Phytochemical Studies on *Periploca aphylla*

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Summary: Eight compounds including stigmasterol, oleanolic acid, ursolic acid, betuline, 2β, 3β-dihydroxyurs-12-en-28-oic acid, 2β, 3β, 23-trihydroxyurs-12-en-28-oic acid, 3β, 6α-dihydroxylup-20(29)-ene and 6α-hydroxylup-20(29)-en-3β-octadecanoate have been isolated from Periploca aphylla. Their structures have been established with the help of spectral data.

Introduction

The genus *Periploca*, belonging to the family Asclepiadaceae, comprises of twelve species. All of these are erect leafless shrubs, distributed in Europe, Asia and tropical Africa. Phytochemical studies on various species of genus *Periploca* including *P. sepium, P. leavigatis,* and *P. gracea* have resulted in the isolation of various triterpenes, lignans and flavonoids [1-3]. *Periploca aphylla* Decne is widely distributed in the northern part of Pakistan and finds various medicinal uses in the indigenous system of medicine [4]. The milky juice is used as an external application to tumors and swellings and is said to be useful in cerebral fever and also as a stomachic [5].

In the present investigations, a methanolic extract of the stems of *P. aphylla* showed positive activity in the brine shrimp lethality test [6]. Further biological screening of the methanolic extract revealed strong α-glucosidase inhibitory as well as antibacterial activity against Gram-psitive (*Bacillus cerus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli, Protens mirabilis, Salmonella typhi*) bacteria [7]. This prompted us to carry out bioassay directed isolation studies on this plant. Herein we describe the isolation of stigmasterol (**1**), oleanolic acid (**2**), ursolic acid (**3**), betuline(**4**), 2β, 3β-dihydroxyurs-12-en-28-oic acid (**5**), 2β, 3β, 23-trihydroxyurs-12-en-28-oic acid (**6**), 3β, 6α-dihydroxylup-20(29)-ene (**7**) and 6α-hydroxylup-20(29)-en-3β-octadecanoate (**8**) respectively. The compounds **1-6** have not previously been reported from *P. aphylla.*

Results and Discussion

Column chromatography of the chloroform soluble fraction of *Periploca aphylla* resulted in the isolation and characterization of known triterpenes, namely oleanolic acid, ursolic acid, betuline, 2β, 3β-dihydroxyurs–12-en-28-oic acid and 2β, 3β, 23-trihydroxyurs-12-en-28-oic acid. In addition, stigmasterol was also obtained and characterized. These compounds have been reported for the first time from *P. aphylla.* None of these were bioactive. However, compounds **7** and **8** obtained from the ethyl acetate fraction showed the enzyme inhibitory activity against α-glucosidase type VI (Sigma G6136) and also antibacterial activity which has also been reported earlier in literature [8].

Experimental

The IR spectra was recorded on JASCO A-302 spectrometer. The mass spectrum were recorded on a Finnigan MAT-112 spectrometer. The NMR spectra ($CDCl_3$) 400 MHz for ^1H and 125 MHz for ^{13}C using TMS as internal standard, silica gel PF_{254}: CC: slilica gel 70-230 mesh.

Plant Material

The whole plant material was collected from Swat and identified as *Periploca aphylla* by Mr. Habib Ahmad. A Voucher specimen is deposited in the herbarium (accession no. 1997-1294) of the Department of Botany, Government Degree College Matta, Swat, Pakistan.

Isolation

The shade dried plant material (25 kg) was extracted three time with MeOH at room temperature. The methanolic extract was evaporated in vacuo, yielding a brownish residue, which was suspended in H_2O and then extracted with *n*-hexane.

The aqueous layer was successively extracted with chloroform and ethyl acetate. The chloroform fraction was chromatographed over silica gel using a mixture *n*-hexane and chloroform in increasing order of polarity. It resulted in the isolation of six pure compounds, stigmasterol, oleanolic acid, ursolic acid, betuline, 2β, 3β-dihydroxyurs-12-en-28-oic acid and 2β, 3β, 23 trihydroxyurs-12-en-28-oic acid. The ethyl acetate fraction which showed potent cytotoxicity was chromatographed over silica gel using mixtures of *n*-hexane and EtOAc in order of increasing polarity as eluent. The fraction obtained from *n*-hexane: EtOAc (19:1) showed strong cytotoxicity, and it was further chromatographed over silica gel using *n*-hexane:EtOAc (9:1) as eluent to yield 6α-hydroxylup-20(29)-en-3β-octadecanoate from the top fractions and 3β, 6α-dihydroxylup-20(29)-ene from the tail fractions.

