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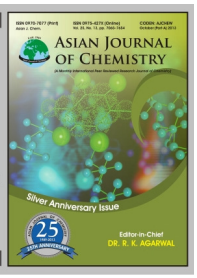

New Ursane Glycoside from the Roots of *Asparagus racemosus*

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One new compound urs-1,12,19- trien-3β-ol-28-oic acid-3β-D-glucopyranosyl (4'-1")-β-D-glucopyranoside (**1**) along with five known compounds (**2-6**) have been isolated from the roots of *Asparagus racemosus*. Their structures have been elucidated with the help of 500/125 MHz NMR using 1D and 2D spectral methods *viz*: ^{1}H and ^{13}C NMR, ESIMS and aided by IR spectroscopy.

Key Words: *Asparagus racemosus*, Liliaceae, Ursane glycoside.

INTRODUCTION

The genus *Asparagus* is usually an undershrubs or herbs, distributed in the temperate and tropical parts of the world. The genus was recently moved from the family Liliaceae (subfamily: Asparagae) to a newly created family Asparagaceae[1]. About 60 species are known to occur worldwide, while only about 22 species are recorded in India. *Asparagus racemosus* wild, a climbing under-shrub with numerous finger-like, clustered tuberous roots is an important member of the genus known for its adaptogenic, galactogogue and aphrodisiac activities[2-7]. It is commonly sold as tonic and is an active ingredient in several Ayurvedic and Unani medicinal preparations. Some constituents were reported from *A. racemosus*[8-15]. It is also used for dry coughs and gastric ulcers. *A. racemosus* was found to be effective for phagocytic activity and killing capacity of macrophages[16]. It is also used successfully for nervous disorders, inflammation, liver diseases and certain infectious diseases. The juice of fresh roots of *A. racemosus* has curative effect in patients with duodenal ulcers. Oral administration of decoction of powered root enhances the immuno-modulatory effect[17]. Also, recent research indicates Shatavari enhances immune function, increases corticosteroid production and promotes cell regeneration[18]. The selection of this herbs was made as it is available in abundance in the local vicinity of Western U.P. (Bareilly district), India and is well known for different curative properties in addition to their immunomodulatory effects. The drug is regularly used by local Indian traditional medicine system practitioners and many other benefits of these herbs are also recognized internationally.

The ethanol extract of the *A. racemosus* was separated by a combination of column chromatography over silica gel and obtained one new urs-1,12,19-trien-3β-ol-28 oic acid- 3β-D-glucopyranosyl (4'-1")-β-D-glucopyranoside (**1**) and five known compounds *n*-octadecanyl hexadecanoate (**2**), *n*-hexacosanyl-*n*-octadecanoate (**3**), β-sitosterol (**4**), 1-lauryl 3-arachidyl glycerol-3-phosphate (**5**), stigmasterol (**6**), were recrystallized to get the pure compound have been identified with 500/125 MHz NMR using 1D and 2D spectral methods *viz*., ^{1}H and ^{13}C NMR, ESI MS and FAB MS aided by IR spectroscopy.

EXPERIMENTAL

All the chemicals and reagents were obtained from SD Fine Chemicals and were of analytical grade. Sodium sulphate was used as drying agent for various solvents used to run the column. All the weighings were done on a single pan metler balance. Melting points were determined on microcontroller based, digital, automatic-photosensing MEPA melting point apparatus (LAB INDIA). Ultraviolet spectra were recorded on Lambda bio 20 spectrometer in methanol. Infrared spectra were recorded on Biored FT IR spectrophotometer using KBr pellets and CCl_4, ν_{max} values are given in cm^{-1}. ^{1}H NMR spectra were screened on advance dry 400, Brucker spectrospin 400 MHz, instrument using $CDCl_3$ and DMSO as solvent and TMS as an

internal standard. Chemical shift values are given in δ (ppm) scale and coupling constant (*J*) in Hz. Notations used throughout as a s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet and br s = unresolved broad singlet. The electrospray mass spectra were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. The samples (dissolved in suitable solvents) were introduced into the ESI source through a syringe pump at the rate of 5 μL/min.

