PERGAMON

Phytochemistry 50 (1998) 481–484

PHYTOCHEMISTRY

# Steroidal saponins from *Asparagus dumosus*

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Revised 15 July 1998

**Abstract**

A new steroidal saponin, dumoside, characterized as (20*S*)-3β,16β-dihydroxy pregn-5-ene-22-carboxylic acid (22,16)-lactone-3-*O*-β-chacotrioside, was isolated from the whole plant of *Asparagus dumosus* Baker and the structure was deduced from spectral data. In addition to dumoside three more steroidal saponins characterized as 3β-dihydroxy pregn-5,16-dien-20-one 3-*O*-β-chacotrioside, 3β,22α,26-trihydroxyfurost-5-ene-3-*O*-β-chacotrioside-26-*O*-β-D-glucopyranoside and its corresponding 22α-*O* methoxy analogue were also isolated for the first time from this source. The structures have been identified with the help of FAB-MS, $^{1}$H NMR, $^{13}$C NMR and extensive 2D NMR spectroscopy, as well as comparison with reported spectroscopic data.


*Keywords:* *Asparagus dumosus*; Liliaceae; Whole plant; Dumoside; Steroidal saponins; Glycoside

## 1. Introduction

*Asparagus dumosus* Baker belonging to the family Liliaceae is commonly found at Karachi, Sindh and Indus delta. The roots of this plant are used as folk medicine, as diuretic, demulcent, aphrodisiac, refrigerant, antiseptic, alterative agent, and are also used as antidysentery and galactogogue (Public Inform. Direct, 1948; Nadkarni & Nadkarni, 1976; Baquar, 1989; Ahmad & Atta-ur-Rahman, 1989; Baquar & Tasnif, 1967). A survey of the literature showed that several *Asparagus* species have already been chemically studied and found to contain steroidal saponins (Hostettmann & Hostettmann-Kaladas, 1990; Kawano, Sakai, Sato & Sakamura, 1975; Konishi & Shoji, 1979; Joshi & Dev, 1988; Sharma & Kumar, 1982; Sati & Sharma, 1985; Sati, Pant & Hostettmann, 1984; Kawano, Sato & Sakamura, 1977; Hostettmann & Marston, 1995), but a chemical study of *A. dumosus* has not yet been made. We are now reporting a new steroidal saponin dumoside (**1**), characterized as (20*S*)-3β,16β-dihydroxy pregn-5-ene-22-carboxylic acid (22,16)-lactone-3-*O*-β-chacotrioside and three known steroidal saponins (3β-dihydroxy pregn-5,16-dien-20-one 3-*O*-β-chacotrioside (**2**), 3β,22α,26-trihydroxyfurost-5-ene-3-*O*-β-chacotrioside-26-*O*-β-D-glucopyranoside (**3**) and its corresponding 22α-*O* methoxy analogue (**4**)) from this species.

## 2. Results and discussion

The butanolic extract of the whole plant of *Asparagus dumosus* Baker by repeated column chromatography on silica gel yielded a mixture showing three spots on TLC. These spots were further purified by HPLC as a result of which four steroidal saponins (**1**–**4**) were obtained. Compound **1** was obtained as a white amorphous substance from the butanolic soluble portion of a methanol extract. The IR spectrum showed a strong absorption band at 1760 cm$^{-1}$ characteristic for a five-membered lactone (Chakravarty, Das & Pakrashi, 1982), besides a broad band between 3500 and 3100 cm$^{-1}$ for hydroxy groups.

