TWO FLAVONE GLUCOSIDES FROM *ANISOMELES MALABARICA* R.Br.

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Abstract – Two apigenin glucosides are reported from the stem parts of ***Anisomeles malabarica***, and identified as apigenin-7-*O*-*β*-D-(4",6"– di-*O*-*p*-coumaroyl) glucoside and its 2",6" – isomer by spectral studies

Introduction

Earlier investigations of ***Anisomeles*** species have led to the isolation and identification of a number of terpenoid and steroid types of compounds (1-5). Perusal of past literature revealed that the genus ***Anisomeles*** elaborates flavonoidal compounds. A number of flavonoids have been reported from ***Anisomeles ovata*** (6-10). ***Only*** one flavone has been reported from ***A.malabarica*** (11). A survey of chemical literature revealed that only limited parts (leaves, aerial parts) of ***Anisomeles malabarica*** have been chemically examined. Hence, it was thought that the chemical investigation of other parts such as stem, root etc., would be worthwhile, which may result in the isolation of newer compounds. Also the plant finds use as a drug and is reported to possess significant activity (12-17).

Phytochemical investigation of the acetone extract of the stem of ***Anisomeles malabarica*** led to the isolation of two compounds by column chromatographic separation designated as 1 and 2. These compounds have been characterized as apigenin –7-O-β-D- (4", 6"-di-O-p-coumaroyl) glucoside and apigenin –7-O-β-D- (2", 6"-di-O-p-coumaroyl) glucoside from their detailed spectral analysis and by comparison with available data from literature. The present communication deals with the isolation and characterization of the compounds 1 and 2. This is the second report of isolation of flavonoidal compounds from ***A.malabarica***

Experimental

All the melting points recorded are uncorrected. Electronic absorption spectra were recorded in Shimadzu UV-VIS 160 spectrophotometer in the range 200- 1100 nm. The IR spectra were recorded in JASCO FT-IR-410 (4000-400) spectrophotometer. Potassium bromide disc was employed for sample preparation. The instrument was calibrated against polystyrene film. Proton magnetic resonance measurements were made on a BRUKER 300 MHz spectrometer. Deuterated organic solvents along with tetramethylsilane (TMS) as the internal standard were used.

Extraction and isolation

Air-dried and cut pieces of stems of *A. **malabarica*** (1 Kg) were thoroughly percolated and first extracted with petroleum ether (60-80°C), and the residual material was then extracted with acetone (4x2.5L) for a period of (4x3hrs) and further the residual material was extracted with methanol. The acetone extract obtained was clarified

by filtration and concentrated under reduced pressure to give a dark green semisolid. Fractions eluted with benzene: ethyl acetate (3:1) yielded a residue which crystallized from methanol as a pale yellow green compound 1, Fractions eluted with benzene: ethylacetate (1:1) on distillation gave another pale yellow green compound 2.

