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b-Sitosterol-3-O-*b*-D-xylopyranoside from the flowers of *Tridax procumbens* Linn.

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Abstract. *Tridax procumbens* Linn belongs to the natural order Compositae and is locally known as 'Ghamra'. It has been found to possess significant medicinal properties. Its leaves are used in bronchial catarrh, dysentery, diarrhoea and to prevent falling of hair. Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties, and are also used to check haemorrhage from cuts, bruises and wounds.

The present work deals with the isolation and identification of steroidal saponin, characterized as *b*-sitosterol 3-O-*b*-D-xylopyranoside, which has been isolated from the flowers of *Tridax procumbens* Linn.

Keywords. *Tridax procumbens* Linn.; Compositae; steroidal saponin; *b*-sitosterol-3-O-*b*-D-xylopyranoside.

1. Introduction

Tridax procumbens Linn.[1–4] (natural order Compositae) is commonly known as *Ghamra* in Hindi. It has been found to have significant medicinal properties. Its leaves are used in the treatment of bronchial catarrh, dysentery and diarrhoea and for preventing hair loss. The juice of its leaves possesses antiseptic, insecticidal and parasiticidal properties. It is also used to check haemorrhage from cuts, bruises and wounds. An aqueous extract of this plant also has marked depressant action on respiration.

Earliar workers[5–7] have already reported the presence of dexamethasone luteolin, glucoluteolin, *b*-sitosterol and quercetin in this plant.

2. Results and discussion

Air-dried and powdered flowers of *Tridax procumbens* Linn. were extracted with 95% hot ethanol and this ethanolic extract was successively extracted with solvents benzene, chloroform, acetone, ethyl acetate and methanol. The concentrated methanol soluble fraction was subjected to column chromatography over alumina. On elution with chloroform : methanol (3 : 2), it gave a compound **I** that analysed for mol. formula $C_{34}H_{58}O_5$, M^+ [546], m.p. 196–198°C. It showed all the characteristic colour reactions of saponin.[8,9]

Compound **I** on acid hydrolysis yielded a glycone **Ia**, $C_{29}H_{50}O$, m.p. 136–138°C and a sugar moiety, D-xylose (R_f = 0·29).

Permethylation by Kuhn procedure[10] followed by acid hydrolysis of saponin **I**, yielded aglycone **Ia** and a methylated sugar identified as 2,3,4-tri-O-methyl-D-xylose showing the presence of D-xylose in pyranose form and also that C-1 –OH group of D-xylose was involved in glycosidation.

Enzymatic hydrolysis of the steroidal saponin **I** with almond emulsion gave aglycone **Ia** and D-xylose (CoPC), indicating *b*-linkage between aglycone **Ia** and D-xylose.

Sodium metaperiodate oxidation of saponin **I** consumed 2·05 moles of periodate and liberated 1·05 moles of formic acid, indicating the presence of sugar and aglycone **Ia** in equimolar ratio (1 : 1) and also confirming that the sugar D-xylose was present in pyranose form.

The IR spectrum of the aglycone **Ia** showed a band at n_{max}^{KBr} 3404·5 cm^{-1}, which indicated the presence of –OH group(s) in it . The aglycone **Ia** was found to form mono acetyl derivative $C_{31}H_{52}O_2$ M^+ [450] m.p. 145–147°C. Estimation of acetyl group (10·23%) by Wiesenberger[11] method as described by Belcher and Godbert[12] indicated the presence of only one –OH group in **Ia**.

The Cr_2O_3/pyridine oxidation of the aglycone **Ia** yielded a ketone $C_{29}H_{48}O$, *M*+ [412], m.p. 162–163°C giving a positive Zimmermann test[13] for the C-3 keto group thereby confirming the presence of one

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–OH group at C-3 and further indicating its nature as secondary in **Ia**.

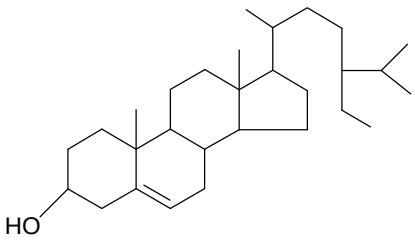
All the above facts help to conclude that the C-3 –OH group of the aglycone **Ia** was linked to the C-1 –OH group of D-xylose via *b*-linkage.

