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ACYLATED KAEMPFEROL GLYCOSIDES FROM *LAURUS NOBILIS* LEAVES

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IN HONOUR OF THE RETIREMENT OF PROFESSOR ANDRÉ CAVÉ

Key Word Index—*Laurus nobilis*; Lauraceae; flavonol glycosides; acylated kaempferol glycosides.

Abstract—*Laurus nobilis* leaves yielded four non-polar flavonoids: kaempferol-3-*O*-α-L-(3″,4″-di-*E*-*p*-coumaroyl)-rhamnoside, kaempferol-3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside, kaempferol-3-*O*-α-L-(2″-*E*-*p*-coumaroyl)-rhamnoside and a new product kaempferol-3-*O*-α-L-(2″,4″-di-*Z*-*p*-coumaroyl)-rhamnoside. Structural elucidation was achieved by UV, 1D- and 2D-NMR experiments, mass spectrometry, acid hydrolysis and saponification.

INTRODUCTION

Apollo's Laurel (*Laurus nobilis* L.), a common Mediterranean aromatic plant, has been analysed for alkaloids [1] and essential oils of its leaves [2–5]. Its leaves also contain glycosylated flavonoids (derived from quercetin and kaempferol), (−)-epicatechin, (+)-catechin, (+)-gallocatechin, (+)-epigallocatechin and procyanidins (B2, B4, B5 and B7) [6]. The present study reports on the isolation of four non-polar flavonoids from leaves [7].

RESULTS AND DISCUSSION

Flavonoids **1**–**4** were isolated from a MeOH–H_2O (7:3) extract of dried leaves. They were identified by 1D- and 2D-NMR experiments (^{1}H NMR, ^{13}C J-MOD, ^{1}H-^{1}H COSY, HMQC and HMBC), FAB-mass spectrometry and by chemical correlations (acid hydrolysis and saponification).

Flavonoids **1**, **2** and **3** all possessed a molecular mass of 724 and flavonoid **4** of 578. Acid hydrolysis (2M H_2SO_4) indicated the aglycone of these heterosides to be kaempferol and saponification proved the presence of rhamnosyl and *p*-coumaroyl moieties. ^{1}H and ^{13}C NMR experiments (Tables 1 and 2) showed that the differences between these kaempferol-3-*O*-α-L-rhamnoside derivatives were due to the number, the position and configuration (*E* or *Z*) of the *p*-coumaroyl residues on the rhamnose moiety. The ^{1}H NMR spectrum and the ^{1}H-^{1}H COSY experiment allowed us to assign accurately all of the glycosidic protons and to determine the positions of acylation by the *p*-coumaroyl residues from the downfield chemical shift of the corresponding proton(s). All the heteronuclear correlations in the HMBC spectrum confirmed these assignments. The double bound *Z* configuration was deduced from the lowest value (12.8 Hz) of the coupling constants of the ethylenic protons and the *E* configuration from the largest one (16.0 Hz). The HMBC correlation between C-3 (δ 135.1) and the anomeric (H-1″) proton (δ 5.53) proved the position of rhamnosyl substitution to be on the 3-hydroxyl group. Finally, the ^{13}C and ^{1}H NMR signals were fully assigned to the four acylated flavonoids, which are thus reported to be: kaempferol-3-*O*-α-L-(3″,4″-di-*E*-*p*-coumaroyl)-rhamnoside (**1**), kaemperol-3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside (**2**), kaempferol-3-*O*-α-L-(2″,4″-di-*Z*-*p*-coumaroyl)-rhamnoside (**3**) and kaempferol-3-*O*-α-L-(2″-*E*-*p*-coumaroyl)-rhamnoside (**4**).