Results

Compound 1 m.p. 225°C; λ_{max} (MeOH) 335,287 sh, (MeOH+$AlCl_3$) 395, (MeOH+$AlCl_3$+HCl) 394, (MeOH+NaOAc) 370; γ_{KBr} cm^{-1}: 3434, 2927, 2364, 1598,1502, 1444, 1355, 1268, 1170, 1072, 827, 757, 626,509 and 472 cm^{-1}. ^{1}H-NMR (DMSO-d_6, assignment, number of protons):δ 6.52 (C-3, 1H), 6.58 (C-6, 1H), 6.70 (C-8, 1H), 7.79-7.81 (C-2' & C-6', 2H), 6.94-6.96 (C-3' & C-5', 2H), 5.90 (C-1" Anomeric proton), 4.60-4.64 (C-2"), 4.28-4.34 (C-3"), 5.20-5.30 (C-4"), 3.94-3.96, (C-5"), 5.59 (C-6"), 6.36-6.41 (4"-p-coumaroyl α-C proton, 1H), 6.25-6.30 (6"-p-coumaroyl α-C proton, 1H), 7.63-7.69 (4"-p-coumaroyl β-C proton, 1H), 7.51-7.56 (6"-p-coumaroyl β-C proton, 1H), 7.42-7.45 (C-2"' & C-6"', 2H), 6.84-6.86 (C-3"' & C-5"', 2H), 7.24-7.26 (C-2"" & C-6"", 2H), 6.70 (C-3"" & C-6"", 2H), 10.0-11.0 (Aromatic hydroxyls at 4', 4"' & 4""), 13.0 (Aromatic hydroxyls at C-5), 5.75 (Aliphatic hydroxyls). ^{13}C-NMR (DMSO-d_6, assignment,):δ 62.24 (C-6"), 67.53 (C-5"), 70.28 (C-3"), 73.06 (C-2"), 76.07 (C-4"), 93.72 (C-8 & C-6), 98.86 (C-1" anomeric carbon), 102.02 (C-3), 104.87 (C-10), 112.42 (6"-p-coumaroyl α-Carbon), 113.28 (4"-p-coumaroyl α-Carbon), 114.74 (C-3' & 5'), 114.9 (C-3"" & 5""), 115.02 (C-3"' & 5"'), 120.13 (C-1'), 123.97 (C-1""), 124.18 (C-1"'), 127.07 (C-2' & 6'), 128.67 (C-2"" & 6""), 128.76 (C-2"' & 6"'), 143.99 (6"-p-coumaroyl β-Carbon), 144.15 (4"-p-coumaroyl β-Carbon), 155.95 (C-9), 158.65 (C-4""), 158.75 (C-4"'), 161.46 (C-7), 160.25 (C-5), 160.42 (C-4'), 163.53 (C-2), 165.78, 165.83 (4" & 6"-p-coumaroyl carbonyl Carbon), 181.0 (C-4 Carbonyl group).

Compound 2 m.p. 245°C; λ_{max} 265 sh, 360 (MeOH), 395 (MeOH+$AlCl_3$), 395 (MeOH+$AlCl_3$+HCl), 366.5 (MeOH+NaOAc); ^{1}H-NMR (DMSO-d_6, assignment, number of protons):δ 6.48 (C-3, 1H), 6.63 (C-6, 1H), 6.67 (C-8,1H), 7.81-7.83 (C-2' & C-6', 2H), 6.91-6.94 (C-3' & C-5', 2H), 5.49 (C-1" Anomeric proton), 5.04-5.11(C-2"), 4.17-4.24 (C-3"), 4.56-4.6 (C-4"), 3.78-3.83, (C-5"), 5.22-5.32 (C-6"), 6.24 -6.29 (2" & 6"-p-coumaroyl α-C proton, 1H), 7.48-7.53 (2" & 6"-p-coumaroyl β-C proton, 1H), 7.24-7.26 (C-2"', C-6"', & C-2"",C-6"", 2H), 6.68 (C-3"', C-5"' & C-3"" & C-6"", 2H), 10.0 (Aromatic hydroxyls at 4', 4"' & 4""), 12.9 (Aromatic hydroxyls at C-5), 5.74 – 5.78 (Aliphatic hydroxyls). ^{13}C-NMR (DMSO-d_6, assignment,):δ 63.73 (C-6"), 70.4 (C-5"), 73.10 (C-4"), 74.35 (C-3"), 76.7 (C-2"), 94.78 (C-8), 99.88 (C-6), 100.19 (C-1" anomeric carbon), 103.2 (C-3), 105.91 (C-10), 113.73 (2 & 6"-p-coumaroyl α-Carbon), 115.92 (C-3' & 5'), 116.2 (C-3"", 5"" & C-3"', 5"'), 121.29 (C-1'), 125.11 (C-1"" & C-1"'), 128.28 (C-2' & 6'), 129.88 (C-2"", 6"" & C-2"', 6"'), 145.17 (2" & 6"-p-coumaroyl β-Carbon), 157.13 (C-9), 159.93 (C-4"" & C-4"'), 162.87 (C-7), 161.52 (C-5), 161.64 (C-4'), 166.83 (C-2), 164.58 (2" & 6"-p-coumaroyl carbonyl Carbon), 182.16 (C-4 Carbonyl group).

