academicJournals

Vol. 7(27), pp. 2040-2042, 17 July, 2013
DOI: 10.5897/JMPR12.1258
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Short Communication

First ever isolation of cytotoxic triterpenoid 2-hydroxydiplopterol from plant source

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Accepted 16 July, 2013

Arisaema jacquemontii **have shown medicinal importance and biological activities particularly, its anticancer activity, so we have decided to keep on investigating the plant in term of its chemical constituents. A chemical investigation on the chloroform extract of *A. jacquemontii* have resulted in the isolation of a cytotoxic, triterpenoid 2-hydroxydiplopterol (1), previously reported from the halotolerant, fungal strain *aspergillus variecolor* B-17. This represents perhaps the first report of cytotoxic compound from plant source. The compound (1) has been identified by multidimensional nuclear magnetic resonance (NMR) spectroscopy and comparison of its spectroscopic data with that reported for the compound. Its exhibited cytotoxicity with IC_{50} value of 22 µM against K 562 cell lines. The cytotoxicity properties of compound 1 were determined by the sulforhodamine B method.**

Key words: *Arisaema jacquemontii,* cytotoxic triterpenoid 2-hydroxydiplopterol, isolation.

INTRODUCTION

The genus *Arisaema* belongs to the family Araceae (Nisar et al., 1978). The genus is reputed for its physiological properties in the folk medicine system (Perry, 1965). *Arisaema jacquemontii* Blume, a herb locally known as "Sap-ki-booti" is abundantly found in northern regions of Pakistan and in Kashmir (Polunim and Stainton, 1984). The herb is also reported to be widely distributed in East Asia, Afghanistan to South East Tibet, upper and lower alpine zone in the drier areas of Himalayas in the range 2400 to 4000 m (Kunkel, 1984). The plant contains calcium oxalate crystals which causes an extremely unpleasant sensation similar to needles stuck into mouth and tongue if they are eaten but are easily neutralized by thoroughly drying or cooking the plant or by steeping it water (Dauglas, 1979). Various parts of the plant found are used in folk medicine system and applied in different form to cure various ailments. The juices from the tubers of the plant are applied to the skin by Khasi and Garo tribes of Meghalaya, India for the treatment of ringworms and other skin diseases (Rao, 1981). A tuber lectin having potent anti-insect and anti-proliferative activity was purified from the plant (Kaur et al., 2006). Earlier, the activity of methyl ethyl ketone and methanol extracts of the plant in *rad 6* and *rad 52* yeast assays have been reported (Habib et al., 1992). Keeping in view its important biological activity (Miglani et al., 1975), investigation is being done on the plant for the last 20 years (Habib and Qadir, 2003). Recent investigations have resulted in the isolation of the cytotoxic triterpenoid, 2-hydroxydiplopterol (compound 1), previously reported from halotolerant fungal strain *Aspergillus variecolor* B-17. Its structure has been identified by comparison of its

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spectroscopic data with that reported for the compound (1) (Wen et al., 2009). To prove the reproducibility of the result, 2-hydroxydiplopterol (compound 1) was re-isolated by applying the same procedure. This represent first ever report of the cytotoxic triterpenoid 2-hydroxydiplopterol (compound 1) from plant source. The cytotoxicity and antioxidant properties of compound 1 were determined by the sulforhodamine B (SRB) (Skechan et al., 1990) and diphenyl-β-picrylhydrazyl (DPPH) (Chen et al., 1999) method, respectively.

MATERIALS AND METHODS

General experimental procedures

Column chromatography and flash chromatography were carried out using silica gel 70-230 and 230-400 mesh. Alumina sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm, E-Merck) were used for chromatography to check the purity of compounds and were visualized under ultraviolet (UV) light (254 and 366 nm) followed by ceric sulphate as spraying reagents. Optical rotations were measured on Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer. Infrared (IR) spectra were recorded on Shimadzu IR-460 spectrometer; EIMS, HRMS and FAB spectra were recorded on Jeol JMS-HX 110 spectrometer. The ^{1}H-NMR spectra were recorded in $CDCl_3$ on Bruker AMX-600 MHz instrument using tetramethylsilane (TMS) as an internal reference. The ^{13}C-NMR spectra were also recorded in $CDCl_3$ on Brukar AMX-150 MHz instrument. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) in Hz.

Plant

The whole plant of *A. jacquemontii* Blume was collected from Nathiagalli, District Abbottabad, Pakistan in August, 2008. The plant was identified by a plant taxonomist at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan where a voucher specimen number XX-278 has been deposited.

