$-MeCO]^+$ (34). On treatment with boiling Ac_2O–pyridine, 1 yielded an acetate, colourless needles, mp 188–189.5° (from $CHCl_3$–MeOH). (Found: C, 59.30; H, 4.52. $C_{24}H_{22}O_{11}$ requires: C, 59.26; H, 4.56%.) UV λ_{max}^{MeOH} nm: 239, 262 (sh), 310. IR ν_{max}^{KBr} cm^{-1}: 1762 (OAc), 1624 (conjugated C–O), 1562 (aromatic C=C). ^1H NMR (100 MHz, DMSO-d_6): δ 2.32, 2.36, 2.42 (each 3H, each *s*, OAc × 3), 3.80, 3.96, 4.05 (each 3H, each *s*, OMe × 3), 6.54 (1H, *s*, H-3), 7.19 (1H, *s*, H-8), 7.24 (1H, *s*, H-3'), 7.55 (1H, *s*, H-6'). ^{13}C NMR (25 MHz, DMSO-d_6): δ 159.4 (*s*, C-2), 111.5 (*d*, C-3), 175.2 (*s*, C-4), 141.6 (*s*, C-5), 139.3 (*s*, C-6), 157.9 (*s*, C-7), 99.0 (*d*, C-8), 153.8 (*s*, C-9), 110.5 (*s*, C-10), 123.0 (*s*, C-1'), 141.2 (*s*, C-2'), 119.1 (*d*, C-3'), 141.2 (*s*, C-4'), 149.3 (*s*, C-5'), 113.2 (*d*, C-6'), 61.0 (*q*, OMe-6), 56.8, 56.5 (each *q*, OMe × 2), 169.1, 168.9, 168.3 (each *s*, OCOMe × 3), 20.7, 20.6, 20.3 (each *q*, OCOMe × 3). MS 70 eV *m/z* (rel. int.): 486 $[M]^+$ (8), 444 $[M-CH_2CO]^+$ (95), 402 $[M-CH_2CO \times 2]^+$ (100), 387 (31), 360 $[M-CH_2CO \times 3]^+$ (26) 345 (52), 181 (10), 167 (21), 153 (14).

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TWO FURTHER ACYLATED FLAVONE GLUCOSIDES FROM *ANISOMELES OVATA*

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Key Word Index *Anisomeles ovata*; Labiatae; aerial parts; apigenin 7-*O*-β-D-(2″,6″-di-*O*-*p*-coumaroyl)glucoside; apigenin 7-*O*-β-D-(4″,6″-di-*O*-*p*-coumaroyl)glucoside.

Abstract—The structures of two new acylated apigenin glucosides are reported from the aerial parts of *Anisomeles ovata*. They were separated as their acetates and identified as apigenin 7-*O*-β-D-(2″,6″-di-*O*-*p*-coumaroyl)glucoside and apigenin 7-*O*-β-D-(4″,6″-di-*O*-*p*-coumaroyl)glucoside by ^1H NMR study of the acetates and by chemical degradative methods. The allocation of the *p*-coumaroyl moieties is also supported by a study of the ^{13}C NMR spectrum of the inseparable mixture of glucosides.

INTRODUCTION

In an earlier communication [1] we have reported the isolation of a new compound anisofolin-A [apigenin 7-*O*-β-D-(3″,6″-di-*O*-*p*-coumaroyl)glucoside] from the aerial parts of *Anisomeles ovata* R. Br. The present communication deals with the characterization of two new compounds **1** and **2** from a study of their acetates.

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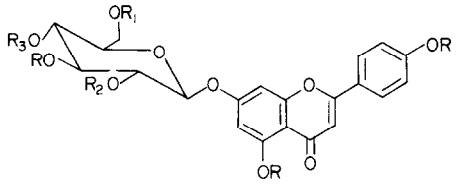
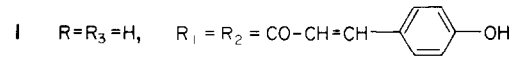
RESULTS AND DISCUSSION

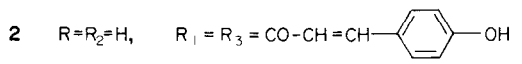
Compounds **1** and **2** are identical in their chromatographic properties and showed a single spot on TLC with various solvent systems. The mixture analysed for $C_{39}H_{32}O_{14}$. UV and IR spectral data of the mixture are similar to that of anisofolin-A and indicated that **1** and **2** are isomers. The quantitative isolation of apigenin 7-*O*-β-D-glucoside and *p*-coumaric acid from the alkaline hydrolysis also support the above conclusion.

Peracetate of the above mixture showed the presence of two close running spots (R_f: 0.33, 0.35; solvent: benzene–acetone, 9:1) which were separated by CC into hexa-acetate **3** (mp 140°) and hexa-acetate **4** (mp 132°). Each of these acetates showed four aromatic acetoxyls [**3**: δ 2.30 (9H), 2.33 (3H); **4**: δ 2.31 (9H), 2.38 (3H)] and two alcoholic acetoxyls [**3**: δ 2.03 (3H), 2.09 (3H); **4**: δ 2.03 (3H), 2.09 (3H)] indicating disubstitution in glucose.