Acetylation of compound 1 with Ac_2O/pyridine yielded a hex acetate **3** as white needles, mp 130° C; ^{1}H-NMR (DMSO-d_6, assignment, number of protons):δ 6.54 (C-3, 1H), 6.58 (C-6, 1H), 6.72 (C-8,1H), 7.96-7.99 (C-2' & C-6', 2H), 7.01-7.07 (C-3' & C-5', 2H), 5.90 (C-1" Anomeric proton), 4.35-4.37 (C-2" & C-3"), 6.0 (C-4"), 4.35-4.37 (C-5"), 5.5-5.65 (C-6"), 6.38-6.48 (4" & 6"-p-coumaroyl α-C proton, 2H), 7.62-7.70 (4" & 6"-p-coumaroyl β-C

proton, 2H), 7.49-7.51 (C-2''' & C-6''', 2H), 7.11-7.17 (C-3''' & C-5''', 2H), 7.25-7.3 (C-2'''' & C-6'''', 2H), 6.74-6.86 (C-3'''' & C-6'''', 2H), 2.25-2.35 (Aromatic acetoxyls), 2.04-2.05 (Aliphatic acetoxyls).

Discussion

Earlier workers have reported (6,7) the isolation of compounds 1 and 2 and the corresponding 3'', 6''-isomer namely anisofolin – A from ***A.ovata***. However compounds 1 and 2 were reported to be isolated as a mixture only. They were characterized by separating their per acetate mixture by CC (7). Hence melting points were not reported for the parent glycosides. Therefore, a confirmation about the structure could be made by resorting to PMR analysis. Also complete PMR and CMR data of the parent glycosides was not available. Data was available for their mixture together. Herein we report the complete data of PMR and CMR analysis of the pure parent glycosides. The two compounds designated as 1 and 2 gave very clear PMR spectra. In the present study, compound 1 was identified as apigenin –7-O-β-D- (4", 6"-di-O-p-coumaroyl) glucoside and compound 2 as apigenin –7-O-β-D- (2", 6"-di-O-p-coumaroyl) glucoside. Table I highlights the comparative study of the PMR data reported earlier for the hexaacetate of apigenin –7-O-β-D- (4", 6"-di-O-p-coumaroyl) glucoside and that of **1**, **2** & acetate of 1 designated **3**

A striking feature of the PMR spectra of 1 is the appearance of two sets of doublet of doublets, one upfield and other downfield comparatively. These are due to the protons attached to the α-carbon of the p-coumaroyl groups. It is reported (7) that when the sugar moiety is disubstituted at the 4" and 6" position, the protons of the two α-carbons appear as a clear doublet of doublet. That implies that their environments are different. Similarly the β-C protons. These protons are deshielded compared to the α proton. Since the PMR of 1 showed this characteristic, this isomer is the 4", 6"-disubstituted one. Therefore, compound 2 is the 2", 6" disubstituted isomer.

PMR of compound 2 showed much simplicity compared to that of 1 in regard to the number of signals in the aromatic region The α and β- protons of the p-coumaroyl unit also appeared as a pair of mutually coupled doublets implying that the environment of the two coumaroyl groups is becoming the same. Therefore, the α protons of both the p-coumaroyl units give one doublet and the β-protons give another doublet downfield.

The 3'', 6''-isomer namely Anisofolin A, mp.245°C is reported (6) to reveal the α and β protons of the p-coumaroyl moiety as a set of four doublets. Hence compound 2 is not the 3'', 6'' isomer.

Further confirmation of the structure was done by recording CMR of the two compounds. The complete assignment of all carbons in 1 and 2 was made. Comparing the CMR spectra of both the compounds confirmed that 1 is the 4" & 6" isomer while, 2 is the 2" & 6" isomer. The CMR of 2 showed less number of signals compared to that of 1. This shows that compound 2 has similarity in its structure with regard to the p-coumaroyl moieties.

