

TORVONIN-B, A SPIROSTANE SAPONIN FROM SOLANUM TORVUM<sup>1</sup>

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**Abstract** - A new steroidal saponin, 'torvonin B' (1) has been isolated from S. torvum leaves and its structure has been established as neosolaspigenin-3-O- $\beta$ -D-fucopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-quinovopyranoside.

In continuation with our work on steroidal saponins from Solanum torvum<sup>2</sup>, now we have isolated a new saponin, designated as torvonin B (1) from the chloroform extract of the leaves of S. torvum.

## RESULTS AND DISCUSSION

Chromatographic fractionation of the chloroform extract of the leaves of Solanum torvum afforded a saponin 1 which showed broad hydroxyl absorption bands in the region of 3700-3200  $\text{cm}^{-1}$  and 1160-1000  $\text{cm}^{-1}$  in its ir spectrum, indicative of its glycosidic behaviour. Its <sup>1</sup>H nmr spectrum displayed six methyl signals, two as singlets at  $\delta$  0.82, 0.84 (H-18 and H-19), four doublets ( $J = 6.5$  Hz) at  $\delta$  1.53, 1.55, 1.64 and 1.71 corresponding to H-21, H-27 and two methyls of two 6-deoxysugars. The appearance of one proton quartet ( $J=7.5$  Hz) at  $\delta$  4.63, a doublet ( $J=11$  Hz) at  $\delta$  3.55 and a double doublet at  $\delta$  4.20 ( $J=11, 2.5$  Hz) corresponding to hydrogens at 16, 26 $\beta$  and 26 $\alpha$  positions, respectively, indicated its spirostane nature with an axially oriented methyl group at C-25 (25S)<sup>3</sup>. The hydroxymethine signals at  $\delta$  3.65 and 4.06 (see experimental) were assigned to the equatorial orientation of 6-hydroxy group and the axial orientation of the 23-hydroxy group<sup>4</sup>, whereas the hydrogen at C-3 at  $\delta$  3.73 as broad multiplet ( $W_{\frac{1}{2}} = 22$  Hz) inferred its axial position. Remaining <sup>1</sup>H nmr signals in the region 0.5 to 3.2 ppm resembled very much with the reported <sup>1</sup>H nmr for neosolaspigenin<sup>4</sup>, thus identifying its genin as neosolaspigenin. The anomeric proton signals observed at  $\delta$  4.75 as a doublet ( $J=8$  Hz) and at  $\delta$  4.81 as broad singlet ( $W_{\frac{1}{2}}=4$  Hz) clearly demonstrated the  $\beta$ -anomeric configuration of the H-1 of the 6-deoxyhexose sugars.

Acid Hydrolysis of 1 resulted in the formation of several products (genin part) which could

not be isolated<sup>5,6</sup> due to paucity of material whereas fucose and quinovose were identified (PC) in sugar part. 1 readily formed a heptaacetyl derivative 2. The mass spectrum of 2 showed characteristic fragment at m/z 503 for the pentaacetyl-fucosylquinovose moiety in addition to peaks at m/z 273, 189, 171, 153 and 111 due to triacetyl- and diacetyl-6-deoxy-hexose sugar moieties.

