April 2008 Volume 4 Issue 1

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 4(1), 2008 [71-73]

Isolation and characterization of triterpene lactones from *Phlogacanthus thyrsiflorus* nees (Acanthaceae)

Okram Mukherjee Singh*,W.Marjit Singh, K.Subharani Devi
Department of Chemistry, Manipur University, Canchipur-795003, Manipur, (INDIA)
Tel : 0385 2435307
E-mail: ok_mukherjee@yahoo.co.in
Received: 11th January, 2008; Accepted: 16th January, 2008

ABSTRACT

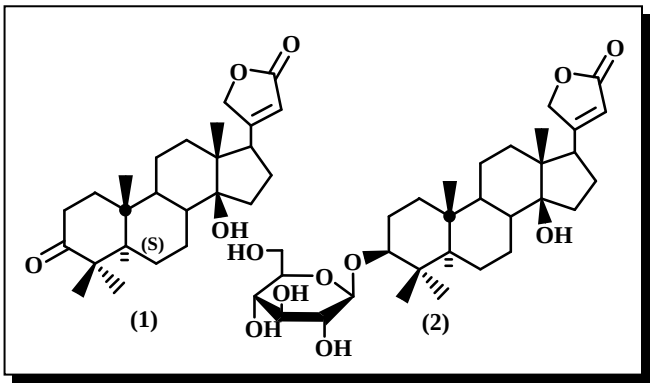
From the leaves of the *Phlogacanthus thyrsiflorus*, two new triterpene lactones **(1)** and **(2)** were isolated and structures of these compounds were determined on the basis of their spectroscopic and analytical data.

KEYWORDS

Butenolide;
Medicinal;
Phlogacanthus thyrsiflorus;
Acanthaceae.

INTRODUCTION

Phlogacanthus Thyrsiflorus Nees, a member of the Acanthaceae family is an important plant as medicine as well as a food item, which grows abundantly in the valley and forest of Manipur. The leaf extract after boiling in water is used in Manipur as traditional folk medicine for curing cough, fever, stomach ulcer and intestinal disorder[1-6]. In continuation of our studies on the isolation, characterization and biological screening of some plant extracts which are used as folk medicines[7], we describe here the isolation and identification of a new butenoliode **(1)** and a lactone glucoside **(2)**. Three known compounds identified as sitosterol, lupeol and betulin that were already reported[8] to be present in the leaves of the *P.Thyrsiflorus.* Structural elucidation of these compounds was based on the analysis of their spectroscopic and spectrometric data and comparison with literature values.



RESULTS AND DISCUSSION

The methanol extract of the leaves yielded butenolides **(1)** and **(2)** after careful column chromatography and crystallization. Compound 1 showed characteristic IR peaks at 1720 and 1697cm^{-1} indicating the presence of α, β-unsaturated γ-lactone and ketonic groups. In the ^{1}H NMR spectra a singlet proton at 5.86 and 4.89 (s, 2H,) suggested the presence of the olefinic proton and methylene protons of the γ-lactone ring. A doublet signal at 2.21 indicated the presence of a methine proton of D-ring. Other methyl, methylene and methane protons are found to spread as a multiplet at 1.02-2.25.The mass spectrum showed peaks at m/z 400, 382, 367 which were described respectively to $[M]^+$, $[M-18]^+$, $[M-(18+15)]^+$.

Similarly compound 2 showed characteristic IR

Full Paper

peaks at 1752cm^{-1} showing the presence of α, β-unsaturated lactone. Furthermore, its IR spectra displayed the strong absorption bands for hydroxyl (3485cm^{-1}), and glycosidic linkage (1066cm^{-1}). In the ^{1}H NMR spectra a singlet proton at 5.96 and 4.90 (s, 2H) was indicative of the presence of the olefinic proton and methylene protons of the γ-lactone ring. The presence of a β- glucoside moiety in **(2)** was indicated from the ^{1}H NMR which displayed signals at 5.01 (1H, d, J=8.0Hz, H-1'), 4.72-4.81(m, 6H, H-glucose moiety) . Other methyl, methylene and methine protons are found to spread as a multiplet at 0.89-2.05. The mass spectrum showed peaks at m/z 565, 547, 532, 395 etc. which were described respectively to $[M]^+$, $[M-18]^+$, $[M-(18+15)]^+$, $[M\text{-Glucosyl}]^+$.

EXPERIMENTAL

General procedures

Optical rotations were recorded on a Perkin-Elmer 241MC polarimeter. The IR spectra were recorded on a Perkin-Elmer 297 and Perkin-Elmer 983 spectrometers. ^{1}H and ^{13}C NMR spectra were determined on a Brucker ACF 300 operating in a field strength of 300 and 75.5MHz, respectively. The Chemical shifts (δppm) and the coupling constants (Hz) are reported in the standard fashion with reference to internal tetramethyl silane (TMS). EI-MS were obtained on a Finnigan MAT spectrometer at 70eV. Elemental analyses were performed on a Heraus CHN-O-Rapid Analyzer.