The following Table II gives a comparative study of the CMR data of 1 and 2 with that reported earlier (7) for the apigenin glucosides.

Table I
Comparative table of PMR data of 1, 2 and 3 and that of the acetate of apigenin glucoside reported from earlier sources (7)

Position of protons	Chemical shift (ppm)			Compound 2	
	Compound 1	Compound 3	Apigenin 7'-O-β-D-(4",6"-di-O-p-coumaroyl) glucoside hexa acetate	Position of protons	Chemical shift (ppm)
C-3	6.52,s, (1H)	6.54	-	C-3	6.48,s (1H)
C-6	6.58,s, (1H)	6.58	-	C-6	6.63,s (1H)
C-8	6.70,s, (1H)	6.72	-	C-8	6.67,s (1H)
C-2' & C-6'	7.79-7.81,d, (2H)	7.96-7.99	-	C-2' & C-6'	7.81-7.83,d (2H)
C-3' & C-5'	6.94-6.96,d, (2H)	7.01-7.07	-	C-3' & C-5'	6.91-6.94,d (2H)
C-1"(Anomeric proton)	5.90	5.9-6.0	-	C-1"(Anomeric proton)	5.49, br.
C-2"	4.60-4.64	4.35-4.37 ,m	-	C-2"	5.04-5.11, br.
C-3"	4.28-4.34	4.35-4.37 ,m	-	C-3"	4.17-4.24,m
C-4"	5.20-5.30	5.9-6.0	-	C-4"	4.56-4.6,d
C-5"	3.94-3.96	4.35-4.37 ,m	-	C-5"	3.78-3.83,m
C-6"	5.59	5.5-5.65	-	C-6"	5.22-5.32,br.
4"-p-coumaroyl α-C proton	6.36-6.41,d, (1H)	6.38-6.48	6.28,d	2" & 6"-p-coumaroyl α-C proton	6.24-6.29,d (2H)
6"-p-coumaroyl α-C proton	6.25-6.30,d, (1H)	6.38-6.48	6.34,d	2" & 6"-p-coumaroyl β-C proton	7.48-7.53 d (2H)
4"-p-coumaroyl β-C proton	7.63-7.69 d, (1H)	7.62-7.70	7.58,d	C-2"' ,C-6"' & C-2"", C-6""	7.24-7.26,d (2H)
6"-p-coumaroyl β-C proton	7.51-7.56 d, (1H)	7.62-7.70	7.70,d	C-3"' , C-5"' & C-3"", C-6""	6.68, distorted .d (2H)
C-2"' &C-6"'	7.42-7.45,d, (2H)	7.49-7.51	-		
C-3"' & C-5"'	6.84-6.86 d, (2H)	7.11-7.17	-		
C-2"" & C-6""	7.24-7.26,d (2H)	7.25-7.3	-		
C-3"" & C-6""	6.70,d, (2H)	6.74-6.86	-		
Aromatic hydroxyls at C-4', C-4"', C-4""	10.0-11.0, br.s	-	-	Aromatic hydroxyls at C-4', C-4"', C-4""	10.0, br.s
Aromatic hydroxyls at C-5	13.0,s		-	Aromatic hydroxyls at C-5	12.9, s
Aliphatic hydroxyls	5.75	-	-	Aliphatic hydroxyls	5.74-5.78
Aromatic acetoxyls	-	2.25-2.35	2.31-2.38		
Aliphatic acetoxyls	-	2.04-2.05	2.03-2.09		

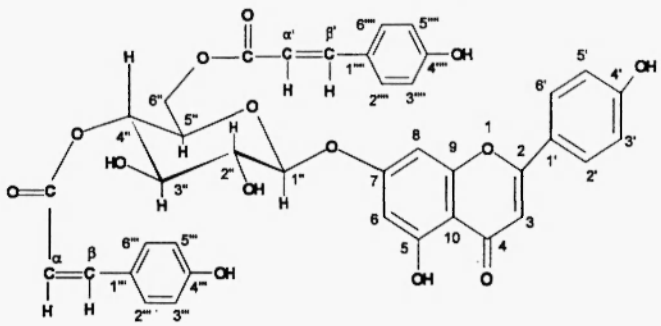
Table 2
Comparative table of CMR data of 1, 2 and that of isomeric mixture of apigenin glucosides reported from earlier sources (7)

