7,3',4'-tetrahydroxyflavone-3-O-α-L-arabinopyranoside.

**Antifeedant activity**

The antifeedant activity was done by dual choice leaf disc bioassay method. Azadirachtin-A was used as a reference antifeedant. The freshly moulted adult beetles of Henosepilachna vigintioctopunctata were collected from brinjal (Solanum melongena) fields. The insects were reared in plastic containers on Solanum nigrum leaves as food. The fourth instar larva of Spodoptera litura collected from Ricinus communis fields was kept in the laboratory on castor leaves. The results of the present study against H. vigintioctopunctata and S. litura display that the antifeedant activity of the compound 1 was concentration dependant with both insect species and shows the appreciable antifeedant activity as compared with azadirachtin-A. Other compounds indicate less or not considerable activity.

**Antifungal activity**

The compounds were screened for its antifungal activity against the fungi Aspergillus niger, Aspergillus fumigatus, Candida albicans and Rhizopus oryzae at 10, 25 and 50 ppm concentrations by filter paper disc method. A commercial fungicide griseofulvin was also tested under similar condition for comparison. The results showed that the compounds 1 and 2 exhibited acceptable antifungal activity against A. niger and C. albicans while the compound 3 displayed moderate activity against C. albicans and R. oryzae as compared with griseofulvin.

**Experimental Section**

IR spectra (KBr) were recorded on a Shimadzu 8201 PC FT; UV spectra on a Perkin-Elmer spectrophotometer; $^1$H NMR spectra at 200 MHz in CDC$_3$ + DMSO-$d_6$ with TMS as an internal standard employing the FT mode; and $^{13}$C NMR spectra on a Brucker WM 400 at 90.56 MHz in CDC$_3$+DMSO-$d_6$ using TMS as an internal standard. The mass spectra were scanned on a Jeol D-300 spectrometer. All mps are uncorrected. TLC were carried out on silica gel G (Merck).

**Plant material.** The roots of A. polystachya were procured from the United Chemicals and Allied Products, Kolkata (India) and identified by the Botanical Survey of India, Allahabad, Circle, Allahabad (India). Reference samples have been deposited at the ethnomedicinal collection of the supplier.

**Isolation.** The air-dried and pulverized plant material (2 kg) was extracted with hot ethanol on a water-bath for about 200 hr (30 days). The ethanol from the percolates (15 L) was removed under reduced pressure to make the volume 300 mL and kept at room temperature for a few days which deposited a dirty white
product. This was separated by filtration, passed through a column of neutral alumina, eluted with benzene and characterized as amoorinin. The ethanol from the remaining volume was removed to yield a residue. The residue was extracted with pet ether, benzene, chloroform, ethyl acetate and methanol respectively. The chloroform extract was column chromatographed Si-gel CC with methanol: chloroform (5:5, v/v) to afford 1 (720 mg). The ethyl acetate extract was column chromatographed on Si-gel with chloroform:methanol (3:7, v/v) and (2:8, v/v) to give 2 (630 mg) and 3 (590 mg).

Compound 1. Colourless crystals, m.p. 189-90°C; IR bands (KBr, cm⁻¹): 3448 (hydroxyl), 1732 (δ lactone), 1726 (ester), 1639 and 819 (trisubstituted double bond), 1509 and 872 (furan), 1287 (epoxide), 908 (exocyclic methylene group) and 822 (glycoside); 1H NMR: δ 0.96, 0.99, 1.09, 1.12 (each s, 12H, 4 × CH3), 1.18-1.92 (complex pattern CH2 and CH3), 1.24 (d, J = 6 Hz, rhamn Me), 3.62 (d, J = 6Hz, 1H, H-3), 3.78 (s, 3H, CO₂CH₃), 3.80 (s, 1H, H-15), 4.50 (d, J = 2Hz, rhamn H-1), 4.92 and 5.09 (each s, 2H, C=CH₂), 5.13 (d, J = 7 Hz, gluc H-1), 5.63 (s, 1H, H-17), 5.92 (dd, J = 6 and 10 Hz, 1H, H-2), 6.13 (d, J = 10 Hz, 1H, H-1), 6.39, 7.22 and 7.39 (each m, 3H, furan-H).

