ANISOFOLIN-A, A NEW ACYLATED FLAVONE GLUCOSIDE FROM ANISOMELES OVATA R. Br.

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Abstract - Aerial parts of Anisomeles ovata R. Br. contain a new acylated apigenin glucoside (0.002%). It was identified as apigenin-7-O-β-D-(3",6"-di-O-p-coumaroyl)-glucoside (anisofolin-A) by ^{13}C nmr, ^{1}H nmr and chemical degradative methods of anisofolin-A and its acetate.

The earlier reports on Anisomeles ovata R. Br. (Syn: Anisomeles indica Linn.) showed the presence of diterpenes[1-4], fatty acids[5,6], triterpenes[7], flavonoids[7] and flavonoid glucosides[8].

Anisofolin-A (I) melted at 245°C and analysed for $C_{39}H_{32}O_{14}$. It yielded apigenin, D-glucose and p-coumaric acid as acid hydrolysis products. The infrared spectrum showed absorption bands at ν_{max}^{nujol} 1660 (γ-pyrone) and 1680 and 980 cm^{-1} for a second α,β-unsaturated carbonyl group in ester linkage. Alkaline hydrolysis of one mole of the glucoside gave one mole and two moles of apigenin-7-O-β-D-glucoside and p-coumaric acid respectively. The former yielded apigenin and D-glucose upon acid hydrolysis with 2N methanolic sulphuric acid. Apigenin was identified by mixed melting point and D-glucose by paper chromatography (Rf: 0.18, BAW (4:1:5)) with authentic samples. The glucosidic linkage is established as β by enzymatic hydrolysis with β-D-glucosidase. Methylation of apigenin-7-O-β-D-glucoside with dimethyl sulphate and potassium carbonate in acetone and subsequent acid hydrolysis of the methyl ether yielded apigenin-5,4'-dimethyl ether confirming the glucosidic linkage at 7. The ultraviolet spectrum of I showed absorption bands at λ_{max}^{MeOH} 225, 270 and 317 nm. The absence of a free hydroxyl at C-7 is indicated by the lack of shift with NaOAc for λ_{max}^{MeOH} 270 nm. A bathochromic shift ($\Delta\lambda$ = 50-65 nm) of the absorption band at λ_{max}^{MeOH} 317 nm with NaOMe and $AlCl_3$ indicated free hydroxyls at 4' and 5 positions, respectively, allocating the p-coumaroyl moieties to D-glucose.

The ^1H nmr spectrum of anisofolin-A (I) showed the presence of 19 protons in aromatic region. The two D_2O exchangeable signals at δ 12.95 and 5.6 are assigned to 5-OH and 4'-OH, respectively. The signals at δ 6.81 (s, 1H); 6.50 (d, J = 2 Hz, 1H), 6.81 (d, J = 2 Hz, 1H), 6.91 (d, J = 8.5 Hz, 2H) and 7.90 (d, J = 8.5 Hz, 2H) are consistent for the protons on C-3; 6, 8; 3',5' and 2',6' of apigenin, respectively[9]. The presence of two p-coumaroyl units was indicated by the four doublets centred at δ 6.32 (d, J = 17 Hz, 1H), 6.42 (d, J = 17 Hz, 1H), 7.60 (d, J = 17 Hz, 1H) and 7.68 (d, J = 17 Hz, 1H) for the two trans-olefinic double bonds in an α,β-unsaturated carbonyl system. The doublets at δ 6.66, 6.79, 7.38 and 7.56 integrated for two protons (J = 8.5 Hz) are assigned to two aryl ring protons of p-coumaroyl units. The broad signal at δ 4.9-5.3 (2H) is assigned to anomeric proton and the proton on the carbon bearing the second p-coumaroyl moiety. The remaining sugar protons are distributed between δ 3.9-4.9 ppm.

The ^1H nmr spectrum of anisofolin-A acetate showed six acetyl signals at δ 2.03-2.36 indicating the presence of six free hydroxyls. The signal at δ 2.03 (6H) indicated the presence of two aliphatic acetyls. The three signals at δ 2.26 (3H), 2.29 (6H) and 2.36 (3H) are allocated to four aryl acetyls; two acetyls in apigenin (5-OAc, 4'-OAc) and one acetyl each in the two p-coumaroyl moieties. The rest of the spectrum is in agreement with that of anisofolin-A (I).

The positions of the two p-coumaroyl units are allocated by a study of the ^{13}C nmr spectra (Table). The assignments were based on the ^{13}C nmr signals of apigenin[10], apigenin-7-O-β-D-glucoside[11] and p-coumaric acid[12] units. The expected shifts are observed only when the spectra of apigenin and its glucoside were recorded in the same solvent. A downfield shift of Δδ 1.2-1.8 ppm was observed for all the carbons except C-5 and C-4' of apigenin when the solvent is changed from DMSO-d_6[10] to DMSO-d_6 + D_2O[12,13]. The magnitude of this shift is less with apigenin-7-O-β-D-glucoside, i.e., Δδ 0.4-0.9, for all the carbons except for C-6, when going from DMSO-d_6[11] to DMSO-d_6 + MeOH-d_4[14]. In view of these solvent shifts only spectra recorded in DMSO-d_6 are taken into consideration for comparison.

