Phenolic constituents from the galls of
Pistacia integerrima Stewart

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Phytochemical investigation of the galls of Pistacia integerrima Stewart (Pistaceae) has yielded three new phytoconstituents characterized as 14′-dihydroxy benzoate (pistiphloro-glucinyl ester) 2, 4′-phenoxy-n-butyl-1′-(3-oxy-5-hydroxy) benzoic acid (pistaciaphenyl ether) 3 and 3′,1,3-dihydroxy-5-phenoxy-1′,5′-dimethoxybenzene (pistici-phloro-glucinyl ether) 4 along with the known compound stigmata-5-en-3β-ol (β-sitosterol) 1. The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

Keywords: Pistacia integerrima Stewart, Pistaceae, galls, phenolic constituents

Pistacia integerrima Stewart (Pistaceae), commonly known as Kakra Shangi, is a moderate-sized deciduous tree, up to 18 m in height, with a short stout bole found in the Himalayas from Indus to Kumaon. Hard, horn-shaped, rugose, hollow gall like excrescences are formed on the leaves and petioles of the plant by an insect of the family Pemphigidae, species. Dry powdered galls have a very astringent and slightly bitter taste and terebinthine odour. The galls are aromatic, astringent and expectorant and are valued in Indian medicine as a remedy for asthma, phthisis and other ailments of the respiratory tract; they are also used to cure dysentery, chronic bronchitis, hiccup, vomiting of children, skin diseases, psoriasis, fever, to increase appetite and to remove bed humors. Sushruta prescribed the galls in combination with other drugs for the treatment of snake bite and scorpion-sting. P. integerrima has depressant action on the central nervous system, analgesic and anti-inflammatory and hypouricemic activities. Monoterpenes, triterpenoids, sterols, dihydromalvalic acid and flavonoids have been reported from the different parts of Pistacia species.

This paper deals with the isolation and characterization of three new phenolic constituents from the galls of P. integerrima.

Results and Discussion

Compound 2, named pistiphloro-glucinyl ester, was obtained as a colourless amorphous mass from chloroform-methanol (99:5:0.5) eluants. It gave green colour with ferric chloride indicating phenolic nature of the compound. Its IR spectrum displayed characteristic absorption bands at 3450 (OH), 1725 (COO), 1640, 1550 and 980 cm⁻¹ (aromatic ring). It had a molecular ion peak at m/z 442 corresponding to the molecular formula C_{27}H_{38}O_5. The base peak was generated at m/z 153 [(OH)C_{12}H_{20}COO]⁻ due to dihydroxy benzoic acid moiety. The ¹H NMR spectrum of 2 showed a three-proton broad multiplet centered at δ 8.21 assigned to aromatic protons H-4, H-2" and H-6". Two multiplets at δ 7.89 and 7.16, integrating for two protons each, and a one-proton multiplet at δ 6.89 were ascribed to the remaining aromatic protons. Four one-proton doublets at δ 5.43 (J=7.29 Hz), 4.31 (J=7.08 Hz) and at δ 4.26 (J=7.05 Hz) and 4.04 (J=7.2 Hz) were associated with C-1' and C-14' oxygenated methylene protons. The remaining methylene protons appeared at δ 2.98 – 1.28. The ¹³C NMR spectrum of 2 exhibited signals at δ 171.34 (C-7) for ester carbon, aromatic carbons between δ 164.06 – 114.05 and oxygenated methylene carbons at δ 64.08 (C-1') and 63.15 (C-14'). The remaining methylene carbons resonated from δ 33.85 to 22.16. The HMBC of 2 showed correlations of C-7 with H-2/H-6 and H-2" and C-14' with H-2"/H-6". On the basis of these evidences, the structure of 2 has been established as 14′phenoxytetradecanyl 3,5-dihydroxybenzoate. It is an unreported aromatic ester isolated from Pistacia or other species for the first time.