Plant material

The matured and tender leaves of the *Phlogacanthus thyrsiflorus* Nees were collected from Imphal and Thoubal districts of Manipur and also from some remote forest areas of Manipur. The plant specimen was identified with the taxonomists (Life Sciences Dept., Manipur University) and authentic books (see reference).

Extraction, isolation and separation

The air-dried and crushed leaves of the plant (about 10kg.) were extracted with Methanol for 75 hours. The extract was concentrated by distilling the solvent. The concentrated extract(160g) was chromatographed over silica gel (60-120 mesh) and eluted with petroleum-ether: ethyl acetate, with the increasing polarity of ethyl acetate. Thus eight column fractions were separated. Rechromatography of the less polar fraction afforded compound (1) and compound (2) and purified finally by crystallization. TLC checked the purities also.

Compound 1: Colourless crystalline solid; mp. 86^{0}C; R_f = 0.807 in 5% ethyl acetate in benzene; $[\alpha]^{26}_D$ -39^o ($CHCl_3$); IR (KBr) νcm^{-1} : 3458, 2919, 2849, 1710, 1697; ^{1}H NMR (300MHz, $CDCl_3$) δppm: 1.02(s, 6H, 2×CH_3,), 1.25 (s, 6H, 2×CH_3), 1.28-1.85 (m, 16H, methylene and methine proton), 2.21 (t, J=8Hz, 1H, H-16),2.25(t, J=8 Hz, 2H, H-4), 4.89(s, 2H, lactonic hydrogen), 5.86 (s, 1H, olefinic hydrogen); ^{13}C NMR ($CDCl_3$): δ216, 174.2, 170.2, 87, 50.6, 45.4, 39.1(C), 22.1, 22.21, 16.5 , 15.2 (CH_3) 38.01, 36.4, 35.2, 32.5, 25.0, 24.8, 24.0, 23.3 (CH_2), 158.0, 157.9, 83.7, 76.9, 76.6, 75.3, (CH). EIMS (70eV): m/z 400[M^+, 25], 382[$M-18^+$, 20], 367 [$M-(18+15)^+$, 25]; Found: C, 73.10; H, 9.50% . Calcd. For $C_{25}H_{36}O_4$: C, 74.96; H, 9.06%.

Compound 2: Light Yellow crystals; mp 204^{0}C; R_f=0.315 in 30% ethyl acetate in benzene;$[\alpha]^{26}_D$ -23^o($CHCl_3$); IR(KBr)ν cm^{-1}: 3414, 2932, 1752, 1712, 1066; ^{1}H (300MHz) δppm: 0.87(s, 3H),1.02-2.05 (m, 31H, methane, methylene and methane proton), 2.21(t, J=8Hz, 1H, H-17), 2.78(t, J=6Hz 1H, H-3β), 4.72-4.81(m, 6H, H-glucose moiety), 4.90(s, 2H, lactonic methylene), 5.01(d, J=8 Hz, 1H), 5.96(s, 1H, olefinic); ^{13}C NMR ($CDCl_3$): δ178.2, 170, 85, 50.6, 44.5, 39.6 (C), 25.1, 25.2, 17.5 and 14.2 (CH_3) 38.01, 36.4, 27.2, 26.5, 26.0, 25.8, 25.0, 23.5 and 23.3(CH_2), 118.0, 89.7, 58.9, 56.9, 52.6, 41.5 (CH). Carbons from glucose: 106, 78.7, 76.4, 74.3, 71.0, 63.2; EIMS (70eV): m/z 565 [M^+, 10], 547 [$M-18^+$, 20], 532 [$M-(18+15)^+$, 15], 395(100); Found: C, 65.05; H, 9.10% . Calcd. For $C_{31}H_{48}O_9$: C, 65.93; H, 8.57%.

ACKNOWLEDGMENT

The authors are thankful to SAIF, NEHU, Shillong for providing the NMR recordings.

REFERENCES

[1] The Wealth of India: A Dictionary of Indian Raw Materials Industrial Products: Supplement Series

(Raw Materials), New Delhi, National Institute of Science Communication, ISBN, **1-3**, **(2001)**.

[2] K.R.Kirtikar, B.D.Basu; Indian Medicinal Plants, India, **1-5**, **(1975)**.

[3] R.P.Rastogi, B.N.Mehrotra; 'Compendium of Indian Medicinal Plants', CDRI Lucknow and Publ and Information Directorate, Lucknow, **1-5**, **(1991)**.

[4] S.C.Sinha; 'Medicinal Plants of Manipur', MASS and SINHA Publications, Manipur, India, **(1996)**.

[5] R.N.Chopra, S.L.Nayar, I.C.Chopra; 'Glossary of Indian Medicinal Plants', CSIR, New Delhi, 190 **(1956)**.

[6] H.B.Singh, R.S.Singh, J.S.Sandhu; 'Herbal Medicine of Manipur, A Colour Encyclopaedia', Daya Publishing House, New Delhi, 31 **(2003)**.

[7] O.M.Singh, K.Subharani, N.I.Singh, M.B.Devi, L. Nivedita; Natural Product Research, **21**, 585 **(2007)**.

[8] S.K.Banerjee, S.Biswas, M.K.Chowdhury; J.Ind. Chem.Soc., **57**, 665 **(1980)**.