The glucose carbons appeared between δ 62.88-99.45 ppm. The position of one of the two p-coumaroyl units is allocated to 6" as the signal due to C-6" moved downfield (δ 62.88, Δδ+2.38) and C-5" upfield (δ 73.54, Δδ-2.76). These shifts are in agreement with the earlier observations made for the acylated flavonoid glycosides at C-6" position[11,13,15,16]. A close comparison of the sugar carbon

signals with that of apigenin-7-O-β-D-glucoside showed that the signals due to C-2", C-4" moved upfield by Δδ-1.97 (δ 71.03) and -1.36 (δ 68.04) ppm, respectively, indicating a possible attachment of the second p-coumaroyl unit at C-3". However, the expected downfield shift of Δδ+2.3 ppm for the carbon bearing the acyl moiety was not observed with C-3" (δ 76.79) signal. But, such a deviation for the carbon bearing the acyl moiety at C-3" and C-4" was earlier observed for Saikosaponin[16] and for kaempferol-3-O-β-(4-O-caffeyl-3-O-β-glucosyl)-glucoside-7-O-rhamnoside[17], whereas the upfield shift for the neighbouring carbons was observed for all the compounds so far reported. Hence, the second p-coumaroyl moiety in anisofolin-A is allocated to C-3" and is characterised as apigenin-7-O-β-D-(3",6"-di-O-p-coumaroyl)-glucoside.

The isolation of acylated apigenin glycosides was restricted to 7 or 8 times[18-22] and a diacylated derivative of apigenin-4'-O-glucoside was reported from Lycopodium clavatum[23]. So far, diacylated apigenin-7-O-β-D-glucosides were not isolated from the nature and anisofolin-A forms the first report.

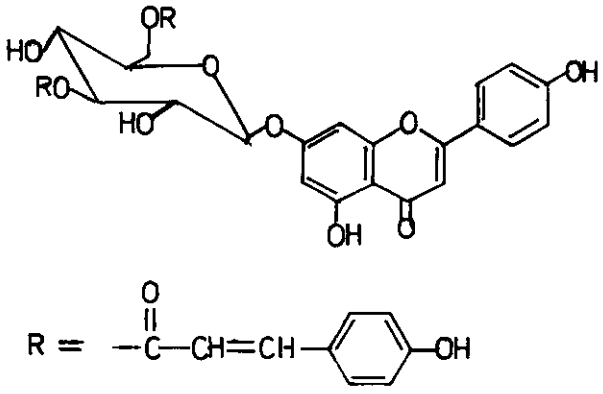

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Table

^{13}C nmr data of the carbons of glucose and p-coumaroyl moieties of flavonoid glucosides and their acetates

	Apigenin-7-O-β-D-glucoside[11] DMSO-d_6	Anisofolin-A DMSO-d_6	Apigenin-7-O-β-D-glucoside hexaacetate $CDCl_3$	Anisofolin-A hexaacetate $CDCl_3$
glucose				
1"	99.30	99.45 (d)	98.21	97.81
2"	73.00	71.03 (d)	71.15	70.80
3"	77.00	76.79 (d)	72.67	72.50
4"	69.40	68.04 (d)	68.39	68.57
5"	76.30	73.54 (d)	72.60	72.50
6"	60.50	62.88 (t)	62.13	62.23
p-coumaroyl				
1"', 1""		125.1 (s), 124.7 (s)		131.5, 131.7
2"', 2"", 6"', 6""		129.9 (d), 129.9 (d)		129.2, 129.5
3"', 3"", 5"', 5""		115.7 (d), 115.7 (d)		122.1, 122.1
4"', 4""		159.6 (s), 159.6 (s)		152.2, 152.5
α, α_1		113.6 (d), 114.6 (d)		116.3, 116.8
β, β_1		144.8 (d), 144.4 (d)		144.7, 145.6
7"', 7""		165.9 (s), 166.2 (s)		165.6, 166.1

Letters in the brackets indicate the multiplicity of the signal in the off-resonance spectrum.

EXPERIMENTAL

All the melting points recorded are uncorrected. ^{13}C nmr spectra at 25.2 MHz and 67.89 MHz are determined on JEOL-FX 100 and Brucker's 270 MHz instruments. ^{1}H nmr spectra are measured on Perkin-Elmer 90 MHz instrument.