A band at n_{max}^{KBr} 1638·0 cm^{-1} in the IR-spectrum of aglycone **Ia** indicated unsaturation in it. On catalytic hydrogenation with Pd/C a dihydro derivative of formula, $C_{29}H_{52}O$, m.p. 150–152°C was obtained, indicating the presence of one double bond. In the ^{1}H-NMR spectrum of **Ia** a one-proton signal appeared at *d* 5·32, which indicated the presence of an olefinic proton at C-6 owing to the double bond between C-5 and C-6 of aglycone **Ia**.

The IR spectrum of aglycone **Ia** showed a band at n_{max}^{KBr} 2924·0 cm^{-1} for an angular methyl group, which when estimated by Ziesel's method (12·47%) confirmed the presence of six methyl groups in it. The H-NMR spectrum of the aglycone **Ia** showed singlets at *d* 0·67 and 0·98 for the angular methyl groups C-18 and C-19, doublets at *d* 0·92, 0·82 and 0·80 and a triplet at *d* 0·84 confirming the presence of C-21, C-26, C-27 and C-29 methyl groups respectively in **Ia**.

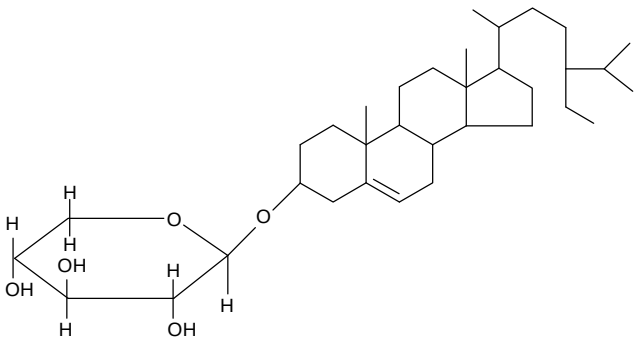
The anomeric proton of the xylose (H-1′) was present as a doublet at *d* 4·5 with *J* = 8 Hz due to axial–axial coupling, thus showing that the xylose is *b*-linked to the aglycone. Further, the ^{13}C-NMR spectrum of the saponin **I** showed a signal at *d* 99·4 assigned to C–1′, indicating *b* configuration for the xylose. If the configuration were *a*, the signal would have appeared at *d* 94·2. This value was calculated taking into account the fact that the anomeric carbon of the methyl-tri-O-acetyl xylopyranoside appears at 96·4 and the shift due to substitution of the methyl group by the aglycone is Δd = 2 ppm.[14]

On the basis of the above deliberations, the steroidal saponin **I** was identified as *b*-sitosterol-3-O-*b*-D-xylopyranoside.



Ia

Chart 1.



I

3. Experimental

3.1 *Plant material*

The flowers of *Tridax procumbens* Linn were collected locally and identified by an expert taxonomist.

3.2 *Extraction and isolation*

The flowers (2·0 kg) of *Tridax procumbens* Linn were air-dried, powdered and extracted with 95% hot ethanol. The ethanolic extract was concentrated under reduced pressure and the residue obtained was subjected to successive extraction with solvents benzene, chloroform, acetone, ethyl acetate and methanol. The methanol soluble part was concentrated and extracted with aqueous *n*-butanol. The *n*-butanol extract was concentrated when it yielded a crude compound, which was subjected to column chromatography over alumina. On elution with chloroform : methanol (3 : 2) and crystallisation from chloroform : methanol (1 : 1), it gave a compound **I** which showed a single homogeneous spot on TLC[15] over silica gel using $CHCl_3$: Me_2CO : HCOOH (9 : 2 : 1) as solvent and I_2 vapours as visualizing agent. Compound **I**: Colourless needles, m.p. 196–198°C, M^+ [546]; IR spectrum n_{max}^{KBr} (cm^{-1}) – 3425·0 (–OH group), 2940 (–CH_2–CH_3 stretch), 1650·3 (C–C stretch), 1367·7 (–$CH(CH_3)_2$ stretch), 1470·0 (C–H bending).

