TORVONIN-B, A SPIROSTANE SAPONIN FROM SOLANUM TORVUM[1]

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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-β-D-fucopyranosyl-(1 → 2)-β-D-quinovopyranoside.

In continuation with our work on steroidal saponins from Solanum torvum[2], 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 cm^{-1} and 1160-1000 cm^{-1} in its ir spectrum, indicative of its glycosidic behaviour. Its ^1H nmr spectrum displayed six methyl signals, two as singlets at δ 0.82, 0.84 (H-18 and H-19), four doublets (J = 6.5 Hz) at δ 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 δ 4,63, a doublet (J=11 Hz) at δ 3.55 and a double doublet at δ 4.20 (J=11, 2.5 Hz) corresponding to hydrogens at 16, 26β and 26α positions, respectively, indicated its spirostane nature with an axially oriented methyl group at C-25 (25S)[3]. The hydroxymethine signals at δ 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[4], whereas the hydrogen at C-3 at δ 3.73 as broad multiplet ($W_{½}$ = 22 Hz) inferred its axial position. Remaining ^1H nmr signals in the region 0.5 to 3.2 ppm resembled very much with the reported ^1H nmr for neosolaspigenin[4], thus identifying its genin as neosolaspigenin. The anomeric proton signals observed at δ 4.75 as a doublet (J=8 Hz) and at δ 4.81 as broad singlet ($W_{½}$=4 Hz) clearly demonstrated the β-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[5,6] 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 pentaacetylfucosylquinovorose 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 ^{13}C nmr spectrum of 1 (Table 1) showed 39 carbon signals which were due to 21 x CH, 9 x CH_2, 6 x CH_3 and 3 x quaternary carbon atoms as depicted from the DEPT spectrum, hence inferring the presence of a disaccharide moiety[7]. The appearance of the signal for C-22 at δ 110.44 which was ca. 1 ppm downfield in comparison with ring-F unsubstituted spirostane[8,9] 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[10]. The signals due to the aglycone were assigned by comparison with reported literature values[8] which lead to the identity of the genin as neosolaspigenin. The assignment of the ^{13}C nmr resonances of the sugar carbon resonances was based upon comparison with the spectra of appropriate methyl-β-D-glycopyranoside[11], the known glycosidation shifts[7,8] and the assignments reported for similar glycosides[12,13]. This infered 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 3β -OH group in the formation of glycosidic bond[8]. 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 $CHCl_3$ (5 x 1 l). $CHCl_3$ 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 $CHCl_3$. The eluates were collected in 500 ml portions.

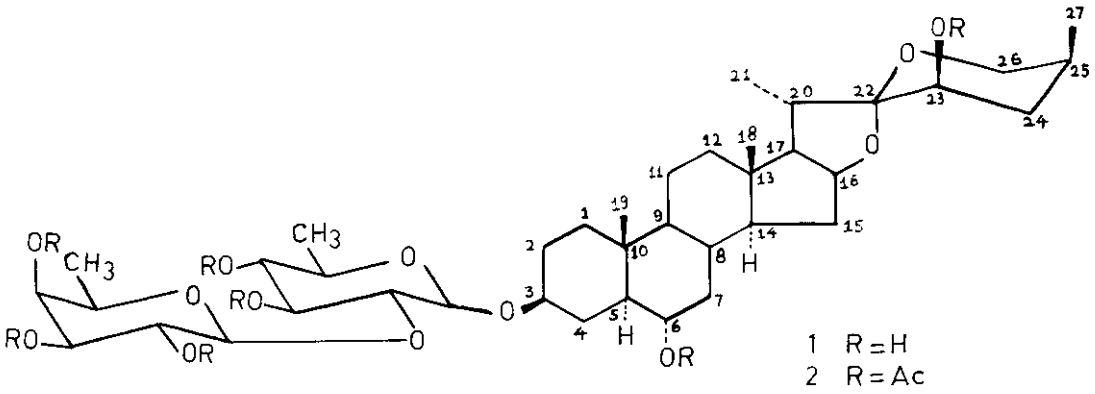

Table 1

^{13}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 $CHCl_3$-MeOH (85:15) gave white residue (4.5 g) which exhibited the presence of two spots with very close Rf values on tlc. The above

residue, on further fractionation on silica gel column using $CHCl_3$-MeOH-H_2O (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-$CHCl_3$).

Torvonin B (1), colourless powder (Anal. Calcd for $C_{39}H_{64}O_3$: C, 63.25; H, 8.64. Found C, 63.22; H.8.65), mp 274°C, $[\alpha]_D$ = -4.5°(C,0.30, Pyridine). Ir ν^{KBr} cm^{-1} 3700-3200 (broad, OH), 1380,1215, 1170, 1160-1000 (broad,C-O-C), 950,930,900 and 830. ^1H Nmr (400 MHz, C_5D_5N) δ 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 = 6.5Hz, H-27), 1.64 (3H, d, J = 6.5Hz, H-6 of sugar), 1.71 (3H, d, J = 6.5Hz, H-6 of sugar), 3.55 (1H, d, J = 11Hz, 26α -H), 3.65 (1H, td, J = 10, 5Hz, 6β -H), 3.73 (1H, m, $W_{½}$ = 22Hz, 3α -H), 3.80 (4H, m, sugar-H), 4.00 (1H, m, sugar-H), 4.06 (1H, t, J = 7Hz, 23α-H), 4.20 (1H, dd, J = 11, 2Hz, 26β -H), 4.28 (1H, t, J = 9Hz, sugar-H), 4.35 (1H, t, J = 11Hz, sugar-H), 4.61 (1H, dd, J=9, 4Hz, sugar-H), 4.63 (1H, q, J=7.5Hz, 16-H), 4.75 (1H, d, J = 8Hz, anomeric-H), 4.81 (1H, brs, $W_{½}$ = 4Hz, anomeric-H); ^{13}C nmr (see table 1).

Acetylation of torvonin B. Compound (1) (10 mg) was acetylated with Ac_2O (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 $C_{53}H_{78}O_{20}$: C, 61.51; H, 7.54; Found C, 61.53; H, 7.53), mp 169°C. Ir ν^{KBr} cm^{-1} 2950, 2845, 1710 (broad), 1456, 1382, 1240-1205 (broad), 956, 935, 860, 845. Ms m/z 1034 (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 H_2SO_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-H_2O, 4:1:5) by comparison with authentic samples while aglycone part showed several spots on tlc due to decomposition of genuine aglycone[4,5].

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