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Short Communication

Structure determination of betulinic acid from the leaves of *Lophira lanceolata* Van Tiegh. Ex Keay (Ochnaceae)

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ABSTRACT

Cupressuflavone a biflavone was earlier isolated from the leaves of *Lophira lanceolata*. From this work Betulinic acid was also isolated from the leaves for the first time. The two compounds were identified on the basis of 1D NMR (^1H , ^{13}C and DEPT), 2D NMR (COSY, HSQC, HMBC and NOESY), IR, MS data and by comparison with reported data. Like that of *Ouretia* this recent isolation suggests that betulinic acid can be used as chemical markers in the Ochnaceae family.

Keywords: betulinic acid, *Lophira lanceolata*, Ochnaceae, Ex Keay.

INTRODUCTION

Lophira lanceolata (Ochnaceae) Van Tiegh. Ex Keay is a tall tree reaching up to 60 feet high and grows in the woody savannah forests, distributed from Sierra Leone to Uganda and Sudan (Pegnyemb et al., 1998; Persinos and Quimby, 1968). Ethnomedicinal uses of the plant include treating: toothache in Cameroon; liver infections in Togo; female sterility, and cough in Nigeria (Ghogomu et al., 1989a). An infusion of young twigs is also reported to be used for treatment of fever, respiratory troubles and to relieve the gripping of dysentery in Nigeria (Persinos et al., 1967). Leaves of *L. lanceolata* were collected from, Sakaru village along Jos road Zaria, in December 2005 and identified at the Herbarium section, Biological Science Department, Ahmadu Bello University, Zaria. A voucher coded 4002B was lodged for verification purposes.

Previous work

The leaves of *L. lanceolata* have yielded a prenylated isoflavone and a benzoylglucoside (Pegnyemb et al., 1998). Several dimeric chalcones, biflavonoids and tetraflavonoids have been reported from the leaves, stem bark and stem heartwood of the plant (Sani et al., 2010; Ghogomu, et al., 1987; Ghogomu, et al., 1989a; Ghogomu, et al., 1989b; Ghogomu, et al., 1990; Pegnyemb et al., 1994). Benzamide has also been reported from the root bark of the plant (Persinos et al., 1967).

Methodology

The air-dried and coarsely powdered leaves (1 Kg) were defatted by soaking in petroleum ether for 10 hours and the extract decanted off. The defatted leaves were then left to completely dry and extracted twice using acetone by soaking. The combined acetone extracts were removed

under pressure to give a highly viscous greenish – brown mass. This crude extract was then refluxed using petroleum ether, benzene and chloroform successively until the solvent in each case was almost colourless. The residue left behind was then treated with hot water, and the water insoluble portion dissolved in acetone and removed under pressure to yield a solid brown residue that tested positive in a flavonoid colour test. The aqueous solution was extracted with ethyl acetate twice, and the solvent concentrated *in vacuo* to give a semi- solid residue that also gave a positive flavonoid test.

The acetone and ethyl acetate fractions were separately fractionated using silica gel column chromatography using methylene chloride followed by a step-wise gradient of CH₂Cl₂-MeOH to give two compounds (1-2). A known biflavonoid cupressuflavone (1) (Sani et al., 2010) and the other, a known triterpenoid; betulinic acid (2) were identified for the first time in the plant and the genus *Lophira*. The two compounds were identified on the basis of 1D NMR (¹H, ¹³C and DEPT), 2D NMR (COSY, HSQC, HMBC and NOESY), IR, MS data and by comparison with reported data, cupressuflavone (1, 36 mg) (Sani et al., 2010; Chari, et al., 1977;), betulinic acid (2, 25 mg) (Macias et al., 1994).

RESULT AND DISCUSSION

Lophira belongs to the Ochnaceae family which comprises of approximately 35 genera and 600 species widely represented in the tropics (do Nascimento et. al., 2008). Biflavonoids such as lanceolatin A and B, are useful chemataxonomic markers in *Lophira* and the Ochnaceae family (Tih et al., 2003). Therefore, the isolation of cupressuflavone (1) confirms that this plant belongs to the genus *Lophira* (Ochnaceae). This report also identifies *L. lanceolata* as a new source of triterpenoids, betulinic acid (2). The presence of triterpenoids in *L. lanceolata* and the isolation of cupressuflavone (1) for the first time from the genus can be used to distinguish between *L. lanceolata* and the closely related *L. alata*, which previous studies on various parts of the two species have showed similar polyflavonoids (Tih et al., 2003). The isolation of triterpenoids in *Ouretia* and now *Lophira* also suggests that triterpenoids can be used as chemical markers in the Ochnaceae family.

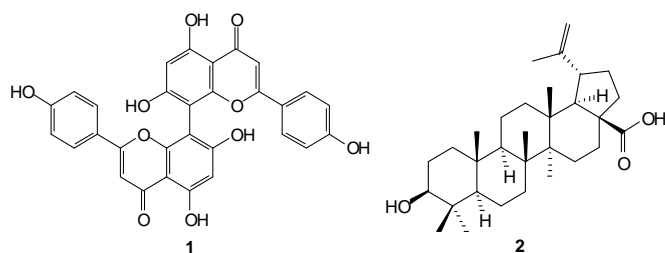


Fig. 1 Structure of isolated compounds. (1) cupressuflavone & (2) betulinic acid.

CONCLUSION

Betulinic acid was isolate from the leaves for the first time, the two compounds identified in this paper were isolated and determined on the basis of 1D NMR (¹H, ¹³C and DEPT), 2D NMR (COSY, HSQC, HMBC and NOESY), IR, MS data and by comparison with reported data.

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