The <sup>13</sup>C nmr spectrum of 1 (Table 1) showed 39 carbon signals which were due to 21 x CH, 9 x CH<sub>2</sub>, 6 x CH<sub>3</sub> and 3 x quaternary carbon atoms as depicted from the DEPT spectrum, hence inferring the presence of a disaccharide moiety<sup>7</sup>. The appearance of the signal for C-22 at δ 110.44 which was ca. 1 ppm downfield in comparison with ring-F unsubstituted spiro-stane<sup>8,9</sup> suggested the presence of an axial hydroxyl at C-23 as equatorial orientation of the 23-OH group led to its appearance at 112.6 ppm<sup>10</sup>. The signals due to the aglycone were assigned by comparison with reported literature values<sup>8</sup> which lead to the identity of the genin as neosolaspigenin. The assignment of the <sup>13</sup>C nmr resonances of the sugar carbon resonances was based upon comparison with the spectra of appropriate methyl-β-D-glycopyranoside<sup>11</sup>, the known glycosidation shifts<sup>7,8</sup> and the assignments reported for similar glycosides<sup>12,13</sup>. This inferred the presence of β-D-fucopyranosyl moiety as the terminal sugar residue which is linked to β-D-quinovopyranosyl moiety via (2 → 1) interglycosidic linkage as C-2 appeared at 6.89 ppm lowerfield. Moreover C-1 and C-3 resonances of the β-D-quinovopyranose were observed at 2.24 and 1.81 ppm higher field thus providing further proof for the above mentioned interglycosidic linkage. The appearance of C-2, C-3 and C-4 resonances at δ 32.23, 79.45 and 32.38 clearly demonstrated the presence of free hydroxyl groups at C-6 and C-23 positions and involvement of 3 β -OH group in the formation of glycosidic bond<sup>8</sup>. Considering all the above evidences, torvonin-B was identified as neosolaspigenin-3-O-β-D-fucopyranosyl-(1 → 2)-β-D-quinovopyranoside (1).

#### EXPERIMENTAL

Plant Material - The leaves of S. torvum were collected from Dehradun, U.P.(India). A voucher specimen is deposited in CIMAP herbarium collection No.249.

Extraction and Purification - The air dried leaves (5.5 kg) were powdered and extracted at room temp. by stirring for 16 h with n-hexane (5 x 7 l) followed by MeOH (5 x 7 l). The MeOH extract was evaporated in vacuo to 500 ml. Water(1 l) was added and the mixture was extracted with CHCl<sub>3</sub> (5 x 1 l). CHCl<sub>3</sub> extract was concentrated to dryness (122 g) and

a part of the extractive (60 g) was chromatographed over silica gel and eluted with hexane and hexane with increasing polarities of benzene and  $\text{CHCl}_3$ . The eluates were collected in 500 ml portions.

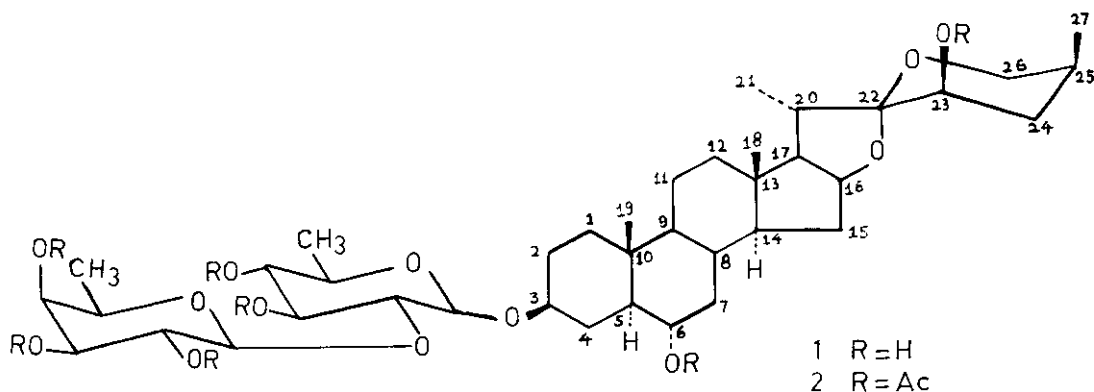


Table 1

 $^{13}\text{C}$  Nmr Chemical Shifts for Torvonin-B (Pyridine- $d_5$ )

Carbon No.	Chemical Shifts	Carbon No.	Chemical Shifts	Carbon No.	Chemical Shifts
1.	37.79	15	33.17	3-Quinovose	
2	32.23	16	81.56	1	103.06
3	79.45	17	64.57	2	83.49
4	32.38	18	17.20	3	76.19
5	51.33	19	13.59	4	75.24
6	69.92	20	41.17	5	72.77
7	41.43	21	16.56	6	18.64
8	34.30	22	110.44	(2+1) fucose	
9	53.91	23	65.35	1	105.59
10	36.75	24	34.49	2	72.68
11	21.22	25	27.28	3	74.16
12	39.95	26	65.35	4	72.58
13	40.86	27	20.52	5	70.63
14	66.48			6	16.79

