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Flavonoid Glucosides from *Biota semipervirens*

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An isoflavone glucoside, tectorigenin-4′-glucoside together with tectoridin, tectorigenin were isolated from leaves of *Biota semipervirens.* The structures of the compounds were established by spectroscopic and chemical methods.

Key Words: *Biota semipervirens,* Cupressaceae, Leaves, Flavonoids, Tectorigenin 4′-glucoside, Tectorigenin, Tectoridin.

INTRODUCTION

The genus Biota elaborates many flavonoids[1–4]. As part of our research programme on Biota species, growing in this region of India[5], we report here the isolation and characterisation of an isoflavone glucoside, tectorigenin-4′-glucoside (**A**) for the first time in this genus as well as from its family along with tectorigenin (**B**) and tectoridin (**C**) from the leaves of plant material. The plant materials are used as disinfectants and insecticides[6].

RESULTS AND DISCUSSION

Compound (**A**), m.p. 185–187°C was analysed for $C_{22}H_{22}O_{11}$ which was supported by $[M]^+$ at m/z 462. It gave a positive Molisch test. IR band at (1660 cm^{-1} is for $\nu C{=}O$), UV (216, 272, 320 nm) and ^1H NMR (δ 8.4, 1H, H-2) spectra established that A is an isoflavone glucoside. The ^1H NMR (DMSO-d_6) displayed, in addition to signals for one methoxyl group and H-2 of an isoflavone nucleus, two doublets at δ 7.1 and 7.5 for H-3′, H-5′ and H-2′ and H-6′. A singlet at δ 6.5 integrating for one proton represented H-8. A doublet at δ 4.9 (j = 7.0 Hz) integrating for a single proton was assigned the H-1″ of a glucose, indicating a β-linkage. A dark green colour with alcoholic ferric chloride and UV shifts with $AlCl_3$, $AlCl_3$-HCl and NaOAc indicated the presence of a 5, 7-dihydroxyl grouping. On acetylation A gave a hexa-acetate. In the ^1H NMR of the acetate all the signals remained unchanged except for the downfield displacement of H-8 to δ 7.15, indicating a free phenolic group only in A-ring. Acid hydrolysis of A gave glucose and an aglycone **B.** The UV, IR, ^1H and ^{13}C NMR spectra of the aglycone and its derivatives were in accord with tectorigenin. Identity of the aglycone was finally confirmed by its m.m.p. with an authentic sample. As tectorigenin has free 5,7 and 4′-hydroxyl positions, the glucose in A must be attached to one of these positions. The attachment of glucose at the 7-position gives tectoridin **C** a known isoflavone glucoside. Bathochromic shifts with $AlCl_3$ and $AlCl_3$-HCl and NaoAc in UV, leaves only the 4′ position for glycosylation. From the above data **A** must be tectorigenin 4′-O-β-D-glucoside. The ^{13}C NMR spectra of **A** and aglycone **B** were also measured. Assignments were based on chemical shift argument and by comparison with closely related compounds.[7, 8] Compound **C**, m.p. 224–245°C, was identified as tetoridin, a 7-O-β-D-glucoside of tectorigenin by spectral studies, acid hydrolysis and m.m.p. determination with an authentic sample. Compound **B** was identified as tectorigenin[9,10], on the basis of spectral data, Co-TLC and m.m.p. determination with authentic sample.

EXPERIMENTAL

Leaves of *Biota semipervirens* were collected from Botanical Garden of Kashmir University. The air-dried leaves 1.5 kg of *Biota sempervirens*, after prior defatting, were extracted with MeOH. The residue after evaporation (40 g) was chromatographed over slica gel using $CHCl_3$, EtOAc and MeOH. The $CHCl_3$ fraction was column chromatographed over slica gel using petrol-EtOAc (7 : 1) and petrol-EtOAc (1 : 1). The fraction obtained here was twice rechromatographed over of silca gel ($CHCl_3$-MeOH) mixture in different proportions and afforded 160 mg. **A** ($CHCL_3$-MeOH, 9 : 1), 100 mg of **C** ($CHCl_3$-MeOH, 9 : 1) and 100 mg **B**. Compound **B** and **C** were identified by comparing with their UV, IR and ^1H NMR spectra with the reported data[9, 10].