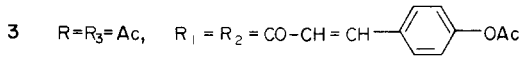




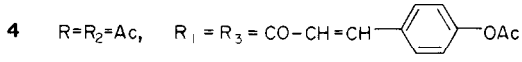
1 R=R₃=H, R₁=R₂=CO-CH=CH-⟨⟩-OH



2 R=R₂=H, R₁=R₃=CO-CH=CH-⟨⟩-OH



3 R=R₃=Ac, R₁=R₂=CO-CH=CH-⟨⟩-OAc



4 R=R₂=Ac, R₁=R₃=CO-CH=CH-⟨⟩-OAc

The chemical shift of the two α-protons of the two *p*-coumaroyl units of hexa-acetate **3** is observed at δ 6.35*d* (J = 17 Hz), whereas the two β-protons are well separated [δ 7.63*d* (J = 17 Hz) and 7.72*d* (J = 17 Hz)]. These chemical shift values are similar to those observed for the acetate of apigenin 4′-*O*-β-D-(2″,6″-di-*O*-*p*-coumaroyl)glucoside [2] where the two α-protons appeared at the same place (δ 6.39*d*, J = 16 Hz) as against the two signals for two β-protons (δ 7.71*d* and 7.76*d*, J = 16 Hz). Hence, in hexa-acetate **3** the sites of acylation are allocated to C-2″ and C-6″.

The ^{1}H NMR spectrum of hexa-acetate **4** showed well separated pairs of doublets centred at δ6.28, 7.58 and 6.34, 7.70 with J = 17 Hz for the olefinic protons of the two *p*-coumaroyl units. As this acetate is not identical with C-3″,C-6″-diacylated anisofolin-A hexa-acetate [1], it can be assigned the structure with C-4″ and C-6″ acylation.

The ^{13}C NMR spectrum (Table 1) of the mixture in DMSO-d_6 markedly differs for the resonances of glucose and *p*-coumaroyl units. The signals of flavone carbons are in agreement with apigenin [3], its 7-*O*-β-D-glucoside [4] and anisofolin-A [1]. The aromatic carbons of *p*-coumaroyl moieties are in agreement with the corresponding carbon signals of anisofolin-A.

The spectrum is complicated for the glucosidic part between δ62.1 and 99.51 and indicated 10 signals. The upfield shift of one of the anomeric carbons at δ97.0 indicates a C-2″ acylation [2] and is allocated to **1**. The second signal at δ 99.51 is in agreement with the corresponding positions reported for apigenin 7-*O*-β-D-glucoside [4] and anisofolin-A [1] indicating the lack of acylation at C-2″ and was allocated to **2**.

The chemical shift of C-6″ carbons in the mixture showed the expected upfield shift and appeared at δ62.1 and 62.8 indicating that the C-6″ position in both compounds was occupied by one *p*-coumaroyl moiety each.

The C-2″, C-6″ diacylated apigenin 7-*O*-β-D-glucoside would show a downfield shift for C-2″ and an upfield shift for the adjacent carbons C-1″ and C-3″. The signals δ97.0 and 73.65 showed an upfield shift of Δδ − 2.45 and − 3.35 and the signal at δ 74.15 moved downfield (Δδ + 1.15). They are assigned to C-1″,C-3″ and C-2″, respectively, in **1**. These and other signals at δ69.8, 73.13 (Δδ − 3.17) and 62.8 (Δδ + 2.30) are in agreement with the values reported for apigenin 4′-*O*-β-D-(2″,6″-di-*O*-*p*-coumaroyl) glucoside [2]. Hence, the first isomer can be assigned structure **1**.

The position of the second acyl moiety in **2** was allocated to the C-4″ position since the position of the C-3″ carbon still indicates acylation on the neighbouring carbons. In the absence of C-2″ acylation, only C-4″ acylation can cause an upfield shift for C-3″. Hence, the signal at δ 73.65 was again allocated to C-3″ in **2**. The anomeric C-1″ was observed at δ 99.51. As C-5″ is situated between two acyl groups at C-4″ and C-6″, a multiple upfield shift is expected and the signal at δ 71.1 was assigned to C-5″. By assigning the remaining signals at δ 71.5 to C-4″ a downfield shift of Δδ + 2.1 was observed

Table 1. ^{13}C NMR data of the carbons of the glucose moiety in flavone glucosides and their acetates

Compound (solvent)	1″	2″	3″	4″	5″	6″
Apigenin-4′-*O*-β-D-(2″,6″-di-*O*-*p*-coumaroyl)glucoside [2] (DMSO-d_6)	97.20	74.00	73.00*	70.10	73.70*	63.40
1 (DMSO-d_6)	97.00	74.15	73.65*	69.80	73.13*	62.80
2 (DMSO-d_6)	99.51	73.13*	73.65*	71.50†	71.10†	62.10
Hexa-acetate **3** ($CDCl_3$)	98.21	71.20	72.57*	69.00	72.75*	62.43
Hexa-acetate **4** ($CDCl_3$)	98.42	71.33	72.66*	69.28	72.85*	62.80

*†Signals are interchangeable.

and is consistent with the previous observations [5]. Hence, **2** has the structure as indicated.