Monoacylated flavonoids are quite usual but diacylation with aromatic acids is not a frequent structural

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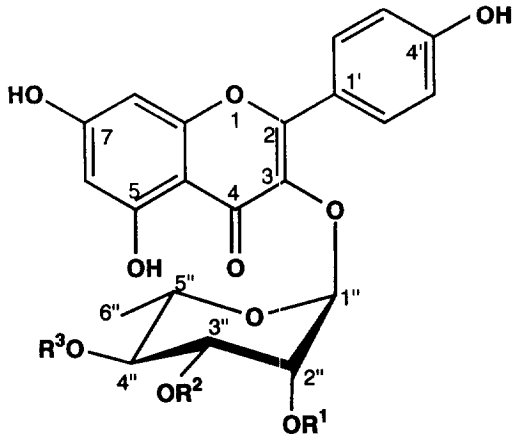
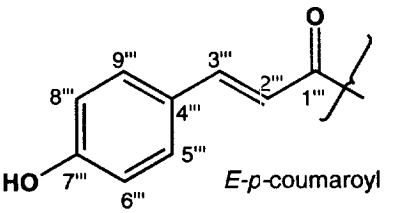
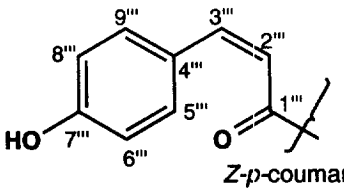


1 R^1=H, R^2=R^3= *E-p*-coumaroyl
2 R^2=H, R^1=R^3= *E-p*-coumaroyl



E-p-coumaroyl



Z-p-coumaroyl

3 R^2=H, R^1=R^3= *Z-p*-coumaroyl
4 R^2=R^3=H, R^1= *E-p*-coumaroyl

feature. Flavonoids **1** and **2** have recently been isolated from *Ocotea vellosiana*, belonging alto to the Lauraceae family [8] and **4** from *Platanus acerifolia* (Platanaceae) [9]. Compound **3** is new and also the first di-*Z*-cinnamoyl flavonoid rhamnoside reported to date in the plant kingdom.

EXPERIMENTAL

General. UV: usual shifts reagents used according to standard procedures [9]. FAB-MS: positive mode, glycerol; NMR: 500 (^{1}H) and 125 (^{13}C) MHz in CD_3OD or DMSO-d_6.

Plant material. Leaves were collected in March 1995 from trees growing in Manas (south-west of France) and were identified by Prof. I. Fourasté.

Extraction and isolation. Dried leaves (3 kg) were extracted at reflux temp. with 2 × 12 l of H_2O–MeOH (3:7). The extracts were bulked and concd under red. press. The water-insoluble green ppt. (122 g) was purified by silica gel filtration. Elution was carried out successively with 3 × 0.5 l hexane, 3 × 0.5 l $CHCl_3$ and 3 × 0.5 l Et_2O. The Et_2O eluates (8.1 g) containing apolar flavonoids were subjected to CC (Büchi 19014, 46 cm × 3.6 cm) on silica gel 60 (Lichroprep 15–25 μm) eluted with CH_2Cl_2–MeOH (19:1). Six frs were selected based on TLC analysis of the initial frs (100 ml): F1 (0.2 g), F2 (2.4 g), F3 (0.8 g), F4 (3.4 g), F5 (0.3 g), F6 (0.8 g). Compound **1** (60 mg) crystallized from F2 in CH_2Cl_2–MeOH (19:1) at room temp.

Flavonoids **2**, **3**, and **4**, respectively, were isolated from F3, F4 and F5 which were purified by prep. CC on Lichroprep (15–25 μm) eluted with CH_2Cl_2–MeOH (19:1). Final purification was achieved by prep. HPLC (Nucleosil C-18, 5 μm, 20 × 250 mm); H_2O–MeOH (8:17) affording 17 mg of **2**, 62 mg of **3** and 10 mg of **4**.