Acid hydrolysis. 1 (400 mg) was hydrolysed with 7% H₂SO₄ (30 mL) to give genin, L-rhamnose and D-glucose. The genin, mp 172-74°C, [α]D-105° (c 0.14 CHCl₃), C₂₅H₃₂O₇ (M⁺470); IR: 3453, 1736, 1728, 1639, 1508, 1289, 908, 878, 824; 1H NMR: 0.93, 0.97, 1.00, 1.08, 1.16-1.90, 3.19 (s, 1H, OH), 3.65, 3.79, 3.82, 4.90, 5.08, 5.61, 5.95, 6.15, 6.39, 7.20, 7.37; 13C NMR: δ 157.2 (C1), 125.2 (C2), 74.3 (C3), 38.4 (C4), 42.4 (C5), 33.1 (C6), 173.7 (C7), 140.6 (C8), 49.5 (C9), 42.9 (C10), 29.8 (C11), 39.2 (C12), 47.4 (C13), 80.5 (C14), 68.9 (C15), 174.3 (C16), 78.9 (C17), 9.9 (C18), 21.6 (C19), 120.5 (C20), 142.3 (C21), 110.3 (C22), 142.7 (C23), 26.6 (C28), 15.6 (C29), 114.9 (C30), 51.7 (C33); MS: 470 (M⁺), 457, 452, 437, 411, 403, 402, 389, 263, 245 was identical to amoorinin (mmp and co-TLC).

Permethylation followed by acid hydrolysis. Permethylation of 1 (100 mg), according to the method of Hakomori, followed by treatment with 5% HCl-MeOH afforded amoorinin (mmp and co-TLC), 2,3,4-tri-O-methyl derivatives of D-glucose and L-rhamnose respectively showing the rutinoside moiety.

Partial acid hydrolysis. The partial acid hydrolysis of 1 (70 mg) yielded L-rhamnose first followed by D-glucose confirming the presence of L-rhamnose as the end sugar.

Takadiastase enzyme treatment. 1 (70 mg) on treatment with takadiastase enzyme afforded L-rhamnose and a prolimonoid glycoside which on further treatment with almond enzyme gave amoorinin and D-glucose supporting the presence of L-glucose and L-rhamnose in 1.

Compound 2. Light yellow crystals, mp 169-70°C; UV max (MeOH): 287, 329(sh); +AlCl₃: 288, 332(sh); +NaOAc: 290, 330(sh) nm; IR bands: 3446 (hydroxyl), 2933 (C-methyl), 2872 and 1168 (methoxyl), 1682, 1493, 1354, 1209, 1152, 905, 812 (furananone nucleus) and 822 (glycoside); 1H NMR(δ) 1.22 (d, J = 6.3Hz, rhamn CH₃), 2.13 (s, 3H, C-8 Me), 2.82 (m, 2H, H-3), 3.81, 3.92 and 3.99 (each s, 2H, 3 × CH₃), 4.45 (d, J = 7.6 Hz, 1H, gluc H-1), 4.56 (d, J = 7.5 Hz, 1H, gluc H-1), 5.12 (d, J = 1.6 Hz, 1H, rhamn H-1), 5.38 (dd, J = 10 and 4 Hz, 1H, H-2), 6.53 (s, 1H, H-6), 7.19 (d, J = 2.5 Hz, 1H, H-3'), 7.32 (dd, J = 9.5 and 2.5 Hz, 1H, H-5'), 7.43 (d, J = 9.5 Hz, 1H, H-6'); 13CNMR: δ 83.49 (C2), 41.48 (C3), 195.9 (C4), 102.89 (C4a), 164.08 (C5), 97.16 (C6), 168.87 (C7), 106.31 (C8), 165.79 (C8a), 128.26 (C1'), 156.97 (C2'), 115.58 (C3'), 159.63 (C4'), 104.15 (C5'). 130.46 (C6'), 56.50, 56.62, 56.69 (4 × OCH₃), 9.98 (CH₃), rhamn: 99.96 (C1), 72.32 (C2), 70.69 (C3), 75.73 (C4), 67.96 (C5), 17.32 (C6), gluc: 94.97 (C1), 74.86 (C2), 76.48 (C3), 69.58 (C4), 76.79 (C5), 65.87 (C6), gluc: 103.06 (C6), 74.86 (C6), 76.56 (C3), 69.85 (C4), 76.53 (C5), 62.03 (C6).