Compound 3, designated as pistaciaphenyl ether,
was obtained as a brown coloured amorphous mass from chloroform-methanol (49:1) eluants. It gave effervescence with sodium bicarbonate and responded positively to ferric chloride test indicating phenolic nature of the compound. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3500 cm\(^{-1}\)), carboxylic group (3390, 1690 cm\(^{-1}\)) and aromatic ring (1575, 990 cm\(^{-1}\)). Its molecular formula was established as C\(_{17}\)H\(_{18}\)O\(_5\) (M\(^+\) m/z 302) on the basis of the mass and \(^{13}\)C NMR spectra. The base peak generated at m/z 153 [HOC\(_6\)H\(_3\)(O)COOH]\(^+\) due to cleavage of O-CH\(_2\) linkage suggested dihydroxy substituted benzoic acid in the molecule. The \(^1\)H NMR spectrum of 3 showed a three-proton broad multiplet at \(\delta\) 8.23 assigned to aromatic H-4, H-2'' and H-3''' protons. Two one-proton double doublets at \(\delta\) 7.58 (J=2.3, 2.7 Hz) and 7.46 (J=2.3, 2.7 Hz) were ascribed to meta-coupled H-2 and H-6, respectively. Two multiplets at \(\delta\) 7.11 (2H) and 6.92 (1H) were attributed to the remaining aromatic protons. Three one-proton double doublets at \(\delta\) 4.28 (J=7.02, 7.08 Hz), 4.23 (1H, dd, J=7.08, 7.08 Hz) and at \(\delta\) 4.16 (J=7.17, 8.37 Hz) and a doublet at \(\delta\) 4.01 (J=6.61 Hz) were accounted to oxygenated C-1' and C-4' protons. Two multiplets at \(\delta\) 1.36 (2H) and 1.32 (2H) were ascribed to C-2' and C-3' methylene protons. The \(^{13}\)C NMR spectrum of 3 exhibited aromatic carbons in the range \(\delta\) 165.80 – 108.50, carboxylic carbon at \(\delta\) 182.11 and oxygenated methylene carbons at \(\delta\) 64.54 (C-1') and 63.89 (C-4') and other methylene carbons at \(\delta\) 33.63 (C-2') and 33.58 (C-3'). The HMBC spectrum of 3 showed correlations of C-7 with H-2/H-6; C-3 with H-2/H-4 and H-2'; and C-1'' with H-2''/H-6'' and H-2'4. On the basis of these spectral data analyses, the structure of 3 has been characterized as 4'-phenoxy-n-butyl-1'-(3-oxyl-5-hydroxy)benzoic acid. This is an unreported aromatic ether isolated from natural or a synthetic source.

Compound 4, named pisticiphloro-glucinyl ether, was obtained as a brown coloured amorphous mass from chloroform-methanol (19:1) eluants. It responded positively to ferric chloride test of phenols. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3490, 3360 cm\(^{-1}\)). Its molecular formula was established as C\(_{18}\)H\(_{24}\)O\(_{5}\) (M\(^+\) m/z 262) on the basis of the mass and \(^{13}\)C NMR spectra. Generation of a prominent ion peak at m/z 153 [C\(_2\)-O fission]\(^+\) suggested that the compound was a trihydroxybenzene. Its \(^1\)H NMR spectrum displayed two three-proton each broad signals at \(\delta\) 7.15 and 7.13 which were attributed to the aromatic protons. A six-proton broad signal at \(\delta\) 3.82 was ascribed to methoxy protons. The \(^{13}\)C NMR spectrum of 4 showed aromatic carbon signals between \(\delta\) 166.64 and 132.26. The methoxy carbons appeared at \(\delta\) 51.20 and 51.16. The HMBC spectrum of 4 exhibited interactions of C-1' with OMe and H-2'/H-6'; and C-5/C-3' with H-4/H-6 and H-2'/H-4'. On the basis of these evidences, the structure of 4 has been characterized as 3'-1,3-dihydroxy-5-phenoxy)-1',5'-dimethoxybenzene. This is a new diphenolic ether isolated from natural or a synthetic source for the first time.

The structure of the known compound 1, obtained from chloroform eluants, has been characterized as stigmata-5-en-3ß-ol (ß-sitosterol)\(^{18}\) on the basis spectral data analysis.

**Experimental Section**

**General experimental procedure**

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). \(^1\)H (300 MHz) and \(^{13}\)C NMR (75 MHz) spectra were recorded by Bruker spectropin NMR instrument in CDC\(_3\) using TMS as internal standard. EIMS were obtained at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed over silica gel (Merck, 60-120 mesh) and thin layer chromatography plates were run on silica gel G-coated TLC plates (Merck).

**Plant Material:** The galls of *P. integerrima* (3 kg) were obtained from Khari Baoli market, Delhi and identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen was deposited in the herbarium of the Faculty of
Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India.

**Extraction and isolation:** The air-dried galls (3 kg) of *P. integrerrima* were coarsely powdered and exhaustively extracted in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated to obtain a dark viscous mass (350 g). Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column chromatography after being dissolved in a little quantity of methanol for preparation of slurry. The slurry was air-dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (90:10, 75:25, 50:50 and 25:75), pure chloroform and finally mixture of chloroform and methanol (99.5:0.5, 99:1, 98:2, 95:5, etc.). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same Rf values) were combined and crystallized. The isolated compounds were recrystallized to get the pure compound(s).

The physicochemical and spectral data of the isolated compounds are reported below.

**β-Sitosterol, 1**

Elution of the column with chloroform gave colourless crystals of 1, recrystallized from methanol, 280 mg (0.0093% yield); Rf: 0.56 (toluene); m.p. 137-38°C; [α]D20: +10° (MeOH, 0.05); EIMS: m/z (%): 414 [M]+ (C25H30O) (67.6).