Structural elucidation. Acid hydrolyses were carried out by dissolving 2 mg of the appropriate compound in 50 μl MeOH and 50 μl 2 M H_2SO_4. After 20 min at 100°, MeOH evapn and addition of 1 ml H_2O, the aq. phase was extracted with 3 × 250 μl Et_2O. The aq. phase was neutralized with Ba_2CO_3 and analysed by HPLC: Nucleosil NH_2, 5 μm, 250 mm × 4.6 mm; MeCN–H_2O (83:17). Sugars were detected by light diffusion detector (Eurosep).

Saponifications were carried out by dissolving 2 mg of flavonoid in 100 μl 1% NaOH. After 5 min at 100°, the mixts were neutralized with 1 N HCl. The aq. phase was extracted with 3 × 50 μl Et_2O and analysed by TLC: silica gel, toluene–Et_2O–10% HOAc (1:1:3), upper phase.

Compound **1** (*kaempferol*-3-O-α-L-(3″,4″-di-E-p-*coumaroyl*)-*rhamnoside*). Yellow powder. Alkaline hydrolysis gave kaempferol-3-*O*-α-L-rhamnoside, and *p*-coumaric acid. UV λ_{max}^{MeOH} nm: 267, 300 sh, 313; ($AlCl_3$) 280, 312, 322 sh, 400 sh; ($AlCl_3$ + HCl) 278 sh, 307, 327 sh, 395 sh; (NaOAc) 274, 317, 325 sh, 368; (NaOAc + H_3BO_3) 268, 303 sh, 317. FAB-MS: *m/z* 725 $[M+H]^+$.

Compound **2** (*kaempferol*-3-O-α-L-(2″,4″-di-E-p-*coumaroyl*)-*rhamnoside*). Yellow powder. Alkaline hydrolysis gave kaempferol-3-*O*-α-L-rhamnoside and *p*-coumaric acid. UV λ_{max}^{MeOH} nm: 268, 300 sh, 313; ($AlCl_3$), 275, 315 sh, 395 sh; ($AlCl_3$ + HCl) 275, 300 sh, 317, 400 sh; (NaOAc) 275, 320 sh, 377; (NaOAc + H_3BO_3) 265, 301 sh, 318. FAB-MS: *m/z* 725 $[M+H]^+$.

Compound **3** (*kaempferol*-3-O-α-L-(2″,4″-di-Z-p-*coumaroyl*)-*rhamnoside*). Yellow powder. Alkaline hydrolysis gave kaempferol-3-*O*-α-L-rhamnoside and *p*-coumaric acid. UV λ_{max}^{MeOH} nm: 266, 301 sh, 313; ($AlCl_3$) 276, 307, 320, 390 sh; ($AlCl_3$ + HCl) 278, 308, 323 sh, 392 sh; (NaOAc) 274, 317, 325 sh, 368; (NaOAc + H_3BO_3) 268, 303 sh, 317. FAB-MS: *m/z* 725 $[M+H]^+$.

Compound **4** (*kaempferol*-3-O-α-L-(2″-E-p-*coumaroyl*)-*rhamnoside*). Yellow powder. Alkaline hydrolysis gave kaempferol-3-*O*-α-L-rhamnoside and *p*-coum-

