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FLAVONOID LACTATES FROM LEAVES OF *MARRUBIUM VULGARE*

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Key Word Index—*Marrubium vulgare*; Labiatae; lactoyl flavones; 7-lactoyl-luteolin and its 2″-*O*-glucuronide and 2″-*O*-glucoside; 7-lactoylapigenin and its 2″-*O*-glucuronide and 2″-*O*-glucoside; 2D-shift correlation NMR spectra.

Abstract—The new natural lactoyl (2-hydroxypropionyl) flavonoids, luteolin and apigenin 7-lactates together with their 2″-*O*-β-glucuronides and 2″-*O*-β-glucosides were isolated from the leaves of *Marrubium vulgare*. The known flavonoids, vicenin II, vitexin, luteolin 7-glucoside, apigenin 7-glucoside, apigenin 7-(6″-*p*-coumaroyl)glucoside, luteolin, chrysoeriol and apigenin were also found. The structures were established by conventional methods of analysis and confirmed by ^{1}H, ^{13}C NMR and MS analysis. 2D-chemical shift correlation NMR was applied in the case of the new flavonoids.

INTRODUCTION

Marrubium vulgare L. (Labiatae) is a much-branched herb from the Egyptian desert–[1–3], which is used in traditional medicine. Earlier phytochemical investigations of *M. vulgare* have led to the characterization of a number of diterpenoids [4, 5] and revealed the presence of apigenin, and luteolin and their 7-glucosides together with quercetin and its 3-glucoside and 3-rhamnoglucoside [6]. In the present communication, we describe the isolation and the structural elucidation of the new lactoyl flavonoids 5,7,3′,4′-tetrahydroxy-7-*O*-lactoylflavone (luteolin 7-lactate) (**10**) and 5,7,4′-trihydroxy-7-*O*-lactoylflavone (apigenin 7-lactate) (**11**). Luteolin 7-[2-glucuronosyllactate] (**2**) and 7-[2-glucosyllactate] (**4**), and apigenin 7-[2-glucuronosyllactate] (**3**) and 7-[2-glucosyllactate] (**5**) were also isolated and identified, together with the known compounds vicenin II (**1**), vitexin (**6**), luteolin 7-glucoside (**7**), apigenin 7-glucoside (**8**), apigenin 7-(6″-*p*-coumaroyl)glucoside (**9**), luteolin (**12**), chrysoeriol (**13**) and apigenin (**14**).

It should be noted that lactoylated (α-hydroxypropionylated) flavonoids are very rare plant constituents and that previously only flavonoid glycosides acylated with lactic acid have been reported. Thus Markham *et al.* in 1978 [7] tentatively identified a luteolin 7-glucuronide-3′-(lactoyl)glucoside-4′-(lactoyl)glucuronide from the liverwort *Riccia fluitans* and more recently Aguinagalde *et al.* [8] characterized kaempferol 3-(lactoyl) glucoside-4-glucoside and 3-(*p*-coumaroyl) feruloyl-diglucoside-7-rhamnoside from *Sisymbrum gilliesii* and *Crambe scaberrima* (Cruciferae) respectively.

RESULTS AND DISCUSSION

The ethanolic leaf extract of *Marrubium vulgare* was shown by preliminary 2DPC screening to contain a complicated flavonoid mixture from which 14 compounds (**1**–**14**) were isolated and purified by cellulose CC followed by polyamide CC and preparative PC. Six of the separated compounds (**2**–**5**, **10**,**11**) are new. The remaining compounds (**1**, **6**–**9** and **12**–**14**) are known and gave chromatographic, UV spectral (Table 1) and hydrolytic data identical with those of vicenin II, vitexin, luteolin 7-glucoside, apigenin 7-glucoside, luteolin, chrysoeriol and apigenin, respectively. The structures of these known compounds (**1**, **6**–**9** and **12**–**14**) were confirmed by MS, ^{1}H and ^{13}C NMR spectral analysis. The ^{1}H and ^{13}C NMR data of some of the known compounds were used to aid the interpretation of the NMR data of the new compounds.