Acid hydrolysis. 2 (300 mg) was refluxed with 7% H₂SO₄ (20 mL) for 5 hr. The resulting aglycone (180 mg) was separated out. The aqueous layer was found to contain L-rhamnose and D-glucose. Aglycone: Light yellow crystals; mp 203-04°C; C₆H₁₂O₆ (M⁺344); UV max (MeOH): 299 and 330 (sh); +AlCl₃: 315, 332 (sh); +NaOAc: 288, 332 (sh) nm; IR bands (KBr): 3456 (hydroxyl), 2931, 2869, 1685, 1498, 1359, 1197, 1172, 1155, 908, 814; 1H NMR: δ 2.12, 2.79, 3.82, 3.99, 4.01, 5.63, 6.48, 7.18, 7.33, 7.48, 13.22 (s, 1H, 1 × OH, exchangeable on D-O shake); 13C NMR: δ 80.46 (C2), 41.29 (C3), 196.09 (C4), 102.99 (C4a), 163.58 (C5), 97.18 (C6), 168.78 (C7), 106.16 (C8), 165.82 (C8a), 128.16 (C1'), 156.50 (C2'), 115.29 (C3'), 159.69 (C4'), 104.88 (C5'), 130.43 (C6'), 56.47, 56.88, 56.93 (3 × OCH₃); MS: 344 (M⁺), 343, 329, 313, 301, 195, 181, 180.

Alkaline degradation. The aglycone (70 mg) in EtOH (18 mL) and 50% KOH (35 mL) was refluxed...
for approximately 8 hr and worked up as usual to give 6-methyl-5-O-methylphloroglucinol and 2,4-di-O-methylbenzoic acid.

**Demethylation.** The aglycone (80 mg) in AcOH (12 mL) was refluxed with 48% HBr (10 mL) and worked up as usual to afford 8-methyl-7,2',4'-tri-O-methyl-5-hydroxyflavanone.

**Permethylation followed by acid hydrolysis of 2.** Permethylation of 2 (100 mg) followed by its hydrolysis (5% HCl-MeOH) as done earlier yielded 2,3,4-tri-O-methyl-D-glucose; 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose and an aglycone (mmp and co-TLC).

**Partial acid hydrolysis.** Partial acid hydrolysis of 2 (50 mg) was performed as usual to give L-rhamnose first followed by D-glucose indicating L-rhamnose as the terminal sugar.

**Compound 3.** Yellow crystals, mp 212-13°C, UV max (MeOH): 258, 358; +271, 359; +AICI 3 +HCl: 260, 360; +NaOAc: 263, 386; +NaOAc+H3BO3: 265, 387 nm; IR bands (KBr): 3452 (hydroxyl), 1649 and 1609 (α, β unsaturated >CO), 2926 (C-Me), 1478, 1362, 1218, 1210, 1158, 911 (substituted flavone nucleus), 822 (glycoside), 723, 712; 1H NMR: (δ) 2.12 (s, 3H, 1-x CH3), 4.43 (d, J=6.5 Hz, 1H, arab H-1), 6.39 (s, 1H, H-6), 7.63 (d, J=9.5 Hz, 1H, H-5'), 7.79 (dd, J=9.5 and 2.5 Hz, 1H, H-6'), 13C NMR: (δ) 158.83 (C2), 134.91 (C3), 177.5 (C4), 104.54 (C4a), 162.3 (C5), 98.97 (C6), 164.35 (C7), 94.95 (C8), 156.52 (C8a), 121.83 (C1'), 115.1 (C2'), 144.69 (C3'), 149.05 (C4'), 116.32 (C5'), 121.6 (C6'), arabinose: 104.2 (C1), 81.03 (C2), 72.48 (C3), 66.97 (C4), 65.06 (C5).

**Acid hydrolysis.** 3 (300 mg) was hydrolysed with 7% H2SO4 (20 mL) as usual to yield 8-C-methylquercetin (mmp and co-TLC) and L-arabinose.

**Permethylation followed by acid hydrolysis.** 3 (100 mg) was permethylated by the method of Hakomori as usual followed by 5% HCl-MeOH hydrolysis to afford 8-C-methylquercetin-5,7,3',4'-tetramethylether (mmp and co-TLC) and 2,3,4-tri-O-methyl-L-arabinose.

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**References**