Compound **A**, m.p. 187°C (cream coloured needles, MeOH), R_f 0.46 ($CHCl_3$-MeOH, 24 : 1), Gibbs test positive $C_{22}H_{22}O_{11}$; UV λ_{max} MeOH nm: 216, 272, 320 (sh); + $AlCl_3$ 282, 325 (sh); + $AlCl_3$-HCl 282, 315 (sh); + NaOAc, 280, 315 (sh), 348; ^1H NMR (400 MHz, DMSO-d_6): δ 3.7, 5.3 (Glc protons), 3.72 (H, s, OMe), 4.9 (1H, d, j = 7 Hz, H-1″), 6.5 (1H, s, H-8), 7.1 (2H, d, j = 8 Hz, H-3′ and H-5′), 7.5 (2H, d, j = 8 Hz, H-2′ and H-6′), 8.4 (1H, s, H-2), 12.46 (1H, br, s, 1 × OH, exch. with D_2O), EIMS m/z (rel. int). 462(2), 300(100), 299(8), 285(25), 282(20), 278(2), 272(1), 257(18), 183(4), 182(2), 168(4), 118(10). Hexaacetate analysed for $C_{34}H_{32}O_{17}$. ^1H NMR (90 MHz, $CDCl_3$) δ 2.04 (12H, s, 4 × OAc), 2.36 (3H, s, Ar, OAc), 2.44 (3H, s, Ar, OAc), 3.83 (3H, s, OMe) 4.20–5.25 (6H, m, Glc protons), 6.99 (2H, d, j = 8 Hz, H-3′ and H-5′), 7.19 (1H, s, H-8, 7.37 (2H, d, J = 8 Hz, H-2′ and H-6′), 7.86 (1H, s, H-2). Compound **A** on hydrolysis with 5% HCl in MeOH gave a sugar and tectorigenin **B**, yellow plates from MeOH, m.p. 226°C analysed for $C_{16}H_{12}O_6$ ($[M]^+$ m/z 300), R_f 0.35 ($CHCl_3$-MeOH, 19 : 1), UV λ_{max}(MeOH) nm: 273, 335 (sh), $AlCl_3$ 281, 330 (sh), 355; +$AlCl_3$-HCl 280, 315 (sh), 360; +NaOAc 280, 336(sh); ^1H NMR (400 MHz, Me_2CO-d_6): δ 3.85 (3H, s, 1 × OMe), 6.5 (1H, s, H-8), 6.9 (2H, d, j = 8 hz, H-3′ and H-5′), 7.4 (2H, d, j = 8 Hz, H-2′ and H-6′), 8.15 (1H, s, H-2), 12.45 (1H, br, s, 1 × OH). The sugar was identified as glucose by Co-Pc (BAW, 4 : 1 : 5) [EtOAc-pyridine-H_2O, 12 : 5 : 4].

^{13}C NMR DATA OF COMPOUNDS A, B AND C AT 67.88 MHZ ($DMSO-D_6$) TMS AS INTERNAL STANDARD, ASSIGNMENTS BASED ON DEPT EXPERIMENTS

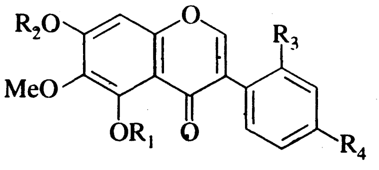
C	A†	B‡
2	154.75	154.50
3	121.07	123.49
4	180.81	180.40
5	152.92	154.27
6	132.46	132.18
7	157.50	157.55
8	94.04	94.25
9	152.51	154.21
10	106.49	106.05
OMe	60.35	60.64
1′	122.09	123.01
2′	133.22	131.17
3′	115.13	115.93
4′	156.65	157.81
5′	115.13	115.93
6′	130.22	131.17

Data from Ref. 11.

†Sugar signals: 100.16 (1″), 77.30 (2″), 76.71 (3″), 69.67 (4″), 73.14 (5″),60.67 (6″)

‡In acetone-ds

Structure



	R_1	R_2	R_3	R_4
A =	H	H	H	OGlc
B =	H	H	H	OH
C =	H	Glc	H	Glc

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