**Pistiphlor-glucinyl ester, 2**

Elution of the column with chloroform-methanol (99.5:0.5) yielded colourless amorphous powder of 2, recrystallized from chloroform: methanol (1:1), 2.5 g (0.083% yield); Rf: 0.41 (chloroform : ethyl acetate:methanol, 9.5:0.5); m.p. 147-49°C; IR (KBr): 3450, 2960, 2845, 1725, 1640, 1550, 1210, 980, 850 cm⁻¹; ¹H NMR: δ 8.21 (3H, brm, H-4, H-2, H-6), 7.89 (2H, m, H-2, H-6), 7.16 (2H, brm, H-3′, H-4′), 6.89 (1H, m, H-4′′), 4.53 (1H, d, J=7.29 Hz, H-2′-a), 4.31 (1H, d, J=7.08 Hz, H2-1′b), 4.26 (1H, d, J=7.05 Hz, H2-14′a), 4.04 (1H, d, J=7.2 Hz, H2-14′b), 1.55 (2H, m, H-2′-c), 1.34 (20H, brs, 10 × CH3), 1.13 (2H, m, H2-13′); ¹³C NMR: δ 149.73 (C-1), 136.98 (C-2), 163.49 (C-3), 142.30 (C-4), 164.06 (C-5), 137.12 (C-6), 171.34 (C-7), 65.08 (C-1′), 63.15 (C-1′′), 33.85 (CH2), 31.47 (CH2), 25.41 (9 × CH2), 22.16 (CH2), 161.23 (C-1″), 125.82 (C-2″, C-6″), 120.12 (C-3″, C-5″), 114.05 (C-4″).

**Pistaciaphenyl ether, 3**

Elution of the column with chloroform-methanol (49:1) gave brown coloured amorphous mass of 3, recrystallized from chloroform : methanol (1:1), 720 mg (0.024% yield); Rf: 0.96 (chloroform:methanol, 7:2:1); m.p.150-52°C; IR (KBr): 3500, 3390, 2950, 2860, 1690, 1650, 1575, 1410, 990, 850 cm⁻¹; ¹H NMR: δ 8.23 (3H, brm, H-4, H-2″, H-3″), 7.58 (1H, dd, J=2.3, 2.7 Hz, H-2), 7.46 (1H, dd, J=2.3, 2.7 Hz, H-6), 7.11 (2H, m, H-3″, H-5″), 6.92 (1H, m, H-4″), 4.28 (1H, dd, J=7.02, 7.08 Hz, H2-1″a), 4.23 (1H, dd, J=7.08, 7.08 Hz, H2-1″b), 4.16 (1H, dd, J=7.17, 8.37 Hz, H2-4″a), 4.01 (1H, d, J=6.61 Hz, H2-4″b), 1.36 (2H, m, H2-2″), 1.32 (2H, m, H2-3″); ¹³C NMR: δ 148.38 (C-1″), 137.02 (C-2″), 165.80 (C-3″), 143.16 (C-4″), 163.21 (C-5″), 137.02 (C-6″), 182.11 (C-7), 64.54 (C-1′″), 33.63 (C-2′″), 33.58 (C-3′″), 63.89 (C-4′″), 162.06 (C-1′″), 136.89 (C-2′″), 120.14 (C-3′″), 108.50 (C-4′″), 120.14 (C-5′″), 137.02 (C-6″). Anal. Calcd: C, 67.5496; H, 5.9602. Found: C, 67.5501; H, 5.9613%. EIMS: m/z (%) 302 [M]+ (C17H13O3) (10.2), 262 (15.7), 198 (26.8), 170 (30.8), 153 (100), 137 (11.5), 125 (25.6), 79 (28.3), 77 (24.2), 43 (78.6).

**Pisticipholoroglucinyl ether, 4**

Elution of the column with chloroform-methanol (19:1) furnished brown coloured amorphous mass of 4, recrystallized from methanol (1:1), 1.7 g (0.057% yield); Rf: 0.88 (chloroform: methanol, 7:3); m.p. 172-75°C; IR (KBr): 3490, 3360, 1645, 1550, 970, 835 cm⁻¹; ¹H NMR: δ 7.15 (3H, brs, H2-2″, H-2″, H2-6″), 7.13 (3H, brs, H2-2″, H2-4″, H-2″), 3.82 (6H, brs, 2 × OCH3); ¹³C NMR: δ 163.52 (C-1′″), 144.53 (C-2″), 162.48(C-3″), 137.15 (C-4″), 166.64 (C-5″), 144.21 (C-6″), 165.27 (C-1′″), 137.81 (C-2′″), 166.64 (C-3″), 132.26 (C-4″), 164.93 (C-5″), 137.81 (C-6′), 51.20 (OCH3), 51.16 (OCH3). Anal. Calcd: C, 64.1221; H, 5.3435. Found: C, 64.1230; H, 5.3439%. EIMS: m/z (%) 262 [M]+ (C14H11O4) (90.7), 203 (41.0), 186 (44.1), 174 (100), 153 (44.4), 121 (43.8), 77 (42.6).

**References**


NOTES


