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Phytochemical Investigations on *Gentiana dahurica*

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The present paper deals with the isolation and structural elucidation of an agigenin along with ursolic acid from aerial parts of *Gentiana dahurica* plant.

INTRODUCTION

Gentiana dahurica commonly known as *Gul-i-Ghafis*[1] is commonly used in Indian system of medicine[2–4]. The extract of this plant is used as a bitter tonic, stomachic and diaphoretic. It is a constituent of the Chinese drug Quinjiao along with *G. macrophylla*, used as bitter tonic. It is reported to have anticonvulsant and antidepressant activity.

This plant is explored for the presence of secoiridoids,[5, 6] iridoids,[5, 6] flavones,[5, 6] alkaloids[6, 7] and triterpenes[8]. In the present study, its aerial parts are again tried to find out some other constituents.

EXPERIMENTAL AND DISCUSSION

The plant material, purchased from the local market Kharibaoli, Delhi, was exhaustively extracted with ethanol under refluxing condition. The ethanolic concentrate (40 g) was extracted with petroleum ether at 60–80°C to remove petrol-soluble fraction (F-1) (5 g). The petrol-insoluble fraction was washed with water to remove water-soluble parts to get water-insoluble fraction (F-2) (12 g).

Isolation of compound (1) from petrol-soluble fraction (F-1)

The above crude product (5 g) after re-dissolving in petroleum ether and concentrating to small volume (20 mL) was left in a refrigerator overnight. A green solid (500 mg) separated out which was filtered and washed with petroleum ether. It gave a positive Liebermann Burchard test. This compound (500 mg) was acetylated with acetic anhydride and pyridine and then processed in the usual way to give the crude acetate (520 mg). It was dried, dissolved in minimum quantity of methanol (1 mL), extracted three times with hot petroleum ether at 60–80°C using 50 mL of petrol each time. All the petroleum extracts were combined together and evoporated to dryness, crystallised from methanol to give a colourless crystalline compound (1), 300 mg, m.p. 283°C.

Characterization of compound (1)

The above compound was characterized on the basis of IR, NMR and mass spectral studies.

IR spectrum of the compound had adsorption λ_{max} nujol 5.8 μ, 5.86 μ and 7.9 μ for the acetoxyl function.

NMR spectrum of the compound revealed methyl groups at δ 0.8 (3H), δ 0.9 (6H), δ 0.92 (3H), δ 1.0 (6H) and δ 1.1 (3H) and one acetoxyl function at δ 2.1. In addition to these, there was a triplet centred at δ 3.46 for proton ∝ to acetoxyl and a signal located at δ 5.2 characterisitc of olefinic proton.

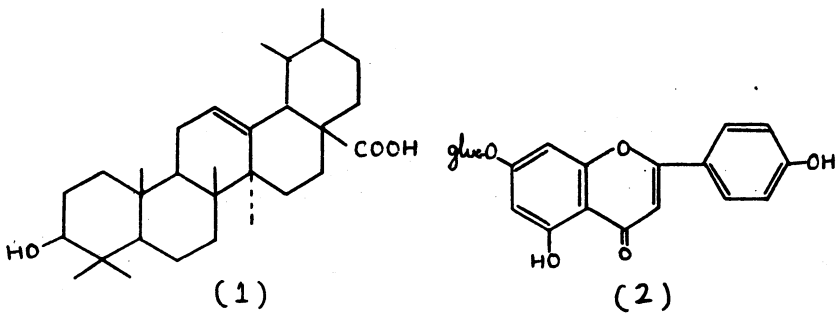
On methylation with diazomethane, it gave acetyl methyl ester, m.p. 236–39°C. Its NMR spectrum showed 7 methyl functions as singlet at δ 0.8 (3H), δ 0.9 (6H), δ 0.92 (3H), δ 0.98 (3H) and δ 1.1 (6H) and one acetoxyl function centered at δ 2.1 as singlet. It showed singlet for ester methoxyl function at δ 3.7. The olefinic proton signal could be located at δ 5.3 and triplet at δ 4.46 arising from proton to acetoxyl function.

On the basis of above physical and spectral data, the acetate was characterized as ursolic acid acetate and so its parent compound is ursolic acid.

A further support to the above findings was obtained form mass spectrum which showed molecular ion peak at m/z 498. Other diagnostic peaks were located at m/z 249 and m/z 248 due to retrodiels alder reaction.

Isolation of compound (2) from water-insoluble fraction (F-2)

It was successively extracted with chloroform and ethyl acetate. The ethyl acetate concentrate (1 g) gave a positive test for phenolic group and flavonoids with Zn-HCl. It was subjected to column chromatography on silica gel G and eluted with petrol (200 mL), benzene (200 mL), benzene ethyl acetate (4 : 1 7 × 100 mL) and (1 : 1, 500 mL) and finally with ethyl acetate. The first two fractions of 100 mL were combined together and evaporated to dryness and crystallised from methanol to give brownish yellow crystalline compound (2), 10 mg, m.p. 300°C.



Structure of compound (1) and (2).

Characterization of compound (2)

It was characterized on the basis of UV spectral studies as apigenin. The UV spectrum of the compound showed two maxima at 269 mμ and 337 mμ with an influxion around 284 mμ. The long wave absorption around 336 mμ is indicative of a flavone (absence of OH group at position 3). Addition of $AlCl_3$ shifted band

I to 350 mμ with an influxion around 385 mμ indicating the hydroxyl group at 5 position. Addition of HCl to the $AlCl_3$ solution did not produce any significant change consistent with absence of OH group at position 3. Addition of sodium acetate shifted band II to 275 mμ indicating the presence of —OH group at position 7 and addition of boric acid and sodium acetate did not produce bathochromic shift of band I indicating the absence of catecholin system. These data suggest that the compound is 5,7,4′-oxygenated. A further identity was established by TLC comparison and mixed melting point technique.

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