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Isolation of Phytoconstituents from the leaves of *Bauhinia variegata* Linn

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ABSTRACT

Fractionation of crude petroleum ether extract of the leaves of *Bauhinia variegata* Linn (Leguminosae) led to the isolation of dotetracont-8, 16,21-triene-13-ol (1), 12,17,18,23,24-pentamethyl dotetracont-13, 17,23-triene-12-ol (2), hexacos-11-en-15, 20-diol (3) and lupeol (4). Their structures were elucidated by spectroscopic methods such as UV, IR, NMR and LCMS. All the compounds were isolated for the first time from this plant.

Keywords: *Bauhinia variegata* Linn; isolation; dotetracont-8, 16,21-triene-13-ol; 12,17,18,23,24-pentamethyl dotetracont-13, 17,23-triene-12-ol; hexacos-11-en-15, 20-diol; lupeol; spectroscopic method.

INTRODUCTION

Bauhinia variegata Linn (leguminosae) is known as Kanchanar in Hindi is a medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India (The Ayurvedic Pharmacopoeia, 2001). The various parts of the plants viz., leaves, flower buds, flower, stem, stem bark, seeds and roots are used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, edema, laxative, anthelmintic, antileprotic, in skin diseases, wound healing, antigoitrogenic, antitumor, in obesity, stomatitis, antidote for snake poisoning, dyspepsia, flatulence and as carminative (Mali et al., 2007). The chemical constituents isolated so far from the plant are β-sitosterol, kaempferol-3-glucoside, tannins (Wealth of India, 1998), carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibers (Sharma et al., 1966), calcium, phosphorus (Sharma et al., 1968), quercetin, rutin, quercitrin, apigenin, apigenin-7-O-glucoside (Spilkova et al., 1992), heptatriacontan-12, 13-diol and dotetracontan-15-en-9-ol (Singh et al., 2006).

In the present work, we have isolated dotetracont-8, 16,21-triene-13-ol (1), 12,17,18,23,24-pentamethyl dotetracont-13, 17,23-triene-12-ol (2), hexacos-11-en-15, 20-diol (3) and lupeol (4) from the petroleum ether extract of dried leaves of *Bauhinia variegata*. All the compounds were isolated for the first time from this plant.

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MATERIAL and METHODS

Plant material

Fresh leaves of *Bauhinia vareigata* were collected, shade dried and authenticated by Dr. Shiddamallayya. N, Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2007-08/15) of the plant is deposited in the Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

General instrument details

UV: Shimadzu UV VIS-1700; IR: JASCO FTIR 5300; LCMS: Agilent 1100 LC-MSD APCI; ^{1}H-NMR (500 MHz): Bruker Avance 500.

Extraction and isolation procedure

Coarsely powdered leaves (750 gm) were extracted with petroleum ether followed by chloroform by the process of continuous extraction (soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator, with the percentage yield being 4.20 % and 0.71 % w/w in term of dry plant material. Crude petroleum ether extract was subjected to column chromatography over silica gel (60-120 mesh) using petroleum ether: benzene (different ratio), benzene (100 %) and benzene: chloroform (different ratio), taking 250 ml fraction each time. From petroleum ether (100 %), fractions 9-13 (BV-1); petroleum ether (100%), fraction 21-30 (BV-2); petroleum ether : benzene: 1:0.2, fractions 42-45 (BV-3); petroleum ether: benzene: 1:2, fraction 142-145 (BV-4) were obtained which on further purification by fractional crystallization yielded compound 1(25 mg), compound 2 (10 mg), compound 3 (25 mg) and compound 4 (07 mg) respectively.

RESULTS and DISCUSSION

The structures of compound isolated were elucidated on the basis of spectral data.

Compound 1 was isolated as white amorphous powder. Its molecular formula was determined as $C_{42}H_{80}O$ on the basis of mass

spectrum by exhibiting a quassi molecular ion at m/z 601 $(M+H)^+$ and molecular weight was established as 600. Its IR spectrum exhibited characteristic band at 1637 cm^{-1} for carbonyl group and a broad band at 3400 cm^{-1} for hydroxyl group. The ^{1}H-NMR spectrum showed a triplet signal at δ 0.83 is due to methyl group adjacent to methylene group and a large singlet signal at δ 1.20 is due to methylene group of a long chain hydrocarbon. The broad signal at δ 4.68 may be due to the hydroxyl group, the corresponding methane proton and the unsaturated protons. The signal at δ 2.36 is due to methylene group adjacent to the hydroxy methine group. With the above data and mass spectrum, the compound 1 is identified as dotetracont-8, 16,21-triene-13-ol.

Compound 2 was isolated as light yellow colored powder. Its molecular formula was determined as $C_{42}H_{80}O$ on the basis of mass spectrum by exhibiting a quassi molecular ion at m/z 601 $(M+H)^+$ and molecular weight was established as 600. Its IR spectrum exhibited characteristic band at 1639 cm^{-1} for carbonyl group and a broad band at 3424 cm^{-1} for hydroxyl group. The ^{1}H-NMR spectrum showed a triplet signal at δ 0.83 is due to methyl group adjacent to methylene proton and the signals at δ 1.37 and δ 1.59 was due to methylene group of a long chain hydrocarbon. There is no signal beyond δ 1.59 indicating the absence of heteroatoms. With the above data and mass spectrum, the compound 2 is identified as 12,17,18,23,24-pentamethyl dotetracont-13, 17,23-triene-12-ol.

Compound 3 was isolated as light brown powder. Its molecular formula was determined as $C_{26}H_{52}O_2$ on the basis of mass spectrum by exhibiting a quassi molecular ion at m/z 397 $(M+H)^+$ and molecular weight was established as 396. Its IR spectrum exhibited characteristic band at 1637 cm^{-1} for unsaturation and a broad band at 3410 cm^{-1} for hydroxyl group. The ^{1}H-NMR spectrum showed signal at δ 0.72 is due to methyl group. Long chain methylene proton signals at d 1.10 and δ 1.34. The signal at δ 2.02 and δ 2.63 were due to the methylene group on either side of the hydroxy methane group. The broad multiplet signal at δ 4.47 is due to hydroxyl group, methane proton and unsaturated protons. With the above data and mass spectrum, the compound 3 is identified as hexacos-11-en-15, 20-diol.

Compound 4 was isolated as colorless powder. Positive test for Liebermann Burchardt test indicated the presence of tetracyclic triterpenoid compound. Its IR spectrum exhibited characteristic bands at 3288 cm^{-1} for hydroxyl group. The ^{1}H-NMR as well as ^{13}C-NMR data were found to be identical with the spectrum of those already reported earlier for lupeol (Lakshmi et al., 1975). It was further confirmed by TLC and CO-TLC method with the reference standard of lupeol. All the compounds were isolated for the first time from this plant.

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