The dried drug (Shatavari roots, 5 kg) was purchased from Natural Drugs and Botanicals, Sahibabad, Ghaziabad (U.P.) and was identified by Dr. M.P. Sharma, Reader and Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi.

Extraction of drugs: The air-dried drug (2 kg) was coarsely powdered and extracted in a Soxhlet apparatus with ethanol (95 %) for 48 h. The combined extracts were then concentrated on a water bath and dried under reduced pressure to get a dark brown mass (250 g).

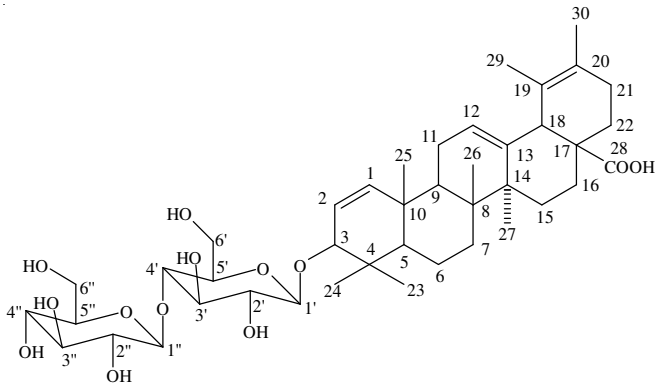
The concentrated extract of the drug was taken in a China dish and heated continuously on a water bath, gradually adding methanol in small portions with constant stirring, till desired consistency obtained. Silica gel (for column) was then added (weighed quantity) slowly with continuous mixing with a steel spatula until desired dry mixture is obtained. The larger lumps were broken-up and finally passed through a sieve (No. 8) to get a slurry of uniform particle size.

Isolation of the compounds from ethanol extract: The viscous dark green mass was adsorbed on silica gel (60-120 mesh) for column after being dissolved in little quantity of methanol for preparation of slurry. The slurry (150 g) was air dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and benzene (90:10, 80:20, 70:30, 60:40, 50:50, 40;60, 50:50, 60:40, 70:30, 80:20.90;10), pure benzene and then mixture of benzene and chloroform (90:10, 80:20, 70:30, 60:40, 50:50, 40;60, 50:50, 60:40, 70:30, 80:20.90;10), pure chloroform and finally the mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 90:10).

Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds urs-1,12,19-trien-3B-ol-28 oic acid-3B-D-glucopyranosyl (4'-1")-B-D-glucopyranoside (**1**), *n*-octadecanyl hexadecanoate (**2**), *n*-hexacosanyl-*n*-octadecanoate (**3**), β-sitosterol (**4**), 1-lauryl 3-arachidyl glycerol-3-phosphate (**5**), stigmasterol (**6**), were recrystallized to get the pure compound (Fig. 1).

Homogeneity of the fractions: The fractions collected were subjected to thin layer chromatography to check homogeneity of various fractions. Chromatographically identical fractions (having same R_f values) were combined together and concentrated. They were then crystallized with suitable solvent system.

Urs-1, 12, 19- trien-3β-ol-28-oic acid-3β-D-glucopyranosyl (4'-1")-β-D-glucopyranoside (1): Elution of the column with chloroform-methanol (4:1) mixture afforded colourless crystals recrystallized from methanol, 137.7 mg