Stigmasterol (1)

The fraction which eluted with *n*-hexane-chloroform (8.5:1.5) was chromatgraphed over silica gel and eluted with hexane-$CHCl_3$ (8:2) to give compound (1) which crystallized from methanol, m.p. 168-169 °C; $[\alpha]_D$ = - 50° ($CHCl_3$): EIMS *m/z*: 412 [$C_{29}H_{40}O$, M^+]. The physical and spectral data coincided with literature [9].

Ursolic acid (2)

The fraction which eluted from *n*-hexane-$CHCl_3$ (8:2) was chromatographed over silica gel using a mixture of *n*-hexane-$CHCl_3$ (7.5:2.5) to give uniform compound (2) from the head fractions which crystallized from methanol, m.p. 279-281 °C; $[\alpha]_D$ + 70° (MeOH); EIMS *m/z*: 456 [$C_{30}H_{48}O_3$, M^+]. The physical and spectral data coincided with literature [10].

Oleanolic acid (3)

The fraction which eluted from *n*-hexane-$CHCl_3$ (8:2) was further chromatographed over silica gel. Elution with *n*-hexane-$CHCl_3$ (7.5:2.5) provided (3) from the tail fractions, m.p. 306-307 °C; $[\alpha]_D$ + 78.9° ($CHCl_3$); EIMS *m/z*: 456 [$C_{30}H_{48}O_3$, M^+]. The physical and spectral data showed complete agreement with the literature [11].

Betuline (4)

The fraction which eluted from *n*-hexane-$CHCl_3$ (6:4) was chromatographed over silica gel and elution with *n*-hexane-$CHCl_3$ (5.5:4.5) provided (4), m.p. 254-255 °C; $[\alpha]_D$ + 12° (MeOH); EIMS *m/z*: 442 [$C_{30}H_{50}O_2$, M^+]. The physical and spectral data coincided with literature [12].

2β, 3β-Dihydroxyurs-12-en-28-oic acid (5)

The fraction which eluted with *n*-hexane-$CHCl_3$ (1:1) was chromatographed over silica gel using a mixture of *n*-hexane: $CHCl_3$ (4:6) to give compound (5) from the head fractions, EIMS *m/z* 472 [$C_{30}H_{48}O_4$, M^+]. The physical and spectral data showed complete agreement with the literature [13].

2β, 3β, 23-Trihydroxyurs-12-en-28-oic acid (6)

The fraction which eluted from *n*-hexane-$CHCl_3$ (1:1) was further chromatographed over silica gel using a mixture of *n*-hexane-$CHCl_3$ (4:6) to afford (6) from the tail fractions, EIMS *m/z* 488 [$C_{30}H_{48}O_5$, M^+]. The physical and spectral data coincided with literature [13].

3β, 6α-Dihydroxylup-20(29)-ene (7)

The fraction which eluted with *n*-hexane:EtOAc (19:1) was chromatgraphed over silica gel using a mixture of *n*-hexane:EtOAc (9:1) to give compound (7) from the top fractions, $[\alpha]_D$ +14° ($CHCl_3$), EIMS m/z: 442 [$C_{30}H_{50}O_2$, M^+]. The physical and spectral data coincided with literature [8].

6α-Hydroxylup-20(29)-en-3β-octadecanoate (8)

The fraction which eluted with *n*-hexane-EtOAc (19:1) was further chromatgraphed over silica gel using a mixture of *n*-hexane-EtOAc (9:1) to give compound (8) from the tail fractions, $[\alpha]_D$ +26° ($CHCl_3$), EIMS m/z: 708 [$C_{48}H_{84}O_3$, M^+]. The physical and spectral data showed complete agreement with the literature [8].

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