Table 1. ^{1}H NMR data of compounds **1–4***

H	1	2	3	4
6	6.24 *d* (1.98)	6.18 *d* (1.75)	6.18 *d* (1.75)	6.18 *d* (2.1)
8	6.41 *d* (1.98)	6.38 *br s*	6.34 *br s*	6.33 *d* (2.1)
2′,6′	7.86 *d* (8.73)	7.77 *d* (8.7)	7.74 *d* (8.8)	7.78 *d* (8.8)
3′,5′	7.07 *d* (8.7)	7.00 *d* (8.7)	6.97 *d* (8.7)	6.94 *d* (8.8)
1″	5.8 *d* (1.32)	5.58 *br s*	5.53 *br s*	5.47 *d* (1.6)
2″	4.44 *m*	5.47 *br s*	5.52 *br s*	5.52 *dd* (1.6; 3.4)
3″	5.40 *dd* (3.15; 10.05)	4.00 *dd* (3.1; 9.7)	4.13 *dd* (3.4; 9.8)	3.93 *dd* (3.4; 9)
4″	5.21 *t* (10.05)	4.82 *t* (9.7)	4.86 *t* (9.8)	3.39 *t* (9)
5″	3.3 *m*	3.2 *m*	3.44 *m*	3.39 *m*
6″	0.87 (6.27)	0.76 *d* (6.15)	0.83 *d* (6.2)	0.98 *d* (5.83)
2‴	6.2 (16.02)	6.31 *d* (15.9)	5.74 *d* (12.8)	6.33 *d* (15.9)
2⁗	6.31 *d* (16.02)	6.44 *d* (15.9)	5.84 *d* (12.8)	
3‴	7.52 *d* (16.02)	7.48 *d* (15.9)	6.86 *d* (12.8)	7.62 *d* (15.9)
3⁗	7.65 *d* (16.02)	7.60 *d* (15.9)	6.89 *d* (12.8)	
5‴,9‴	7.42 *d* (8.58)	7.61 *d* (8.7)	7.64 *d* (8.6)	7.44 *d* (8.7)
5⁗,9⁗	7.45 *d* (8.58)	7.59 *d* (8.7)	7.66 *d* (8.6)	
6‴,8‴	6.78 *d* (8.58)	6.82 *d* (8.7)	6.75 *d* (8.6)	6.78 *d* (8.7)
6⁗,8⁗	6.88 *d* (8.58)	6.79 *d* (8.7)	6.72 *d* (8.6)	

* At 500 MHz in CD_3OD except for compound **1** which was dissolved in DMSO-d_6.
J (Hz) in parentheses.

Table 2. ^{13}C NMR data of compounds **1–4***

C	1	2	3	4
2	157.0	157.0	159.3	159.2
3	133.1	132.6	135.1	135.6
4	177.1	176.8	179.2	179.4
5	160.9	160.9	163.1	163.2
6	98.8	97.5	99.8	100.0
7	165.4	165.5	166.6	166.3
8	93.7	93.8	95.0	94.9
9	156.4	156.4	158.6	158.6
10	103.6	103.3	105.7	105.8
1′	120.0	119.9	122.4	122.5
2′	130.2	130.3	131.8	131.8
3′	115.5	115.2	116.7	116.7
4′	159.7	160.1	161.8	161.7
5′	115.5	115.2	116.7	116.7
6′	130.2	130.3	131.8	131.8
1″	100.4	98.9	101.1	100.5
2″	67.8	70.9	72.9	73.4
3″	70.6	65.7	68.5	70.7
4″	69.4	72.4	74.3	73.6
5″	67.1	67.7	69.8	72.1
6″	16.7	16.7	17.6	17.8
1‴,1⁗	165.7	165.6	167.0; 167.4	168.3
2‴,2⁗	113.0; 113.4	113.34; 113.7	115.8	114.9
3‴,3⁗	145.1	144.4; 144.6	145.9	147.1
4‴,4⁗	124.6; 124.5	124.7	127.5	127.2
5‴,9‴; 5⁗,9⁗	130.1	130.28; 130.1	133.9	131.3
6‴,8‴; 6⁗,8⁗	115.5	115.51	116.1	116.8
7‴,7⁗	159.7	159.8; 159.7	160.1	161.3

* At 500 MHz in DMSO-d_6 for **1** and CD_3OD for **2**, **3** and **4**.

aric acid. UV λ_{max}^{MeOH} nm: 266, 293 sh, 314; ($AlCl_3$) 275, 302, 322, 393 sh; ($AlCl_3$+HCl) 273, 303 sh, 323, 395 sh; (NaOAc) 275, 323 sh, 372; (NaOAc+H_3BO_3) 265, 303 sh, 314. FAB-MS: 578 *m/z* $[M+H]^+$.

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