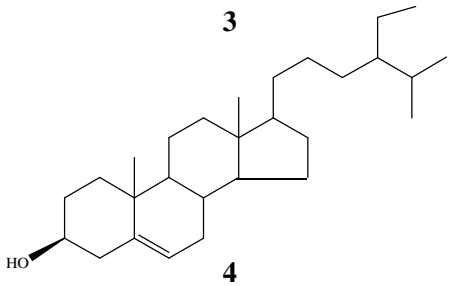
1

$CH_3(CH_2)_{14}COOCH_2(CH_2)_{16}CH_3$

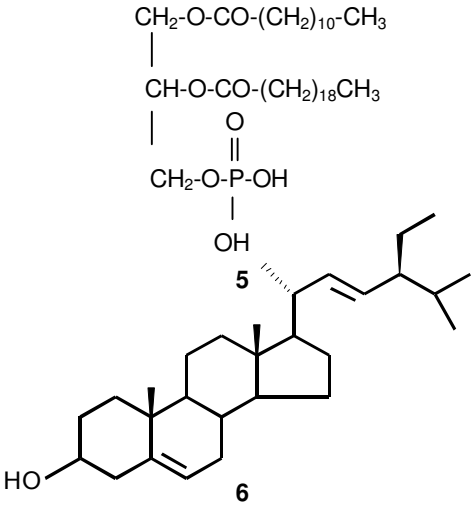
2

$CH_3(CH_2)_{16}CO\text{-}OCH_2(CH_2)_{24}\text{-}CH_3$

3



4



5

6

Fig. 1. Compounds (**1-6**) isolated from ethanolic extract of *A. racemosus* roots

(0.0077 % yield); R_f : 0.67 (benzene-chloroform-methanol : 3:4:1); m.p.: 315-316 ºC (decomposed); UV ν_{max} (MeOH): 243 nm (log ε 5.6); IR (KBr, ν_{max}, cm^{-1}): 3447, 3380, 3260, 2922, 2845, 1698, 1636, 1457, 1396, 1260, 1019, 795; ^1H NMR (DMSO-d_6) : δ 6.61 (1H, d, *J* = 6.8 Hz, H-1), 6.57 (1H, dd, *J* = 6.8, 4.8 Hz, H-2), 5.65 (1H, dd, *J* = 8.0 Hz, H-12), 5.17 (1H, d, *J* = 3.6 Hz, H-1'), 4.78 (1H, brs, H-1"), 4.52 (1H, m, H-5'), 4.50 (1H, m, H-5"), 4.36 (1H, m, H-2'), 4.33 (1H, m, H-2"), 4.11 (1H, d, *J* = 4.8 Hz, H-3a), 4.09 (1H, m, H-3'), 4.06 (1H, m, H-3"), 3.63 (1H, m, H-4'), 3.57 (1H, m, H-4"), 3.12 (2H, brs, H2-6'), 3.04 (2H, brs, H2-6"), 2.92 (1H, d, *J* = 3.6

