A new monoterpenoid-glucoside from Indian Illicium griffithii

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A new monoterpenoid-glucoside isolated from Indian Illicium griffithii fruits has been characterised as p-menth-1(7),4(8)-dien-3-0-β-D-glucoside by spectral data and chemical studies.

The genus Illicium (Illiciaceae) comprises large number of species occurring in India, China in the rainfed forest at an altitude of about 2500 m and have aromatic essential oil in their fruit1. The fruit consists of 11-12 follicules and each follicule has one seed. The chemical composition and pharmaceutical studies of Illicium species has been the subject of numerous studies2.5. Anethole is the major compound from I. verum and safrole is from I. Parviflorum. Illicium religiosus contains illicin, an haemostatic principle and fruit of this plant is a Japanese home remedy6. We report herein the isolation and structure elucidation of a new monoterpenoid glucoside from methanolic extract of the fruits of I. griffithii.

The methanolic extract of Indian I. griffithii fruits yielded a new monoterpenoid-glucoside p-menth-1(7), 4(8)-dien-3-O-β-D-glucoside 1. Elemental analysis and mass spectrum (FABMS : m/z 315 [M+H]+) of compound 1 showed it to have the molecular formula C16H26O6. The IR absorption bands at 3430, 1475 and 906 cm⁻¹ were indicative of hydroxyl, exocyclic methylene and isopropylidene group.

The 'H NMR spectrum revealed the presence of exocyclic methylene protons at δ 4.62 and 4.79 (each brs) and a double doublet at δ 4.10 (J=11, 4 Hz) indicative of an oxymethine proton. Two methyl singlets at δ 1.67 and 1.72 were assigned for isopropylidene group. Further, signals in the 'H NMR and 13C NMR spectra clearly indicated the presence of a glucose moiety. A one proton doublet at δ 4.92 (J=8 Hz) was assigned to the β-anomeric proton of glucose which was further supported by the chemical shift in the 13C NMR spectrum at δ 102.4 (C-1'). Signals at δ 150.1, 106.7 were attributed to exocyclic methylene group (C-1 and C-7 respectively) and at δ 129.8 and 121.6 for C-8 and C-4 carbons respectively. Other signals are noted in experimental section.

The acid as well as enzymatic hydrolysis10 of 1 afforded two products, a sugar which was identified as glucose by paper chromatography and an aglycone moiety, which was identified as p-menth-1(7),4(8)-dien-3-ol by spectral analysis (IR, EIMS, 'H NMR and 13C NMR). The aglycone is a new p-menthane monoterpenes. 'H and 13C NMR showed the presence of one exomethylene group, one secondary hydroxyl group at C-3, methylene protons, three tetra substituted olefinic carbon, one oxymethine, two methyl and methylene carbons. The stereochemistry at C-3 hydroxyl was fixed as β-equitorial on the basis of C-3 oxymethine coupling values in its 'H NMR spectrum. It is worth mentioning that p-menthane monoterpenes abundantly occur in nature18. Thus from the above spectroscopic and chemical data, structure of compound 1 was established as p-menth-1(7),4(8)-dien-3-O-β-D-glucoside.

Experimental Section

Mps were determined on a Toshniwal apparatus and are uncorrected. The IR spectra were recorded on a Perkin Elmer 399B spectrometer as KBr pellets. The 200 MHz 'H and 13C NMR spectra of 1 and 2 were obtained on a Bruker spectrometer in CDCl3 or CD3OD with TMS as internal standard. EIMS were obtained on a JEOL D-300 mass spectrometer at 70 eV. CC and TLC were carried out on silica gel (Ranbaxy). The spots were visualized by exposure to I2 vapours and/or by spraying with 5 % vanillin-H2SO4, followed by heating at 105° for 5 min.
Plant material

The fruits of *Illicium griffithii* were collected from Shaergon region of West Kameng district, Arunachal Pradesh and identified by Dr S P Jain, Botany Department, CIMAP where a voucher specimen has been maintained.

Extraction and isolation of compounds

The air dried and powdered fruits (300 g) were extracted with hexane (3 x 0.75 L) and with MeOH (3 x 0.75 L) in a percolator at room temp., successively. After filtration, hexane and methanolic extracts were evaporated to dryness under reduced pressure below 60° to get hexane extract (7.5 g) and methanolic extract as a dark brown mass (15 g). The MeOH extract was chromatographed on a silica gel column using CHCl₃, CHCl₃-MeOH [1 : 2, 5, 10 and 20% v/v] mixtures. The eluates were monitored by TLC and grouped into six fractions.