Extraction procedure

The plants were dried under shade. The dried plant material (4 kg) was chopped and cut into small pieces and then grinded to the powdered form. The powdered material was soaked in chloroform for 10 days. The chloroform extract was separated by filtration and evaporated to dryness on rotary evaporator under reduced pressure. This afforded 163 g of chloroform extract. The extract was subjected to the column chromatography over silica gel (mesh size 70-230 and 63-200 µm). The column was eluted with increasing polarities of hexane and acetone. The fraction obtained with hexane/acetone (9.5:0.5) was chromatographed over flash silica column with hexane/acetone (9.0:1.0) as the eluting solvent system. This afforded 10 mg, a pure compound, 2-hydroxy diplopterol (compound 1).

RESULTS AND DISCUSSION

The high-resolution mass spectrometry (HRMS) of compound 1 showed a molecular ion peak at m/z 444.4076,

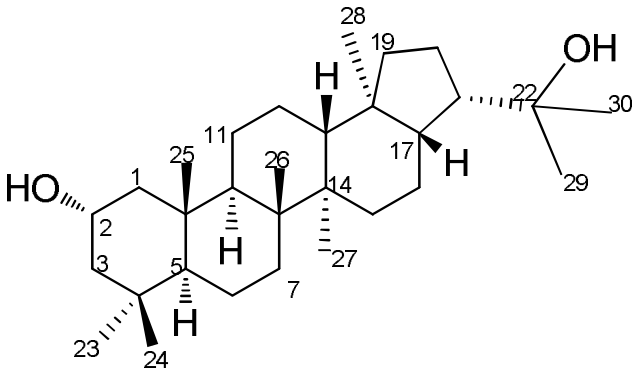

Figure 1. Structure of compound 1.

in agreement with the molecular formula $C_{30}H_{52}O_2$ (Calculated 444.4073), indicating the presence of five double bond equivalents, which accounted for five rings. A strong IR absorption band at 3344 cm^{-1} indicating OH function. The ^{1}H-NMR spectrum ($CDCl_3$ 600 MHz) of compound 1 showed six singlets (δ 0.92, 0.83, 0.76, 0.95, 0.85 and 0.95) each integrating for three protons due to C-23, C-24, C-25, C-26, C-27 and C-28 methyls. Two 3H singlets at δ 1.20 and 1.17 were due to the secondary C-29 and C-30 methyl protons, respectively. The C-2 methine proton resonated at δ 3.87 as broad triplet. Its downfield chemical shift value was indicative of the presence of a hydroxyl function on the carbon. The ^{13}C-NMR spectrum of compound 1 showed 30 carbon resonances in the molecule. The chemical shifts of all the carbons are similar to the values reported for 2-hydroxydiplopterol (compound 1) (Wen et al., 2009). Distortionless enhancement by polarization transfer (DEPT) spectrum showed the presence of eight methyls, ten methylenes, six methines and six quaternary carbons. The combination of ^{1}H and ^{13}C-NMR data suggested that compound 1 has diplopterol type structure (Figure 1) as ^{1}H and ^{13}C-NMR chemical shift values of compound 1 were similar to those of 2-hydroxydiplopterol reported in the literature (Wen el al., 2009). The ^{1}H and ^{13}C-NMR chemical shift values were assigned with the help of COSY-45, HMQC and HMBC spectral data.

Cytotoxicity and antioxidation

Cytotoxicity of compound 1 was checked by SRB assay; 150 µl of the cell suspension were plated in 96-well plates at a density of 2 × 10^5 cells per ml. Two microliters of test sample in methanol was added to each well and culture was incubated for 24 h. The cells were fixed with 12% trichloroacetic acid and cell layer stained with 0.4% SRB. The absorbance of SRB solution was measured at 515 nm. Dose response curves were generated and IC_{50} values were calculated from the linear portion of log dose response curves.

Antioxidant activity of compound 1 was checked by DPPH radical scavenging assay. Five microliters of sample was dissolved in DMSO and mixed with 95 µl of DPPH in ethanol. The concentration of DPPH was maintained at 300 mM with variable concentrations of sample. The mixture was dispensed in 96-well plate and incubated with at 37°C for 30 min. The absorbance at 520 nm was measured by microtitre plate reader (Spectro Max plus 384, Molecular Devices, CA, USA), and percent radical scavenging activity was determined in comparison with DMSO-treated control (3-t-butyl-4-hydroxyanisole). IC_{50} value denotes the concentration of compound 1 required to scavenge 50% of the DPPH free radicals.

ACKNOWLEDGEMENTS

The authors acknowledged the cooperation of the department of wild life, Khyber Pakhtunkhaw, Pakistan in plant collection and financial support from the Higher Education Commission, Government of Pakistan.

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