Compound **10** was obtained as a dark yellow amorphous powder with chromatographic and UV spectral properties (Table 1) similar to those for 7-*O*-substituted luteolin [9]. However, it exhibited a M_r of 358 in positive FABMS, $[MH]^+$: 359 and showed in its EIMS an [M −15] ion peak at *m/z* 343, a base peak at *m/z* 286, corresponding to the luteolin molecular ion and a pattern of fragmentation typical for luteolin [10]. On acid hydrolysis **10** yielded luteolin (co-PC, UV spectral data, Table 1, EIMS and ^{1}H NMR analytical data) and lactic acid (coPC). Consequently, in **10** the lactic acid must be bonded to the luteolin molecule through a hydrolysable linkage, probably of the acyl type.

The ^{1}H NMR spectrum of **10** revealed a lactoyl pattern of signal at δ 1.25 (*d*, $J = 7$ Hz, methyl lactoyl protons) and at 4.15 (*q*, $J = 7$ Hz, methine lactoyl proton). In addition, a pair of *meta* coupled aromatic proton doublets ($J = 2.5$ Hz) localized at δ6.38 and 6.56 was also recorded and was assigned to the the 6-H and 8-H protons of 7-*O*-substituted luteolin [11]. The ^{13}C NMR spectrum of **10**, finally confirmed its structure, whereby a pattern of carbon signals typical for 7-*O*-acylated (or 7-*O*-glycosylated) [12] luteolin revealed its presence, together with three carbon signals at δ173.6, 64.2 and 20.1, assignable to the carbonyl, methine and methyl carbons of the lactoyl moiety [13], respectively. Consequently, compound **10** is 7-*O*-lactoyl-luteolin.

Compound **2** was obtained as dark yellow amorphous powder and appeared on PC as dark purple spot, under UV light which changed to orange-yellow on fuming with ammonia vapour. Its R_f-values and UV absorption

Table 1. Chromatographic and UV data of the flavonoids **1–14**

No.*	Chromatographic properties R_fs (x 100)				UV spectral data λ_{max}(nm)				
	H_2O	HOAc	BAW	HOAc‡	MeOH	NaOAc	NaOAc–H_3BO_3	NaOMe	A
14	00	11	88	82	266, 335	272, 300, 373	270, 300†, 340	275, 321, 390	2
8	10	25	74	68	269, 333	267, 355, 385	268, 337	270, 389	2
9	08	23	79	72	270, 320	270, 322	268, 320	272, 390	2
1	23	55	33	70	272, 333	282, 393	283, 339	275, 361, 402†	2
6	12	30	66	64	271, 335	268, 390	280, 338	277, 330, 397	2
3	32	46	28	—	270, 333	268, 387	267, 335	271, 385	2
5	29	42	43	—	270, 334	269, 399	268, 335	270, 390	2
11	08	22	67	69	269, 330	267, 355, 388	269, 337	246, 274, 392	2
13	00	05	88	79	245, 269, 347	279, 320†, 393	270, 347		2
12	00	08	76	76	253, 267, 349	268, 384	262, 370, 426†	266, 330†, 400	2
7	08	18	54	62	255, 267†, 346	259, 265†, 360, 398	260, 370	264, 300, 398	2
2	30	44	30	—	253, 269†, 346	258, 268†, 348, 400	258, 368	265, 298†, 395	2
4	22	32	40	—	253, 267, 344	255, 265†, 352, 404	259, 370	262, 300†, 400	2
10	05	20	55	65	254, 268†, 290, 347	269, 321†, 378	260, 370	268, 300†, 400	2

*Compounds are arranged in an order which allows easier comparison.

†Inflection.

‡See Experimental.