Compound 1		Compound 2		Isomeric mixture of apigenin glucoside	
C-position	Chemical shift in δ	C-position	Chemical shift in δ	C-position	Chemical shift in δ
C-6"	62.24	C-6"	63.73	C-6"	62.10,62.40
C-5"	67.53	C-5"	70.4	C-5"	71.10,73.13
C-3"	70.28	C-4"	73.10	C-3"	73.65
C-2"	73.06	C-3"	74.35	C-2"	73.13,74.15
C-4"	76.07	C-2"	76.7	C-4"	71.50,69.80,
C-8	93.72	C-8	94.78	C-8	94.90 (2C)
C-6	93.72	C-6	99.88,	C-6	99.20 (2C)
C-1" (anomeric carbon)	98.86	C-1" (anomeric carbon)	100.19	C-1" (anomeric carbon)	99.51
C-3	102.02	C-3	103.2	C-3	103.1 (2C)
C-10	104.87	C-10	105.91	C-10	105.5 (2C)
6"-p-coumaroyl α-C proton	112.42	2" & 6"-p-coumaroyl & α-C proton	113.73	6"-p-coumaroyl α-C proton	113.5 (2C)
4"-p-coumaroyl α-C proton	113.28	-	-	4"-p-coumaroyl α-C proton	113.9 (2C)
C-3' & 5'	114.74	C-3' & 5'	115.92,	C-3' & 5'	115.9 (4C)
C-3"" & 5""	114.9	C-3"", 5"" & C-3'" & 5'"	116.2	C-3"" & 5""	115.8 (4C)
C-3'" & 5'"	115.02	-	-	C-3'" & 5'"	115.7 (4C)
C-1'	120.13	C-1'	121.29	C-1'	121.0 (2C)
C-1""	123.97	C-1"" & C-1'"	125.11	C-1""	125.1 (2C)
C-1'"	124.18	-	-	C-1'"	124.9 (2C)
C-2' & 6'	127.07	C-2' & 6'	128.28	C-2' & 6'	128.5 (4C)
C-2"" & 6""	128.67	C-2"", 6"" & C-2'" & 6'"	129.88	C-2"" & 6""	130.3 (4C)
C-2'" & 6'"	128.76	-	-	C-2'" & 6'"	129.9 (4C)
6"-p-coumaroyl β-carbon	143.99	2" & 6"-p-coumaroyl β-carbon	145.17	6"-p-coumaroyl β-carbon	145.0 (2C)
4"-p-coumaroyl β-carbon	144.15	-	-	4"-p-coumaroyl β-carbon	145.2 (2C)

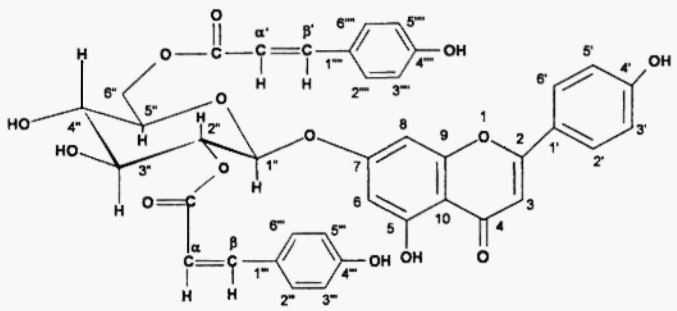
C-9	155.95	C-9	157.13	C-9	156.9 (2C)
C-4""	158.65	C-4"" & C-4""	159.93	C-4""	159.8,159.9 (2C)
C-4""	158.75	-	-	C-4""	159.7 (2C)
C-7	161.46	C-7	162.87	C-7	162.6 (2C)
C-5	160.25	C-5	161.52,	C-5	162.0 (2C)
C-4'	160.42	C-4'	161.64	C-4'	161.3 (2C)
C-2	163.53	C-2	166.83	C-2	164.3 (2C)
4"& 6"-p-coumaroyl carbonyl	165.78,165.83	2" & 6"-p-coumaroyl carbonyl	164.58	4"& 6"-p-coumaroyl carbonyl	165.5,166.1 (2C)
-	-	-	-	2" & 6"-p-coumaroyl carbonyl	165.9,166.3 (2C)
C-4,(C=O group)	181.0	C-4 (C=O group)	182.16	C-4, (C=O group)	181.9 (2C)