Fraction 5 (1.35 g) eluted with CHCl₃-MeOH (10%) was rechromatographed on a SiO₂ column using CHCl₃ and increasing proportions of CHCl₃-MeOH (1 to 10%) to give compound 1 (40 mg).

*p*-Menth-1(7),4(8)-diene-3-0-β-D-glucoside 1: Colourless amorphous powder; [α]₂⁰° = -59.6° (1, MeOH); IR (KBr) : 3430, 1640, 1475, 1415 and 910 cm⁻¹; EIMS m/z (rel. int.) : 152 [M⁺]⁺ (15). ¹H NMR (200 MHz, CDC₁₃) : δ 1.69 (3H, brs, H-9), 1.84 (3H, brs, H-10), 4.63, 4.75 (1H each, brs, H₆-7, H₇-7), 4.10 (1H, dd, J=11, 4 Hz, H-3), 4.92 (1H, d, J=8 Hz, H-1' of glc), 3.20-3.80 (10H, m, aliphatic H and OH), 1.96-2.10 (6H, m, H-2, H-5, H-6). ¹³C NMR (50 MHz, CDCl₃) : δ 150.1 (C-1), 28.7 (C-2), 85.3 (C-3), 129.8 (C-4), 41.6 (C-5), 47.5 (C-6), 106.7 (C-7), 121.6 (C-8), 19.8 (C-9), 20.2 (C-10), 102.4 (C-1'), 75.4 (C-2'), 78.7 (C-3'), 71.6 (C-4'), 78.2 (C-5'), 63.3 (C-6'); Anal. Found : C, 61.90; H, 8.47. Calcd. for C₁₉H₂₅O₆ : C, 61.14; H, 8.24%.

Acid hydrolysis of 1. Compound 1 (10 mg) was heated at around 90° in 0.5 mL of 10% aq.-methanolic (1:1) HCl for 1 hr. The reaction mixture was diluted with 5 mL of MeOH, neutralized with Ag₂CO₃ and evaporated. The residue was dissolved in a minimum volume of MeOH and the aglycone deposited on cooling was filtered and checked by TLC. The filtrate was concentrated and subjected to PC. Sugar component of glycoside showed the spot with the same Rₚ values as that of D-glucose (Rₚ 0.16 by n-BuOH-AcOH-H₂O: 4:1.5, top layer, 0.57 by n-BuOH-C₆H₅N-H₂O; 6:2:3, top layer).

Enzymatic hydrolysis of 1. A solution of compound 1 (10 mg and β-D-glucosidase in H₂O, 6 mL) was kept at 37° for 10 hr, then water was added and the reaction mixture was extracted with EtOAc. The extract was recrystallised from MeOH to afford colourless amorphous powder 2. Its spectral data was similar with that of p-menth-1(7),4(8)-diene-3-ol. The 4q layer was evaporated to dryness and glucose was identified by PC.⁰°

*p*-Menth-1(7),4(8)-diene-3-0-β-D-glucoside 2: Colourless amorphous powder; [α]₂⁰° = -41.5° (1, MeOH); IR (KBr) : 3400, 1640, 1475, 1415 and 910 cm⁻¹; EIMS m/z (rel. int.) : 152 [M⁺]⁺ (15). ¹H NMR (200 MHz, CDC₁₃) : δ 1.69 (3H, brs, H-9), 1.84 (3H, brs, H-10), 4.63, 4.75 (1H each, brs, H₆-7, H₇-7), 4.10 (1H, dd, J=11, 4 Hz, H-3), 4.92 (1H, d, J=8 Hz, H-1' of glc), 3.20-3.80 (10H, m, aliphatic H and OH), 1.96-2.10 (6H, m, H-2, H-5, H-6). ¹³C NMR (50 MHz, CDC₁₃) : δ 149.2 (C-1), 25.6 (C-2), 77.2 (C-3), 132.6 (C-4), 42.2 (C-5), 49.4 (C-6), 108.5 (C-7), 124.6 (C-8), 21.3 (C-9), 20.8 (C-10).

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References