The FAB mass spectrum (negative ion mode) of **1** gave molecular ion peak at $m/z$ 797 $[M-H]^-$ corresponding to the molecular formula $C_{40}H_{62}O_{16}$. On acid hydrolysis, **1** afforded D-glucose and L-rhamnose which were identified by TLC with authentic samples. The $^{1}$H NMR spectrum of **1** exhibited three anomeric

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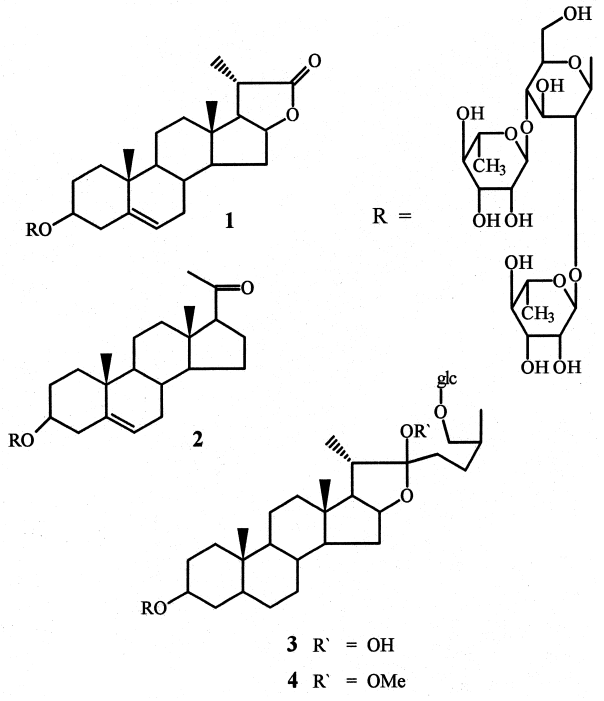
0031-9422/98 $ - see front matter 
PII: S0031-9422(98)00566-4




proton signals of sugar components at $\delta$ 4.49 (*d*, $J = 7.80$ Hz), 4.83 (*d*, $J = 1.76$ Hz) and 5.20 (*d*, $J = 1.68$ Hz). Its $^{13}C$ NMR spectrum showed three anomeric carbon resonances at 100.6, 102.3 and 103.1. The ion peaks at *m/z* 651 $[M - H - Rha]^-$, at *m/z* 505 $[M - H - Rha - Rha]^-$ and at *m/z* 343 $[M - H - Rha - Rha - Glc]^-$ in the FAB mass spectrum also showed the presence of three sugars. The D-glucose and two L-rhamnoses as sugar moieties in **1** were confirmed with the help of FAB mass spectrometry and comparative study of $^1H$ NMR and $^{13}C$ NMR values (Nakano, Murakami, Takaishi, Tumimatsu & Nohara, 1989).

Comparison of $^{13}C$ NMR data of the sugars moieties in **1** with methyl β-D-glucopyranoside indicated that the C-2 and C-4 of the glucosyl moiety were shifted downfield to $\delta$ 80.32 and 79.38 from 74.80 and 70.30 respectively. The significant glycosylation shifts clearly showed that two terminal rhamnopyranoses were linked to the C-2 and C-4 positions of the inner glucopyranose. These linkages were further confirmed by HMBC spectrum [Fig. 1(a)]. The C-5 signals of two rhamnose units were at $\delta$ 69.79 and 70.78 respectively, indicating their α-configurations (Seo, Tomita, Tori & Yoshimura, 1978).

The $^{13}C$ chemical shift values of **1** other than the sugar moieties having three characteristic methyl signals suggest that the aglycone pertains to a pregnane type steroid. The $^{13}C$ NMR spectrum of **1** exhibited two carbon signals of the aglycone moiety directly attached to oxygen atom at $\delta$ 78.09 and at $\delta$ 84.78. The carbon signals at $\delta$ 122.38, 142.06 and at $\delta$ 183.99 indicated the olefinic and carbonylic functionalities in the molecule. In the $^1H$ NMR spectrum of **1** the $^1H$-signals at $\delta$ 3.60 (*m*, H-3) and at $\delta$ 5.01 (*dt*, H-16) were