In our study, the complete assignment of carbons for the two compounds 1 and 2was done.

Structure of 1 and 2.



Apigenin 7-O-β-D- (4", 6"-di-O-p-coumaroyl) glucoside 1



Apigenin 7-O-β-D- (2", 6"-di-O-p-coumaroyl) glucoside 2

Conclusion

Phytochemical investigation of the stem parts of *Anisomeles malabarica* R.Br. has led to the isolation of two apigenin glucosides .This is the second report of isolation of flavonoidal compounds from of *Anisomeles malabarica* R.Br.

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References

1. H. Immer, J. Polonsky, R. Toubiana and H.D. An., Tetrahedron, , **21**, 2117, (1965).
2. K.K. Purushothaman, R. Bhima Rao and K. Kalyani, Indian J. Chem., **13**, 1357, (1975).
3. Percy S. Manchand and John F. Blount , J. Org. Chem., **42**, 24, 3824, (1977).
4. Geeta Devi, R.S. Kapil and S.P. Popli, Indian. J. Chem., **16B**, 441, (1978).
5. L.Jagan Mohan Rao, G.N. Krishna Kumari and N.S. Prakasa Rao, Journal of Natural Products, 47, 6, 1052, (1984).
6. L Jagan Mohan Rao, G.N. Krishna Kumari and N.S. Prakasa Rao, Heterocycles, **19**, 1655, (1982).
7. L.Jagan Mohan Rao, G.N. Krishna Kumari and N.S. Prakasa Rao, Phytochemistry, **22**, 1058, (1983).
8. L.Jagan Mohan Rao, G.N. Krishna Kumari and N.S. Prakasa Rao, Phytochemisry, **22**, 1522, (1983).
9. L.Jagan Mohan Rao, G.N.Krishna Kumari and N.S. Prakasa Rao, Journal of Natural Products, **46**, 4, 595, (1983).
10. L Jagan Mohan Rao, G.N. Krishna Kumari and N.S. Prakasa Rao, Journal of Natural Products, **48**, 1, 150, (1985).
11. Geeta Devi, R.S. Kapil and S.P. Popli, Indian J.Chem. **17B**, 84, (1979).
12. P. Brindha, B. Rukmani and K.K.Purushothaman, Bull.Med. Ethnobot. Research. **74**, (1981).

13. S. Rajasekaran, C.R. Jawahar, K. Radhakrishnan, P.K. Ratheeshkumar, L. Sarada Amma and P. Pushpagadam, Aryavaidyam, 5, (4), 224, (1992).

14. R. Valsaraj, P. Pushpagadam, V.W. Smitt, A. Adsersen and V. Nymar, Journal of Ethnopharmacology, **58**, (2), 75, (1997).

15. M.A. Kalharo, Z. Kapadia and Y. Badar, Hamdard Medians, **39, 78**, (1998).

16. M.A. Kalharo, Z. Kapadia and Y. Badar, Journal of Faculty of pharmacy of Gazi University, **14**, 37, (1999).

17. A. Saraswathy, R. Vimala and A. Senthil Selvakumari, Abstract of Proceedings of National Seminar on Recent Trends in Isolation, Characterization, Synthesis and Biological Studies of Organic Compounds, 5th and 6th March 2004, GRI, Gandhigram. pp 40.

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