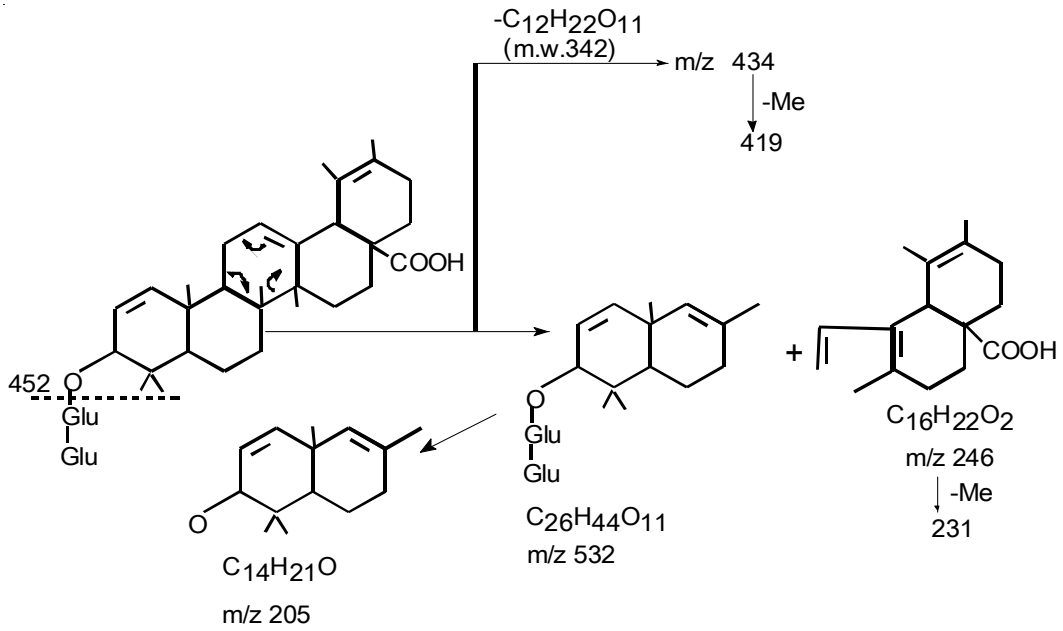


Fig. 2. Mass fragmentation pattern of compound (**1**)

Hz, H-18 a), 2.61 (2H, brs, H2-11), 2.37 (2H, m, H2-21), 2.02 (1H, m, H-5 a), 1.96 (3H, brs, Me-29), 1.82 (2H, brs, H2-22), 1.68 (3H, brs, Me-30), 1.68 (2H, m, H2-6), 1.63 (2H, m, H2-7), 1.39 (2H, m, H2-15), 1.17 (6H, brs, Me-25, Me-23), 1.15 (3H, brs, Me-26), 1.07 (3H, brs, Me-27), 0.95 (2H, m, H2-16), 0.85 (3H, brs, Me-24); ^{13}C NMR (DMSO-d_6): δ 138.96 (C-1),, 109.16 (C-1'), 103.81 (C-1"), 164.27 (C-2), 74.85 (C-2'), 74.85 (C-2"), 73.89 (C-3), 64.18 (C-3'), 66.21 (C-3"), 42.61 (C-4), 65.07 (C-4'), 63.89 (C-4"), 53.29 (C-5), 81.73 (C-5'), 82.08 (C-5"), 19.71 (C-6), 62.11 (C-6'), 62.77 (C-6"), 32.25 (C-7), 41.33 (C-8), 47.81 (C-9), 37.23 (C-10), 23.19 (C-11), 129.75 (C-12), 139.38 (C-13), 42.11 (C-14), 29.16 (C-15), 20.17 (C-16), 48.21 (C-17), 53.83 (C-18),142.13 (C-19), 143.41 (C-20), 36.22 (C-21), 34.18 (C-22), 27.37 (C-23), 19.28 (C-24), 16.51 (C-25), 21.35 (C-26), 22.89 (C-27), 183.12 (C-28), 25.16 (C-29), 21.36 (C-30); +ve ion ESI MS *m/z* (rel. int.): 648 $[M]^+$, ($C_{42}H_{64}O_{13}$) (7.5), 295 (8.1), 183 (8.3).

Acid hydrolysis of 1: Compound 1 was refluxed with 2 mL of 1 mol/L hydrochloric acid: dioxane (1:1, V:V) in awater water bath for 4 h. The reaction mixture was evaportaed to dryness and partitioned with chloroform and water four times, extyract concentrated. The chloroform extract contained the aglycone portion, while the water extract contained β-D glucose.

RESULTS AND DISCUSSION

Compound **1**, named asparagusursenyl glycoside, was obtained as colourless crystals from chloroform-methanol (4:1) eluants. It responded positively to triterpenic glycosides. It showed UV absorption maxima at 243 nm which is a characteristic of highly conjugated system. Its IR spectrum exhibited absorption band for hydroxyl group (3447, 3380 and 3260 cm^{-1}), carboxylic group (1698 cm^{-1}) and unsaturation (1638 cm^{-1}) in the molecule. Its ESIMS spectrum displayed molecular ion peak at *m/z* 776 corresponding to molecular formula of pentacyclic triterpenic diglycoside, $C_{42}H_{64}O_{13}$. It indicated eleven double bond equivalents; five of them were adjusted in the pentacyclic carbon framework, three in the vinylic linkages, one in the carboxylic group and the remaining two in the glycosidic moieties. The retro-Diels-Alder fragmentation of **1** generated ion peaks at *m/z* 242, 205 $[M\text{-}246\text{-}C_{12}H_{21}O_{10}]^+$ and 231 $[246\text{-}Me]^+$ indicating that the molecule contained one each vinylic linkage in rings A, C and E. The other prominent ions arose at *m/z* 434 $[M\text{-}C_{12}H_{22}O_{11}$, m.u. 342$]^+$ and 419 $[434 - Me]^+$ indicating that a diglycoside moiety was attached to the molecule. The fragmentation pattern of 1 is shown in Fig. 2.