3.3 *Acid hydrolysis of saponin* **I**

Saponin **I** (60 mg) was refluxed with 20 ml of 7% H_2SO_4 for 6 h, cooled and filtered to afford aglycone **Ia** (*b*-sitosterol), confirmed by mixed melting point and superimposable ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis.[16–20] Colourless needles, m.p. 136–138°C, M^+ [414]. IR spectrum n_{max}^{KBr} (cm^{-1}) 3404·5 (–OH group), 2924·0 (–CH_3 stretch), 1635·0

(C=C stretch), 1455·0 (C–H bending). FABMS *m/z* [414] M^+ 399, 396, 381, 329, 303, 301, 275, 273, 272, 271, 255, 253, 231, 229 and 213.

^{13}C-NMR: 37·0 (C-1), 29·5 (C-2), 79·9 (C-3), 38·7 (C-4), 140·2 (C-5), 121·9 (C-6), 31·7 (C-7), 32·2 (C-8), 50·5 (C-9), 36·6 (C-10), 21·0 (C-11), 39·6 (C-12), 42·2 (C-13), 56·6 (C-14), 24·2 (C-15), 28·1 (C-16), 55·9 (C-17), 11·6 (C-18), 19·2 (C-19), 36·0 (C-20), 18·6 (C-21), 34·0 (C-22), 26·0 (C-23), 45·7 (C-24), 29·2 (C-25), 19·6 (C-26), 18·9 (C-27), 23·0 (C-28), 11·8 (C-29).

^{1}H-NMR: 1·01 (2H, *m*, H-1), 1·37 (2H, *m*, H-2), 3·82 (1H, *m*, H-3), 2·62 (2H, *m*, H-4), 5·32 (1H, *t*, H-6), 1·93 (2H, *m*, H-7), 1·54 (1H, *m*, H-8), 0·94 (1H, *m*, H-9), 1·44 (2H, *m*, H-11), 1·69 (2H, *m*, H-12), 1·10 (1H, *m*, H-14), 1·51 (2H, *m*, H-15), 4·61 (2H, *m*, H-16), 1·74 (1H, *m*, H-17), 0·67 (3H, *s*, H-18), 0·98 (*s*, 3H, H-19), 1·90 (1H, *m*, H-20), 0·92 (3H, *d*, *J* = 2·9 Hz, H-21), 1·62 (2H, *m*, H-22), 1·65 (2H, *m*, H-23), 1·58 (1H, *m*, H-24), 1·56 (1H, *m*, H-25), 0·82 (3H, *d*, *J* = 7 Hz, H-26), 0·80 (3H, *d*, *J* = 7 Hz, H-27), 1·52 (2H, *m*, H-28), 0·84 (3H, *t*, H-29).

The neutralized and concentrated aqueous hydrolysate showed the presence of D-xylose [PC, solvent B : A : W (4 : 1·5), R_f value = 0·29].

3.4 *Permethylation of saponin* **I**

The saponin **I** (10 mg) was treated with methyl iodide (7 ml) and Ag_2O (40 ml) in DMF (5 ml) in a 150 ml conical flask and kept for 4 days at room temperature. The contents were filtered and the residue was washed with DMF. The filtrate was concentrated under reduced pressure to give a viscous mass which on hydrolysis with HCI gave aglycone **Ia** and methylated sugar. The aglygone **Ia** was separated and the aqueous hydrolysate was neutralized with $BaCO_3$. $BaSO_4$ formed was filtered off and the filtrate was concentrated under reduced pressure. The sugar was examined by paper chromatography[21] using Whatman filter paper No. 1, B : A : W (4 : 1 : 5) as solvent system and aniline hydrogen phthalate as spraying reagent. The sugar was identified as 2,3,4-tri-O-methyl-D-xylose.

3.5 *Periodate oxidation of saponin* **I**

The saponin **I** (25 mg) in H_2O (10 ml) was mixed with $NaIO_4$ (250 mg) and the solution was kept in the dark for 48 h. Ethylene glycol was added to decompose excess $NaIO_4$ and the solution was hydrolysed with 10% MeOH–HCl (45 min). It was then filtered and upon neutralization the filtrate did not show the presence of any monosaccharide.

3.6 *Enzymatic hydrolysis of saponin* **I**

The saponin **I** (40 mg) was dissolved in MeOH mixed with almond emulsion (30 ml) in a 100 ml conical flask fitted with a stopper. The contents were allowed to stand at room temperature for 48 h and then filtered. The concentrated hydrolysate was examined by paper chromatography for the sugar moiety using Whatman filter paper No. 1 and B : A : W (4 : 1 : 5) as the solvent system. The sugar was identified as D-xylose.

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