Table 1
$^{13}C$ NMR data for compounds **1**–**4** (in $CD_3OD$)

| C No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| C-1 | 38.56 | 38.49 | 38.58 | 38.58 |
| C-2 | 30.74 | 30.75 | 30.77 | 30.77 |
| C-3 | 79.32 | 79.33 | 79.33 | 79.33 |
| C-4 | 39.54 | 39.59 | 39.57 | 39.57 |
| C-5 | 142.06 | 142.32 | 141.95 | 141.99 |
| C-6 | 122.38 | 122.41 | 122.57 | 122.59 |
| C-7 | 33.01 | 33.26 | 32.78 | 32.82 |
| C-8 | 32.64 | 31.58 | 32.81 | 32.35 |
| C-9 | 51.78 | 52.20 | 51.76 | 51.78 |
| C-10 | 38.04 | 38.07 | 38.07 | 38.07 |
| C-11 | 21.52 | 21.83 | 21.98 | 21.97 |
| C-12 | 39.09 | 36.64 | 40.87 | 40.85 |
| C-13 | 42.57 | 47.29 | 41.85 | 41.86 |
| C-14 | 55.90 | 57.89 | 57.77 | 57.77 |
| C-15 | 34.05 | 36.04 | 33.20 | 33.19 |
| C-16 | 84.77 | 147.17 | 82.47 | 82.59 |
| C-17 | 60.13 | 156.39 | 65.05 | 65.05 |
| C-18 | 14.02 | 16.13 | 16.82 | 16.81 |
| C-19 | 19.81 | 19.73 | 19.87 | 19.84 |
| C-20 | 37.54 | 199.47 | 41.20 | 41.22 |
| C-21 | 18.10 | 27.13 | 16.08 | 16.04 |
| C-22 | 183.99 | | 114.05 | 112.34 |
| C-23 | | | 36.98 | 31.40 |
| C-24 | | | 29.02 | 28.83 |
| C-25 | | | 35.02 | 35.02 |
| C-26 | | | 76.03 | 76.60 |
| C-27 | | | 17.31 | 17.87 |
| 3-*O*-glc | | | | |
| C-1 | 100.54 | 100.56 | 100.54 | 100.57 |
| C-2 | 80.32 | 80.22 | 80.22 | 80.30 |
| C-3 | 76.60 | 76.59 | 76.58 | 76.58 |
| C-4 | 79.38 | 79.34 | 79.41 | 79.43 |
| C-5 | 78.09 | 78.08 | 78.06 | 78.10 |
| C-6 | 62.10 | 62.05 | 62.91 | 62.95 |
| Rha | | | | |
| C-1 | 102.29 | 102.28 | 102.27 | 102.29 |
| C-2 | 72.47 | 72.46 | 72.45 | 72.47 |
| C-3 | 72.23 | 72.23 | 72.21 | 72.24 |
| C-4 | 73.80 | 73.78 | 73.78 | 73.80 |
| C-5 | 69.79 | 69.78 | 69.78 | 69.79 |
| C-6 | 17.87 | 17.86 | 17.89 | 17.89 |
| Rha′ | | | | |
| C-1 | 103.06 | 103.06 | 103.03 | 103.08 |
| C-2 | 72.47 | 72.46 | 72.45 | 72.45 |
| C-3 | 72.28 | 72.23 | 72.21 | 72.21 |
| C-4 | 74.03 | 74.01 | 74.00 | 74.03 |
| C-5 | 70.78 | 70.75 | 70.75 | 70.77 |
| C-6 | 17.97 | 17.96 | 17.99 | 17.99 |
| 26-*O*-glc | | | | |
| C-1 | | | 104.60 | 103.38 |
| C-2 | | | 75.21 | 75.18 |
| C-3 | | | 78.20 | 78.23 |
| C-4 | | | 71.81 | 71.85 |
| C-5 | | | 77.89 | 77.97 |
| C-6 | | | 62.07 | 62.08 |

Spectra were obtained in $CD_3OD$; $\delta$ in ppm, ref. TMS.