Its ^{1}H NMR spectrum showed two one-proton doublets at δ 6.61 (J = 6.8 Hz) and δ 5.17 (J = 3.6 Hz) assigned to vinylic H-1 and anomeric H-1', respectively and two one-proton double doublets at δ 6.57 (J = 6.8, 4.8 Hz) at δ 6.61 (J = 6.8 Hz) and d 5.17 (J = 3.6 Hz H-2 and H-12, respectively. A one-proton broad singlet appeared at δ 4.78 accounted to anomeric H-1". Four one-proton multiplets appeared at δ 4.52, 4.50, 4.36 and 4.33 attributed to hydroxy methine protons H-5', H-5", H-2' and H-2", respectively. A one-proton doublet appeared at δ 4.11 (J = 4.8 Hz) was ascribed to H-3α. The remaining carbinol protons of the sugar moiety appeared between δ 4.09-3.57. Two two-proton broad signal appeared at δ 3.12 and 3.04 accounted for hydroxy methylene protons H2-6' and H2-6" respectively. A one-proton doublet appeared at δ 2.92 (J = 3.6 Hz) ascribed to H-18α. A two-proton broad singlet at δ 2.61 accounted for H2-11 adjacent to the vinylic linkage. A two-proton multiplet at δ 2.37 was assigned to H2-21. A one proton multiplet appeared at δ 2.02 assigned to H-5α. Two three proton broad signals appeared at δ 1.96 and 1.68 attributed to Me-29 and Me-30, attached to the vinylic carbons, respectively. A six proton broad signal appeared at δ 1.17 was ascribed to tertiary methyl Me-25, Me-23 protons. Three broad signals at δ 1.15, 1.07 and 0.85, integrated for three protons each, were accounted correspondingly to tertiary C-26, C-27 and C-24 methyl protons. The remaining methylene protons resonated between δ 1.82 - 0.95. The ^{13}C NMR spectrum of 1 showed important signals for C-28 carboxylic carbon at δ 183.12, vinylic carbons at δ 138.96 (C-1), 164.27 (C-2), 129.75

(C-12), 139.38 (C-13), 142.13 (C-19) and 143.41 (C-20), carbinol carbon at δ 73.89 (C-3), anomeric carbons at δ 109.16 (C-1') and 103.81 (C-1''), hydroxyl methylene carbons at δ 62.11 (C-6'' and 62.77 (C-6''), hydroxymethine carbons between δ 82.00-63.89 and the remaining methine and methylene carbons in the range δ 53.89-19.71. The methyl carbon appeared at δ 27.37 (C-23), 19.28 (C-24), 16.51 (C-25), 21.35 (C-26), 22.89 (C-27), 25.16 (C-29) and 21.36 (C-30). Acid hydrolysis of 1 yielded β-D-glucose (TLC comparable). On the basis of spectral data analysis and chemical reactions the structure of 1 has been formulated as urs-1, 12, 19- trien-3β-ol-28-oic acid-3β-D-glucopyranosyl (4-1'')-β-D-glucopyranoside. This is a new ursane-type triterpenic diglycoside isolated from a natural source for the first time.

Conclusion

One new compound as Urs-1, 12, 19- trien-3β-ol-28-oic acid-3β-D-glucopyranosyl (4'-1'')-β-D-glucopyranoside along with five known compounds have been isolated.

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