(Table 1) were similar to those for luteolin 7-*O*-glycosides [9]. However, it exhibited a M_r of 534 as shown by negative FABMS, $[M-H]^-$: 533. On normal acid hydrolysis, **2** yielded luteolin (coPC, UV spectral data, ^1H and ^{13}C NMR), lactic and glucuronic acids (coPC), while on controlled acid hydrolysis or enzymic hydrolysis by β-glucuronidase, it yielded 7-*O*-lactoyl luteolin (**10**), (coPC, UV spectra, MS and ^1H NMR). These data led to the suggestion that **2** is luteolin acylated at its 7-position by 2-*O*-β-glucuronosyl lactic acid. The ^1H NMR spectral analysis of **2** supported this view and revealed an anomeric sugar proton doublet ($J=9$ Hz) at δ5.28, assignable to the β-glucuronide 1-H proton. The spectrum, also showed a doublet ($J=7$ Hz) and a quartet ($J=7$ Hz) located at δ1.18 and 4.18, respectively, which were assigned to the methyl and methine protons of the lactoyl moiety in the molecule of **2**. A COSY-90 NMR experiment of **2** showed a cross peak joining these two signals, thus confirming their correlation in the lactoyl moiety. The same COSY spectrum correlated the sugar protons (H-1 up to H-5) and showed that they are localized at δ3.2 (*q*, $J=9$ Hz, H-2); 3.3 (*t*, $J=9$ Hz, H-3); 3.6 (*t*, $J=9$ Hz, H-4); 4.0 (*d*, $J=9$ Hz, H-5); 5.28 (*d*, $J=9$ Hz, H-1), thus confirming that none of the sugar carbons which bear these protons is substituted, except for the anomeric carbon with its low field shifted anomeric proton. The unambiguous assignments of the glucuronide protons have been achieved also on the basis of a 2D-C/H correlation NMR experiment, carried out for **2**. The ^1H NMR spectrum of **2** revealed, in addition a pattern of aromatic proton signals typical for 7-*O*-substituted luteolin [11]. Consequently, compound (2) is identified as luteolin 7-*O*-[2-*O*-β-glucopyranuronosyl-lactate].

The ^{13}C NMR spectrum of **2** showed three carbon signals at δ172.6, 65.9 and 21.0, which were assigned to the carbonyl, oxygenated methine and methyl carbons of the lactoyl moiety. In this spectrum, the signal localized at δ99.9 was assigned to the anomeric β-glucuronide carbon attached to the methine carbon of the lactoyl moiety. The remaining glucuronide carbons (C-2 to C-6) revealed their presence at δ71.8, 73.7, 75.7, 76.3 and 169.8, respectively, thus proving that this moiety is substituted only at its anomeric carbon [14, 15], consequently it cannot be an intermediary between the luteolin and lactoyl moieties. Furthermore the chemical shift values of the glucuronide carbons (C-1 to C-6) ensure the pyranose form of this moiety. Substitution of the 7-position of the luteolin moiety of **2** follows from the upfield position of the C-7 signal, on comparison with the chemical shift value of the corresponding carbon in the spectrum of luteolin itself (Table 2). These data finally confirm the structure of **2** as luteolin 7-*O*-[2-glucopyranuronosyl-lactate].

Compound **4** was isolated as a dark yellow amorphous powder with chromatographic and UV absorption characteristics (Table 1) of a 7-*O*-glycosylated luteolin. A M_r of 520 was indicated by negative FABMS, $[M-H]^-$:

Table 2. ^{13}C NMR data of some of the flavonoids of *M. vulgare*

	Compounds								
C	**1**	**6**	**8**	**9***	**7**	**10**	**2**	**4**	**12**
2	164.1	164.1	164.2	164.2	164.5	165.0	164.6	164.5	164.5
3	102.6	102.6	103.0	103.1	103.5	101.8	103.0	103.3	103.6
4	182.3	182.2	181.7	181.9	181.8	181.0	181.9	181.9	182.1
5	158.5	160.4	161.5	161.2	161.1	161.2	161.0	161.0	161.9
6	107.5	98.3	99.4	99.4	99.8	99.5	99.8	99.9	99.4
7	161.2	162.7	162.8	162.7	163.1	163.7	163.1	163.0	165.0
8	105.3	104.7	94.9	94.7	95.0	94.9	94.8	94.8	94.8
9	155.1	156.1	156.8	156.9	157.0	157.3	157.0	157.1	157.9
10	103.8	104.2	105.4	105.4	105.6	102.6	105.4	105.4	104.8
1′	121.5	121.7	120.9	121.0	121.7	120.0	121.0	121.5	122.0
2′	129.0	129.4	128.3	128.5	113.9	112.7	113.5	113.7	113.8
3′	115.8	115.9	115.9	115.9	145.8	145.4	146.0	145.8	146.2
4′	160.7	162.6	161.0	161.2	149.8	150.1	150.5	150.3	150.2
5′	115.8	115.9	115.9	115.9	116.2	116.1	116.2	116.3	116.3
6′	129.0	129.4	128.3	128.5	119.3	118.9	119.2	119.2	119.3
1″	74.0	73.5	100.1	99.5	100.3	173.6	172.6	172.6	
2″	71.9	71.0	73.1	72.9	73.3	64.2	65.9	65.7	
3″	78.8	78.8	76.5	76.1	76.6	20.1	21.0	20.9	
4″	70.5	71.0	69.9	69.9	70.0				
5″	81.8	82.0	77.2	74.1	77.5				
6″	60.5	61.4	60.8	63.4	61.0				
1‴	73.3						99.9	100.0	
2‴	70.8						71.8	73.1	
3‴	77.8						73.7	76.4	
4‴	69.1						75.7	69.6	
5‴	80.8						76.3	77.2	
6‴	61.3						169.8	60.6	