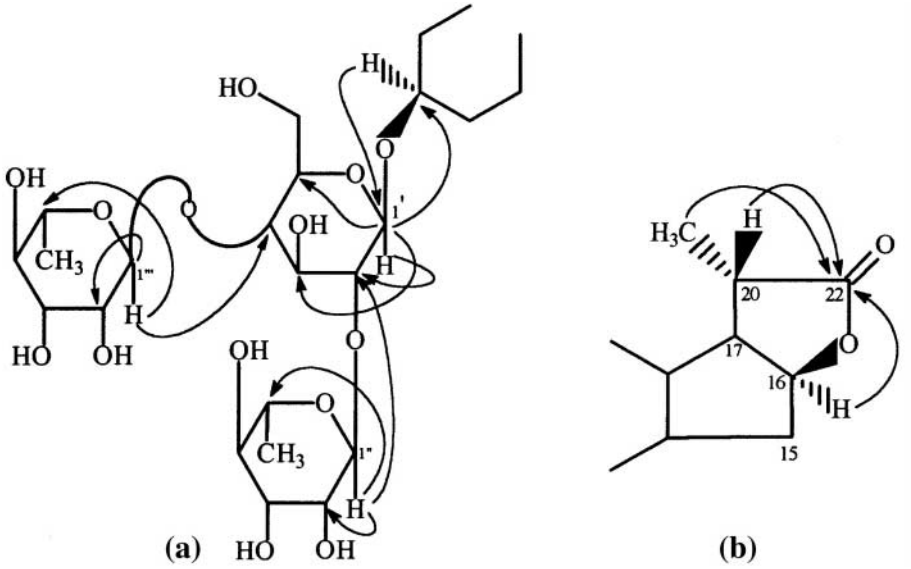



Fig. 1. Long-range $^{1}H$–$^{13}C$ couplings observed in the HMBC experiment.

assigned to carbon at $\delta$ 78.09 (C-3) and at $\delta$ 84.78 (C-16), respectively, with the help of a HETEROCOSY experiment. The glycosidic linkage to the aglycone and the presence of lactone ring were confirmed with the help of HMBC experiment [Fig. 1(b)].

The aglycone of **1** named as vespertiline was isolated for the first time from *Solanum vespertilio* (Gonzalez, Freire, Francisco, Salazar & Sua'rez, 1973). We are reporting the $^{13}C$ NMR chemical shifts of vespertiline in glycosidic form. With the help of above discussion the structure of compound **1** was established as (20*S*)-3β,16β-dihydroxy pregn-5-ene-22-carboxylic acid-(22, 16)-lactone-3-*O*-β-chacotrioside and named as dumoside. The compounds **2**–**4** were isolated for the first time from this species and characterized with the help of spectral data (Tables 1 and 2). The structures of **2**–**4** were confirmed by comparing their spectroscopic data with the reported data (Nakano et al., 1989; Aquino, Behar, Simone, Agostino & Pizza, 1986). From the best of our knowledge, compound **1** is a new natural product, where as **2**–**4** are reported in this communication as new compounds from this source.

## 3. Experimental

### 3.1. General

$^{1}H$ NMR spectra were recorded on Bruker-AM 400 and $^{13}C$ NMR spectra were recorded on Bruker AM-300 with TMS as internal standard. Mass spectra were measured on JMSHX-110 (Jeol), for FAB-MS, sulphur glycerol was used as matrix. IR spectra were obtained on JASCO A-320. Precoated silica gel plates (Merck,

Table 2
Partial $^{1}H$ NMR data for compounds **1-4** (in $CD_3OD$)