Extraction and isolation - Dried aerial parts of Anisomeles ovata R.Br. (9 kg) were successively extracted with n-hexane and methanol. Methanol extract was fractionated into chloroform, acetone and methanol. Acetone fraction was chromatographed on a column of silica gel using benzene and acetone mixtures as solvents. Anisofolin-A is isolated from benzene + acetone (7:3) fraction and crystallized

from methanol as pale yellow powder, mp 245°C, Found: C, 64.35; H, 4.05; $C_{39}H_{32}O_{14}$ requires C, 64.65; H, 4.51%; λ_{max}^{MeOH} (log ε): 225 (4.56), 270 (4.36), 317 (4.43); (MeOH + NaOMe) 242sh, 265sh, 310sh, 365; (MeOH + $AlCl_3$) 227sh, 277sh, 300, 315sh, 380; (MeOH + $AlCl_3$+HCl) 227sh, 277sh, 300, 315sh, 380; (MeOH + NaOAc) 315, 375 nm; ν_{max}^{nujol}: 3100-3450b, 2800-3000s, 1680w, 1660w, 1590s, 1445s, 1370s, 1170m, 1075m, 975w, 825m, 720w cm^{-1}; ^{13}C nmr δ DMSO-d_6: 164.2s(C-2), 102.9d(C-3), 181.8s(C-4), 161.2s(C-5), 99.44(C-6), 162.3s(C-7), 94.7d(C-8), 156.7s(C-9), 105.3s(C-10), 120.8s(C-1'), 128.44(C-2',6'), 115.7d(C-3',5') and 161.0s(C-4'). Acetylation of anisofolin-A with Ac_2O/pyridine yielded a hexaacetate as white needles, mp 148°C; Found: C, 62.78; H, 4.64; $C_{51}H_{44}O_{20}$ requires C, 62.72; H, 4.54%; ν_{max}^{nujol}: 1750s, 1710m, 1640m and 980m cm^{-1}; ^{13}C nmr δ $CDCl_3$: 161.2(C-2), 102.3(C-3), 176.0(C-4), 150.6(C-5), 109.5(C-6), 159.8(C-7), 112.7(C-8), 158.2(C-9), 108.3(C-10), 128.4(C-1'), 127.4(C-2',6'), 122.1(C-3',5') and 153.1(C-4').

Alkaline hydrolysis of anisofolin-A: 50 mg of anisofolin-A was refluxed with 10 ml of 1% methanolic potassium hydroxide for 2 hours on a boiling water bath and neutralised, methanol was removed under vacuum and extracted with ether and ethyl-acetate successively. From the ether extract p-hydroxycinnamic acid was obtained as colourless needles, mp 210-211°C, (lit. mp 210°C), yield 20 mg; Found: C, 65.90; H, 4.85; $C_9H_8O_3$ requires C, 65.84; H, 4.91%.

The ethyl acetate extract, upon crystallization from methanol yielded apigenin-7-O-β-D-glucoside, as pale yellow needles, mp 239°C, yield 25 mg; Found: C, 58.70; H, 4.56; $C_{21}H_{20}O_{10}$ requires C, 58.33; H, 4.66%.

Acid hydrolysis of anisofolin-A: 50 mg of anisofolin-A was hydrolysed with 24 ml of 2N methanolic sulphuric acid for 6 hours on a boiling water bath. Then solvent was removed under vacuum and 50 ml water was added. It was repeatedly extracted with ether. The combined ether extracts was washed with sodium carbonate and water. The dried ether extract upon crystallization from methanol yielded apigenin as yellow needles, mp 346°C, (lit. mp 347°C) and was identified by mmp and ir, yield 15 mg. The bicarbonate extract was neutralised and extracted with ether. It showed the presence of p-coumaric acid on thin layer chromatography with an authentic sample. The filtrate from the acid hydrolysis was neutralised with barium carbonate and filtered. The filtrate was evoporated to dryness under vacuum. The residue was extracted into methanol and concentrated. It showed the presence of D-glucose on ascending paper chromatography (Rf: 0.18, BAW (4:1:5))

on Whatman No. 1.

Acid hydrolysis of apigenin-7-O-β-D-glucoside: 10 mg of glucoside was hydrolysed as above. The presence of apigenin and D-glucose were identified on thin layer (silicagel) and paper (Whatman No. 1) chromatographies, respectively.

Isolation of apigenin-5,4'-dimethyl ether: 10 mg of apigenin-7-O-β-D-glucoside in dry acetone (20 ml) was refluxed with 0.2 ml of dimethyl sulphate and 2 g of potassium carbonate on a steam bath for 48 hours and filtered. Acid hydrolysis of the methyl ether with 2N methanolic sulphuric acid yielded apigenin-5,4'-dimethyl ether identified by comparison with an authentic sample on thin layer chromatography.

Enzymatic hydrolysis of apigenin-7-O-β-D-glucoside: 5 mg of glucoside was hydrolysed with β-D-glucosidase in 5 ml of acetate buffer at 37°C for 24 hours. Then the reaction mixture was extracted with ether. Apigenin was identified as the only compound on thin layer chromatography with an authentic sample.

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