Hence, these two isomers differ in the point of attachment of the second *p*-coumaroyl moiety to the glucose unit and are, respectively, characterized as apigenin 7-*O*-β-D-(2″,6″-di-*O*-*p*-coumaroyl)glucoside and apigenin 7-*O*-β-D-(4″,6″-di-*O*-*p*-coumaroyl)glucoside from ^{13}C NMR data of the mixture and a study of the acetates. Compounds **1** and **2** may be assigned the trivial names anisofolin-B and anisofolin-C.

The isolation of 2″,- 3″- and 4″-isomers of 6″-acylated apigenin-7-*O*-β-D-glucoside supports the earlier observation that the first preferred position for acylation is OH-6″ in glucose [6], and the remaining three hydroxyls are equally preferred.

EXPERIMENTAL

All mps are uncorr. ^{13}C NMR spectra at 68.79 MHz were determined at 270 MHz.

Extraction and isolation. Dried aerial parts of *Anisomeles ovata* R. Br. (9 kg) were successively extracted with *n*-hexane and MeOH. The MeOH extract was fractionated into $CHCl_3$ Me_2CO and MeOH. The Me_2CO fraction was chromatographed on a column of Si gel using C_6H_6–Me_2CO mixtures. Compounds **1** and **2** were isolated from the 3:2 fraction. (Found: C, 64.40; H, 4.05; $C_{39}H_{32}O_{14}$ requires C, 64.65; H, 4.45%.) UV λ_{max}^{MeOH} nm: 317, 270 sh, 225; (MeOH + NaOMe) 310 sh, 365, 242 sh, 265 sh; (MeOH + $AlCl_3$) 227 sh, 277 sh, 300, 315 sh, 380; (MeOH + $AlCl_3$ + HCl) 227 sh, 277 sh, 300, 315 sh, 380; (MeOH + NaOAc) 300 sh, 315, 175. ^{13}C NMR (DMSO-d_6, assignment, number of carbons): δ164.3 (C-2, 2C), 103.1 (C-3, 2C), 181.9 (C-4, 2C), 162.0 (C-5, 2C), 99.2 (C-6, 2C), 162.6 (C-7, 2C), 94.9 (C-8, 2C), 156.9 (C-9, 2C), 105.5 (C-10, 2C), 121.0 (C-1′, 2C), 128.5 (C-2′, C-6′, 4C), 115.9 (C-3′, C-5′, 4C), 161.3 (C-4′, 2C), 124.9, 125.1 (C-1‴, C-1⁗, 4C), 129.9, 130.3 (C-2‴, C-6‴, C-2⁗, C-6⁗, 8C), 115.7, 115.8 (C-3‴, C-5‴, C-3⁗, C-5⁗, 8C), 159.7, 159.8, 159.9 (C-4‴, C-4⁗, 4C); 113.5, 113.9 (C-α, α_1, 4C), 145.0, 145.2 (C-β, β_1, 4C); 165.5, 165.9, 166.1, 166.3 (C-7‴, C-7⁗, 4C).

The mixture was acetylated with pyridine–Ac_2O to yield a mixture of **3** and **4**. ^{13}C NMR ($CDCl_3$, assignment, number of carbons): δ 161.4 (C-2, 2C), 102.6 (C-3, 2C), 176.1 (C-4, 2C), 151.0 (C-5, 2C), 109.7 (C-6, 2C), 160.1 (C-7, 2C), 112.4 (C-8, 2C), 158.4 (C-9, 2C), 108.5 (C-10, 2C), 128.7 (C-1′, 2C), 127.5 (C-2′, C-6′, 4C), 122.3 (C-3′, C-5′, 4C), 153.4 (C-4′, 2C), 131.7 (C-1‴, C-1⁗, 4C), 129.3, 129.5 (C-2‴, C-6‴, C-2⁗, C-6⁗, 8C), 122.2, 122.3 (C-3‴, C-5‴, C-3⁗, C-5⁗, 8C), 152.2, 152.4 (C-4‴, C-4⁗, 4C), 116.5, 116.6, 117.2 (C-α, α_1, 4C), 144.8, 144.9, 145.7, 145.9 (C-β, β_1, 4C), 164.8, 165.3, 166.1, 166.2 (C-7‴, C-7⁗, 4C).

Separation of **3** *and* **4**. The mixture separated on CC by elution with C_6H_6 and C_6H_6–Me_2CO mixtures (99:1, 98:2, 97:3, 95:5, 9:1) into hexa-acetate **3** (mp 140°) and hexa-acetate **4** (mp 132°). (Found: C, 63.01; H, 4.39; $C_{51}H_{44}O_{20}$ requires C, 62.72; H, 4.54%.)

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