* ^{13}C NMR of the *p*-coumaroyl moiety: δ166.4 (C=O) 113.7 (C-α), 144.9 (C-β), 124.9 (C-1), 130.0 (C-2 and C-6), 159.7 (C-4), 116.1 (C-3 and C-5).

519. The data received from the hydrolytic procedures (normal and controlled acid hydrolysis and β-glucosidase enzymatic hydrolysis) carried out for **4** suggested, together with the above given data that it is luteolin 7-*O*-[2-*O*-β-glucosyl-lactate]. The recorded ^{1}H NMR spectrum of **4** lent support to this view and revealed an anomeric β-glucose proton doublet ($J = 7.5$ Hz) at δ5.05 and a lactoyl pattern of proton signals at δ1.2 (*d*, $J = 7$ Hz) and 4.15 (*q*, $J = 7$ Hz), assignable to the methyl and oxygenated methine protons of the lactoyl moiety. Other signals in this spectrum were consistent with the proposed structure of **4** as luteolin 7-*O*-[2-*O*-β-glucosyl-lactate], which was further confirmed by 2D-shift correlation experiments.

In the ^{13}C NMR spectrum of **4**, the presence of β-glucopyranoside and lactoyl moieties followed from the anomeric carbon signal at δ100.0, the five glucose carbon signals in the area from 60.6 to 77.2 and from the signals of the lactoyl carbonyl, methine and methyl carbons at 172.6, 65.7, and 20.9 respectively. Also the luteolin carbon signals recorded in this spectrum possessed chemical shift values closely similar to those reported for 7-*O*-glycosylated or 7-*O*-acylated luteolin, thus finally confirming the structure of **4** as luteolin 7-*O*-[2-glucopyranosyl-lactate].

A minor constituent (**11**) appeared on PC as dark purple spot under UV light changing to lemon-yellow when fumed with ammonia vapour. It possessed R_f-values and UV absorption which resembled those of apigenin 7-*O*-glycosides [9]. However, it exhibited a M_r of 342 as given by positive FABMS, $[MH]^+$: 343 and by negative FABMS, $[M-H]^-$: 341. The spectra also showed an apigenin molecular ion, in each (271 and 269, respectively). Acid hydrolysis of **11** afforded apigenin (co-PC, UV absorption, Table 1 and EIMS) and lactic acid (coPC). These data led to the suggestion that **11** is 7-*O*-lactoylapigenin. The ^{1}H NMR of **11** supported this suggestion and disclosed five aromatic proton signals at δ6.4, 6.62, 6.75, 6.92 and 7.95, arranged in a 7-*O*-substituted apigenin-like-pattern of signals [11]. The spectrum also showed the typical lactoyl proton signal pattern (δ1.2, *q*, $J = 7$ Hz, methyl and 4.10, *d*, $J = 7$ Hz, oxygenated methine), thus proving the structure of **11** as 7-*O*-lactoylapigenin. Final confirmation of the structure of **11** was accomplished by synthesis, using lactic acid anhydrosulphite [16] as an agent for acylating the 7-*O*-monosodium salt of apigenin [17] in dry acetone. Purification of the synthetic product by repeated prep. PC afforded a pure sample of 7-*O*-lactoylapigenin which was found to be identical, with respect to R_f-values, UV absorption, positive FABMS and ^{1}H NMR with the natural product (**11**).