| H No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| H-3... | 3.40*m* | 3.41*m* | 3.37*m* | 3.37*m* |
| H-6... | 5.38*m* | 5.39*m* | 5.36*m* | 5.36*m* |
| H-16... | 5.01 *dt*, *J* = 9.12, 4.56 Hz | 6.89 *dd*, *J* = 3.36, 1.86 Hz | 4.33*m* | 4.33*m* |
| H-18... | 0.76*s* | 0.92*s* | 0.82*s* | 0.83*s* |
| H-19... | 1.05*s* | 1.07*s* | 1.04*s* | 1.04*s* |
| H-20... | 2.58*q*, *J* = 7.60 Hz | | 2.16*q*, *J* = 6.90 Hz | 2.16*q*, *J* = 6.95 Hz |
| H-21... | 1.29*d*, *J* = 7.6 Hz | 2.25*s* | 0.99*d*, *J* = 6.95 Hz | 0.97*d*, *J* = 6.25 Hz |
| H-27... | | | 0.93*d*, *J* = 6.73 Hz | 0.93*d*, *J* = 6.73 Hz |
| H-1′... | 4.49*d*, *J* = 7.80 Hz | 4.48*d*, *J* = 7.76 Hz | 4.48*d*, *J* = 7.82 Hz | 4.48*d*, *J* = 7.82 Hz |
| H-1″... | 5.20*d*, *J* = 1.68 Hz | 5.19*d*, *J* = 1.68 Hz | 5.19*d*, *J* = 1.55 Hz | 5.19*d*, *J* = 1.55 Hz |
| H-1‴... | 4.83*d*, *J* = 1.76 Hz | 4.82*d*, *J* = 1.75 Hz | 4.82*d*, *J* = 1.75 Hz | 4.82*d*, *J* = 1.75 Hz |
| H-1⁗... | | | 4.22*d*, *J* = 7.82 Hz | 4.26*d*, *J* = 7.82 Hz |
| Rha-$CH_3$ | 1.25*d*, *J* = 8.76 Hz | 1.25*d*, *J* = 6.20 Hz | 1.22*d*, *J* = 6.20 Hz | 1.22*d*, *J* = 6.25 Hz |
| Rha-$CH_3$ | 1.23*d*, *J* = 8.76 Hz | 1.23*d*, *J* = 6.20 Hz | 1.24*d*, *J* = 6.20 Hz | 1.24*d*, *J* = 6.25 Hz |

Spectra were obtained in $CD_3OD$; $\delta$ in ppm, ref. TMS.

silica gel 60, $F_{254}$, 0.25 mm and Rp-18 $F_{254}$, 0.25 mm) were used for TLC.

### 3.2. Plant material

The whole fresh plant material of *Asparagus dumosus* Baker (12 kg) was collected in April and identified by Prof. Dr. M. Qaiser, a voucher specimen is deposited in the Herbarium of the Department of Botany, University of Karachi, Pakistan (KUH-6347). The plant was dried under shade and finally chopped.

### 3.3. Extraction and isolation

The dried material of *A. dumosus* was extracted repeatedly with distilled MeOH (25 liters) at room temperature then concentrated *in vacuo*. The resulting residue was dissolved in $H_2O$, extracted with EtOAc followed with *n*-BuOH (5 liters). The combined *n*-BuOH layers were concentrated under red. press. to a brownish gummy residue (95 g) which was subjected to CC on silica gel with $CHCl_3$, $CHCl_3$–MeOH, and MeOH. The fraction eluted with MeOH–$CHCl_3$ (25:75, v/v) contained a mixture of compounds **1**, **2**, **3** and **4**. The compounds **1** (8.2 mg), **2** (17.8 mg), **3** (24.9 mg) and **4** (24.9 mg) were purified by HPLC with MeOH–$H_2O$ (7.2:2.8) using RP C-18 bondapak column with flow rate 3 ml/min and pressure was max. 500 Psi.

Dumoside (**1**) White amorphous (8.2 mg), mp. 185.5–187°C; $[\alpha]_D^{25}$ −13.3° (MeOH;0.36) IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500–3100, 1760. FAB-MS *m/z*: 797 $[M-H]^-$, 651 $[M-H-Rha]^-$, 505 $[M-H-Rha-Rha]^-$, 343 $[M-H-Rha-Rha-Glc]^-$, $^{13}C$ NMR: see Table 1. $^1H$ NMR see Table 2.

### 3.4. Acid hydrolysis of dumoside *1*

Compound **1** (6.0 mg) was dissolved in MeOH (1 ml) and 20% HCl (3 ml). The reaction mixture was refluxed for 6 hr. After cooling, the MeOH was evaporated *in vacuo*. The reaction mixture was neutralized with $Ag_2CO_3$, then filtered and concentrated under red. pres. The residue obtained contained D-glucose and L-rhamnose as sugar moieties identified by comparative TLC $H_2O$–MeOH–HOAc–EtOAc (1:2:1:6) with authentic sample.

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