An important fraction obtained by eluting with  $\text{CHCl}_3$ -MeOH (85:15) gave white residue (4.5 g) which exhibited the presence of two spots with very close  $R_f$  values on tlc. The above

residue, on further fractionation on silica gel column using  $\text{CHCl}_3$ -MeOH- $\text{H}_2\text{O}$  (60:17:10, organic layer as eluent, 100 ml each fraction) yielded torvonin B containing fractions (17-19) which afforded torvonin B (105 mg) on crystallization (MeOH- $\text{CHCl}_3$ ).

Torvonin B (1), colourless powder (Anal. Calcd for  $\text{C}_{39}\text{H}_{64}\text{O}_3$ : C, 63.25; H, 8.64. Found C, 63.22; H, 8.65), mp  $274^\circ\text{C}$ ,  $[\alpha]_D = -4.5^\circ$  (C, 0.30, Pyridine). Ir  $\nu^{\text{KBr}}$   $\text{cm}^{-1}$  3700-3200 (broad, OH), 1380, 1215, 1170, 1160-1000 (broad, C-O-C), 950, 930, 900 and 830.  $^1\text{H}$  Nmr (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ )  $\delta$  0.82 (3H, s, H-18), 0.84 (3H, s, H-19), 1.53 (3H, d, J = 6.5 Hz, H-21), 1.55 (3H, d, J = 6.5 Hz, H-27), 1.64 (3H, d, J = 6.5 Hz, H-6 of sugar), 1.71 (3H, d, J = 6.5 Hz, H-6 of sugar), 3.55 (1H, d, J = 11 Hz,  $26\alpha$  -H), 3.65 (1H, td, J = 10, 5 Hz,  $6\beta$  -H), 3.73 (1H, m,  $W_{1/2} = 22$  Hz,  $3\alpha$  -H), 3.80 (4H, m, sugar-H), 4.00 (1H, m, sugar-H), 4.06 (1H, t, J = 7 Hz,  $23\alpha$  -H), 4.20 (1H, dd, J = 11, 2 Hz,  $26\beta$  -H), 4.28 (1H, t, J = 9 Hz, sugar-H), 4.35 (1H, t, J = 11 Hz, sugar-H), 4.61 (1H, dd, J = 9, 4 Hz, sugar-H), 4.63 (1H, q, J = 7.5 Hz, 16-H), 4.75 (1H, d, J = 8 Hz, anomeric-H), 4.81 (1H, brs,  $W_{1/2} = 4$  Hz, anomeric-H);  $^{13}\text{C}$  nmr (see table 1).

Acetylation of torvonin B. Compound (1) (10 mg) was acetylated with  $\text{Ac}_2\text{O}$  (0.2 ml) and pyridine (0.2 ml) at room temperature for 12 h and after usual work up it gave hexaacetate 2 (12 mg) (Anal. Calcd for  $\text{C}_{53}\text{H}_{78}\text{O}_{20}$ : C, 61.51; H, 7.54; Found C, 61.53; H, 7.53), mp  $169^\circ\text{C}$ . Ir  $\nu^{\text{KBr}}$   $\text{cm}^{-1}$  2950, 2845, 1710 (broad), 1456, 1382, 1240-1205 (broad), 956, 935, 860, 845. Ms m/z 1034 ( $\text{M}^+$ ), 914, 503, 397, 283, 273, 189, 171, 153, 111, 42.

Acid Hydrolysis of torvonin B. Compound (1) (20 mg) was treated with 7% methanolic  $\text{H}_2\text{SO}_4$  (5 ml) for 5 h under reflux and worked up as usual. The aq. portion revealed the presence of fucose and quinovose on PC (BuOH-AcOH- $\text{H}_2\text{O}$ , 4:1:5) by comparison with authentic samples while aglycone part showed several spots on tlc due to decomposition of genuine aglycone<sup>4,5</sup>.

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