Compound **3**, another minor constituent, exhibited chromatographic and UV spectral properties (Table 1) similar to those of apigenin 7-*O*-glycosides [9]. Its M_r was determined to be 518 by negative FABMS, $[M-H]^-$: 517. On normal acid hydrolysis, it yielded apigenin (co-PC and UV spectral data, Table 1), lactic and glucuronic acids (co-PC), while on β-glucuronidase enzymic hydrolysis or controlled acid hydrolysis, it yielded 7-lactoylapigenin (**1**), (coPC and UV absorption) and glucuronic acid (coPC). The ^{1}H NMR spectrum of **3** proved the presence of β-glucuronide, (anomeric proton at δ5.2, *d*, $J = 9$ Hz; other sugar protons, *m*, between 3.15 and 3.9) and lactoyl (methyl at δ1.18, *d*, $J = 7$ Hz and oxygenated methine at 4.18, *q*, $J = 7$ Hz) moieties. The spectrum also showed the normal proton signal pattern of 7-*O*-substituted apigenin. Consequently, **3** is apigenin 7-*O*-[2-glucopyranuronosyl-lactate].

Compound **5** is another minor constituent for which the chromatographic, UV spectral data (Table 1), negative FABMS (M_r 504, $[M-H]^-$: 503) and the results of the hydrolytic procedures suggested a structure of apigenin 7-*O*-[2-glucopyranosyl-lactate]. The ^{1}H NMR spectrum of **5** revealed the presence of the lactoyl, glucoside and 7-*O*-substituted apigenin proton signal patterns, thus confirming its structure as apigenin 7-*O*-[2-glucopyranosyl-lactate].

These new natural flavonoids are of special interest because the flavone molecules are directly acylated at one of the phenolic hydroxyls and also because the acyl moiety itself (2-hydroxypropionic acid or lactic acid) is quite rare.

EXPERIMENTAL

^{1}H NMR chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts relative to DMSO-d_6 and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 6000 Hz for ^{1}H and 22000 Hz for ^{13}C, 32K data points and a flip angle of 45°. FABMS were recorded on a MM-7070 instrument (VG Analytical). PC was carried out on Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) HOAc (HOAc–H_2O, 3:17); (3) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); (4) *n*-BuOH–2M NH_4OH (1:1, top layer); (5) HOAc (HOAc–H_2O, 3:2); (6) C_6H_6–*n*-BuOH–pyridine–H_2O (1:5:3:3, top layer). Solvent systems 2 and 3 were used for PPC on Whatman No. 3 MM. Solvent systems 3 and 6 were used for sugar analysis.

Plant material. Plants of *Marrubium vulgare* L. were collected from the El-Salloum desert, north west of Egypt, during April 1985 and identified by Dr William A. Girgis (Head of the Ecology Dept., Desert Research Institut, Cairo). Vouchers are deposited at the herbarium of the NRC, Cairo.

Isolation and identification. Fresh leaf material was extracted with EtOH–H_2O (3:1). The concd extract was applied to a microcrystalline cellulose column and eluted with H_2O, whereby the non-phenolic (2DPC, $FeCl_3$ spray reagent) components were desorbed. The elution process was continued with EtOH and traced under UV light. The successive eluates, were individually dried *in vacuo* and subjected to 2DPC, whereby six different flavonoid fractions (I–VI) were obtained. Repeated crystallization of fraction I, from aq. EtOH gave pure **1**. A combination of polyamide CC (6 S for CC, Riedel-De Häen AG, seelze-Hannover, F.R.G.; eluent used H_2O–EtOH mixtures of decreasing polarities) and prep. PC of fraction II afforded pure samples of **2** and **3**. Compounds **4** and **5** were individually isolated from fraction III through cellulose CC, using *n*-BuOH saturated with H_2O. Polyamide CC of fraction IV, using MeOH–C_6H_6–H_2O mixture (30:19:1) for elution, followed by prep. PC of the desorbed flavonoids produced compounds **6**, **7** and **8**. Samples of **9**, **10** and **11** were separated from fraction V by applying cellulose CC, using *n*-BuOH saturated with H_2O as eluent. The aglycones **12**–**14** were successively desorbed from a polyamide column of fraction VI, by EtOAc saturated with H_2O.

Known compounds, vicenin II (**1**), vitexin (**6**), luteolin-7-glucoside (**7**), apigenin 7-glucoside (**8**), apigenin 7-*O*-(6″-*p*-coumaroyl)glucoside (**9**), luteolin (**12**), chrysoeriol (**13**) and apigenin (**14**) were identified by standard procedures. R_fs and UV data: Table 1. The M_r of **1**: 594, pos. FABMS (MH^+: 595); (**6**): 432, pos. FABMS (MH^+: 433); (**7**): 448, pos. FABMS $[MH]^+$: 449); (**8**): 432, pos. FABMS ($[MH]^+$ 433); (**9**): 578, negative FABMS ($[M-H]^-$: 577); (**12**): 286, EIMS; (**13**): 300, EIMS and (**14**): 270, EIMS. ^{1}H NMR of **1**: aglucone moiety: δ6.76 (*S*, H-3),

6.92 (*d*, $J = 8$ Hz, H-3′ and H-5′), 8.00 (*d*, $J = 8$ Hz, H-2′ and H-6′); sugar moieties: δ4.84 (*br s*, $W_{1/2} = 16$ Hz, H-1″ and H-1‴), 3.08–3.88 (*m*, 12 sugar protons). **6**: aglucone moiety: δ6.27 (*s*, H-8), 6.78 (*s*, H-3), 6.89 (*d*, $J = 8$ Hz, H-3′ and H-5′), 8.00 (*d*, $J = 8$ Hz, H-2′ and H-6′); sugar moiety: δ4.72 (*d*, $J = 7.5$ Hz, H-1″), 3.30–3.88 (*m*, 6 sugar protons). **7**: aglucone moiety: δ6.44 (*d*, $J = 2.5$ Hz, H-6), 6.81 (*d*, $J = 2.5$ Hz, H-8), 6.88 (*s*, H-3), 6.94 (*d*, $J = 8$ Hz, H-5′), 7.45 (*dd*, $J = 8$ and 2.5 Hz, H-2′ and H-6′); sugar moiety: δ5.10 (*d*, $J = 7.5$ Hz, H-1″), 3.30–3.80 (*m*, 6 sugar protons). **8**: aglucone moiety: δ6.42 (*d*, $J = 2.5$ Hz, H-6), 6.81 (*d*, $J = 2.5$ Hz, H-8), 6.88 (*s*, H-3), 6.94 (*d*, $J = 8$ Hz, H-3′ and H-5′), 7.94 (*d*, $J = 8$ Hz, H-2′ and H-6′); sugar moiety: δ5.05 (*d*, $J = 7.5$ Hz, H-1″), 3.15–3.50 (*m*, 6 sugar protons). **9**: Aglucone moiety: δ6.44 (*d*, $J = 2.5$ Hz, H-6), 6.72 (*s*, H-3), 6.72 (*d*, $J = 2.5$ Hz, H-8), 6.82 (*d*, $J = 8$ Hz, H-3′ and H-5′), 7.94 (*d*, $J = 8$ Hz, H-2′ and H-6′); *p*-coumaroyl moiety: δ6.25 (*d*, $J = 16$ Hz, H-α), 6.62 (*d*, $J = 8$ Hz, H-3‴ and H-5‴), 7.35 (*d*, $J = 8$ Hz, H-2‴ and H-6‴), 7.51 (*d*, $J = 16$ Hz, H-β); sugar moiety: δ5.18 (*d*, $J = 7.5$ Hz, H-1″), 4.49 (*d*, $J = 12$ Hz, H-6″), 4.20 (*dd*, $J = 12$ and 5 Hz, H-6″), 3.80 (*t*, $J = 12$ Hz, H-5″), 3.20–3.50 (*m*, three sugar protons). (12): δ6.19 (*d*, $J = 2.5$ Hz, H-6), 6.46 (*d*, $J = 2.5$ Hz, H-8), 6.85 (*s*, H-3), 6.89 (*d*, $J = 8$ Hz, H-5′), 7.40 (*dd*, $J = 8$ and 2.5 Hz, H-2′ and H-6′). **13**: δ6.18 (*d*, $J = 2.5$ Hz, H-6), 6.47 (*d*, $J = 2.5$ Hz, H-8), 6.72 (*s*, H-3), 6.88 (*d*, $J = 8$ Hz, H-5′), 7.49 (*m*, H-2′ and H-6′), 3.92 (*s*, 3 methoxyl protons). **14**: δ6.18 (*d*, $J = 2.5$ Hz, H-6), 6.47 (*d*, $J = 2.5$ Hz, H-8), 6.92 (*d*, $J = 8$ Hz, H-2′ and H-6′), 6.90 (*s*, H-3), 7.92 (*d*, $J = 8$ Hz, H-2′ and H-6′). For ^{13}C NMR of compounds (**1**, **6**, **7**–**9** and **12**–**14**) see Table 2.

7-O-*Lactoyl-luteolin* (**10**). R_fs: Table 1. M_r 358, pos. FABMS (MH^+: 359). UV spectral data: Table 1. Compound **10** was hydrolysed by 1.5 M HCl (methanolic, 100°, 7 hr) to luteolin (**12**) and lactic acid (coPC). ^{1}H NMR of **10**, luteolin moiety: δ6.38 (*d*, $J = 2.5$ Hz, H-6), 6.56 (*d*, $J = 2.5$ Hz, H-8), 6.74 (*s*, H-3), 6.84 (*d*, $J = 8$ Hz, H-5′), 7.40 (*dd*, $J = 8$ and 2.5 Hz, H-2′ and H-6′); lactoyl moiety: δ1.25 (*d*, $J = 7$ Hz, H-3″), 4.15 (*q*, $J = 7$ Hz, H-2″). ^{13}C NMR of **10**: Table 2.

Luteolin 7-O-[2-O-β-*glucopyranuronosoyl-lactate*] (**2**) R_fs: Table 1. M_r 534, negative FABMS ($[M-H]^-$: 533). UV spectral data: Table 1. Normal acid hydrolysis (2 M aq. Hcl, 100°, 7 hr) yielded luteolin (**12**), lactic and glucuronic acids (coPC). Controlled acid hydrolysis (0.1 M aq. HCl, 100°, 30 min) yielded **10**. Treatment with β-glucuronidase (0.05 M acetate buffer, pH 5.1, 37°, 24 hr) gave **10**. ^{1}H NMR of **2**; luteolin moiety: δ6.46 (*d*, $J = 2.5$ Hz, H-6), 6.70 (*s*, H-3), 6.82 (*d*, $J = 2.5$ Hz, H-8), 6.88 (*d*, $J = 8$ Hz, H-5′), 7.40 (*dd*, $J = 8$ and 2.5 Hz, H-2′ and H-6′); lactoyl moiety: δ1.18 (*d*, $J = 7$ Hz, H-3″), 4.18 (*q*, $J = 7$ Hz, H-2″); glucuronide moiety: δ5.28 (*d*, $J = 9$ Hz, H-1‴), 4.00 (*d*, $J = 9$ Hz, H-5‴), 3.20–3.50 (*m*, three glucuronide protons overlapped with H_2O signals). ^{13}C NMR of **2**: Table 2.

Luteolin 7-O-[2-O-β-*glucopyranosyl-lactate*] (**4**). R_fs: Table 1. M_r 520, negative FABMS ($[M-H]^-$: 519). UV spectral data: Table 1. Normal acid hydrolysis gave luteolin (**12**), lactic and glucose (coPC). Controlled acid hydrolysis gave 7-lactoylutolin (**10**). Enzymic hydrolysis with β-glucosidase yielded **10**. ^{1}H NMR of **4**, luteolin moiety: δ6.43 (*d*, $J = 2.5$ Hz, H-6), 6.70 (*s*, H-3), 6.80 (*d*, $J = 2.5$ Hz, H-8), 6.88 (*d*, $J = 8$ Hz, H-5′), 7.40 (*dd*, $J = 8$ and $J = 2.5$ Hz, H-2′ and H-6′); lactoyl moiety: δ1.20 (*d*, $J = 7$ Hz, H-3″), 4.15 (*d*, $J = 7$ Hz, H-2″); glucoside moiety: δ5.05 (*d*, $J = 7.5$ Hz, H-1‴), 3.20–3.70 (*m*, six sugar protons). For ^{13}C NMR of **4** see Table 2.

7-O-*Lactoyl-apigenin* (**11**). R_fs: Table 1. M_r 342, negative FABMS ($[M-H]^-$: 341), pos. FABMS (MH^+:343). UV spectral data: Table 1. Compound **11** was hydrolysed by 1.5 M HCl (methanolic, 100°, 7 hr) to apigenin (**14**) and lactic acid (coPC). ^{1}H NMR of **11**: apigenin moiety: δ6.40 (*d*, $J = 2.5$ Hz, H-6), 6.62 (*d*, $J = 2.5$ Hz, H-8), 6.75 (*s*, H-3), 6.92 (*d*, $J = 8$ Hz, H-3′ and H-5′), 7.95 (*d*, $J = 8$ Hz, H-2′ and H-6′); lactoyl moiety: δ1.20 (*d*, $J = 7$ Hz, H-3″), 4.10 (*q*, $J = 7$ Hz, H-2″). Synthesis: redistilled thionyl chloride (1.5 mol) was added slowly to stirred soln of lactic acid (1.0 mol) in dry Et_2O (400 ml) at $-10°$. The system was allowed to warm to 0° and then stirred for 16–20 hr, under red. pres. (100–200 mm), followed by 2 hr at 25°. Removal of the solvent under red. pres. afforded oily lactic acid anhydrosulphite which was purified by treatment with Ag_2O [14]. The obtained pure lactic anhydrosulphite (1.0 mol) was added to a suspension of excess dried apigenin 7-mono-sodium salt, ($\simeq 4.0$ mol) in dry Me_2CO or tetrahydrofuran (250 ml). The mixture was refluxed for 2 hr, left to cool to room temp., dried *in vacuo* and subjected to repeated prep. PC to afford a pure sample of 7-*O*-lactoylapigenin, which was found to be identical with **11**, (coPC, UV spectral data, FABMS and ^{1}H NMR).

Apigenin 7-O-[2-O-β-*glucopyranuronosyl-lactate*] (**3**). R_fs: Table 1. M_r 518, negative FABMS ($[M-H]^-$: 517). UV spectral data: Table 1. Normal acid hydrolysis gave apigenin (**14**), lactic and glucuronic acids (co-PC). Controlled acid hydrolysis gave 7-lactoylapigenin **11**. Enzymatic β-glucuronidase hydrolysis gave **11**, ^{1}H NMR of **3**, apigenin moiety: δ6.40 (*d*, $J = 2.5$ Hz, H-6), 6.70 (*s*, H-3), 6.77 (*d*, $J = 2.5$ Hz, H-8), 6.88 (*d*. $J = 8$ Hz, H-3′ and H-5′), 7.90 (*d*, $J = 8$ Hz, H-2′ and H-6′); Lactoyl moiety: δ1.18 (*d*, $J = 7$ Hz, H-3″), 4.18 (*d*, $J = 7$ Hz, H-2″); glucuronide moiety: δ5.20 (*d*, $J = 9$ Hz, H-1‴), 3.90 (*d*, $J = 9$ Hz, H-5‴), 3.15–3.60 (*m*, three glucuronide protons overlapped with H_2O porotons).

Apigenin 7-O-[2-O-β-*glucopyranosyl-lactate*] (**5**). R_fs: Table 1. M_r 504, negative FABMS ($[M-H]^-$: 503). UV spectral data: Table 1. Normal acid hydrolysis gave apigenin (**14**), lactic acid and glucose (coPC). Controlled acid hydrolysis yielded **11**. Enzymatic β-glucosidase treatment gave **11**. ^{1}H NMR of **5**, apigenin moiety: δ6.40 (*d*, $J = 2.5$ Hz, H-6), 6.64 (*s*, H-3), 6.76 (*d*, $J = 2.5$ Hz, H-8), 6.84 (*d*, $J = 8$ Hz, H-3′ and H-5′), 7.95 (*d*, $J = 8$ Hz, H-2′ and H-6′); lactoyl moiety: δ1.20 (*d*, $J = 7$ Hz, H-3″), 4.10 (*d*, $J = 7$ Hz, H-2″); glucoside moiety: δ5.10 (*d*, $J = 7.5$ Hz, H-1‴), 3.30–3.70 (*m*, six sugar protons/overlapped with H_2O protons).

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