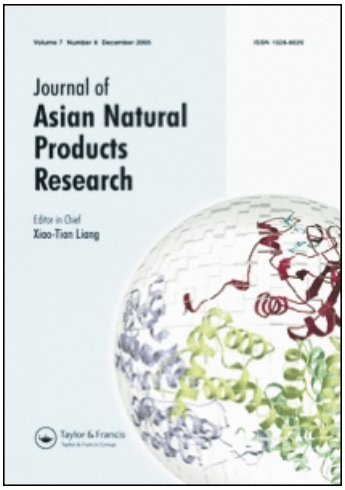

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Two new 4′-substituted flavones isolated from the fruit peels of *Citrus limon* (L.) Burm.f. (Rutaceae) have been characterized as 4′-(9′-ethylene-16′-methylnon-9′,15′-dien-7′,11′-oate)-5,7-dimethoxyflavone (limonflavonyl lactone A, **1**) and 4′-(9′-ethylene-16′-methylnon-9′,15′-dien-7′,11′-oate)-5,7,3′-trimethoxyflavone (limonflavonyl lactone B, **2**) on the basis of spectral data and chemical analyses. Both the flavones are reported for the first time from a plant source.

Keywords: *Citrus limon*; Rutaceae; flavones; limonflavonyl lactone A; limonflavonyl lactone B

1. Introduction

Citrus limon (L.) Burm.f. (Rutaceae) is an important medicinal plant of India. The true homeland of the lemon is northwestern India. It is cultivated all over India, particularly in Uttar Pradesh, Maharastra, Tamil Nadu, and Karnataka. Today California and Arizona have become the leading sources of lemons in the western hemisphere [1,2]. In the traditional systems of Indian medicine, the fruit peel is utilized as a stomachic, carminative, diaphoretic, astringent, febrifuge, and diuretic agent. It regulates skin moisture, softens hard and rough skin, and has a cleaning effect on the oily skin [1]. Phytochemical investigation of *C. limon* peels showed the presence of β-sitosterol, flavonoids, coumarins, glycosides, and volatile oils [3–7]. Biological activities, viz. antihypertensive [8,9], antihyperlipidemic, antidiabetic [10] and anticancer [11], of various extracts of the plant fruits have been reported. The present paper describes the isolation and characterization of two unknown 4′-substituted flavones from the fruit peels of *C. limon* of unexplored north Indian Delhi region.

2. Results and discussion

Limonflavonyl lactone A (**1**) was obtained as a colorless powder from chloroform: methanol (99:1) eluants. It responded positively to the tests of flavonoids. The UV spectrum of **1** displayed absorption maxima at 257 and 330 nm, indicating flavone type nature of the molecule [12,13]. There was no shift of the absorption maxima with the shift reagents suggesting the absence of hydroxyl groups in the flavone nucleus [14]. Its IR spectrum displayed characteristic absorption bands for δ-lactone group (1736 cm^{-1}) and flavone carbonyl group (1690 cm^{-1}). The MS spectrum of **1** exhibited a molecular ion peak at *m/z* 474 relating to a dimethoxy flavone with a δ-lactone unit, $C_{29}H_{30}O_6$. The generation of the important ion fragments at *m/z* 180 and 294 due to retro-Diels Alder fragmentation of ring C and then at *m/z* 134 [180-OCH_3, CH_3]$^+$ and 193 [$C_{4'}$-$C_{7'}$ fission, $C_{12}H_{17}O_2$]$^+$ indicated the existence of two methoxy groups in ring A and a C_{12}-carbon chain with δ-lactone in ring B. The ^{1}H NMR spectrum of **1** exhibited two deshielded one-proton doublets at δ 6.28 and

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6.40 with coupling interactions of 1.8 Hz each assigned to aromatic ring A *meta*-coupled H-6 and H-8, respectively. A set of AA′, BB′ signals in the aromatic regions as one-proton double doublets at δ 8.01 and 7.98 with coupling interactions of 9.6 and 1.3 Hz each and at δ 6.13 and 6.10 with $J = 9.6$, 1.5 Hz were ascribed correspondingly to *ortho*- and *meta*-coupled protons of the *para*-substituted B-ring. A one-proton broad signal at δ 6.25 was accounted to flavone H-3. Two one-proton double doublets at δ 5.49 ($J = 6.6$, 2.3 Hz) and 5.08 ($J = 9.3$, 9.3 Hz) were attributed to vinylic H-10′ and H-15′, respectively. Two one-proton doublets at δ 4.61 ($J = 6.6$ Hz) and 4.58 ($J = 6.6$ Hz) were associated with the oxygenated methylene 11′-protons of δ-lactone. Four three-proton broad signals at δ 3.84 and 3.81 and at δ 1.67 and 1.61 were accommodated to C-5 and C-7 methoxy and to C-17′ and C-18′ methyl protons located on the C-16′ vinylic carbon. The remaining methylene and methine protons appeared at δ 1.74 (H_2-12′), 1.25 (H_2-13′), 2.14 and 2.11 (H_2-14′) and at 2.17 (H-8′). The ^{13}C NMR spectrum of **1** showed important signals for δ-lactone carbons at δ 173.5 (C-7′), flavone carbonyl carbon at δ 177.6 (C-4), vinylic carbon at δ 144.9 (C-9′), 139.1 (C-10′), 123.6 (C-15′) and 142.1 (C-16′), methoxy carbons at δ 55.8 and 50.8 and methyl carbons at δ 17.7 (C-17′) and 16.7 (C-18′). The presence of olefinic carbons at δ 163.6 (C-2) and 105.1 (C-3) (Table 1) supported the flavone character of **1** [17,18]. The DEPT spectrum of **1** indicated the existence of 4 methyl, 4 methylene, 10 methine and 11 quaternary carbons. The ^{13}C NMR spectral values of **1** were compared with the related flavones [15,16]. The aromatic H-8 signal showed HMBC correlations with C-6, C-7, C-9, and C-10. The presence of undecanoate group at C-4′ in ring B was determined by correlations of H-8′ with C-3′, C-4′, and C-5′. The vinylic proton H-10′ showed correlations with C-9′, C-8′, C-11′, and C-12′. The above data established the structure of **1** as 4′-(9′-ethylene-16′-methylnon-9′,15′-dien-7′,11′-oate)-5,7-dimethoxyflavone. Compound **2**, named limonflavonyl lactone B, was obtained as a pale yellow powder from chloroform: methanol (99:1) eluants. It gave positive tests of flavonoids. The UV spectrum of **2** showed absorption maxima at 273, 335 nm suggesting flavone-type nature of the molecule [12,13]. There was no significant shift of the λ_{max} maxima with the shift reagents indicating the absence of hydroxyl group in the flavone moiety [14]. Its IR spectrum exhibited characteristic absorption bands for δ-lactone (1725 cm^{-1}) and flavone carbonyl (1690 cm^{-1}) groups. The MS spectrum of **2** showed a molecular ion peak at *m/z* 504 corresponding to a trimethoxyflavone linked with a C_{12}-moiety, $C_{30}H_{32}O_7$. The prominent ion fragments generated at *m/z* 180 and 324 due to retro-Diels Alder fragmentation of ring C and at *m/z* 136 [180-OCH_3—CH_3]$^+$, 193 [$C_{4'}$—$C_{7'}$ fission, $C_{12}H_{17}O_2$], and 205 [C_2—$C_{1'}$ fission]$^+$ supported the location of two methoxy groups in ring A, one methoxy group in ring B and attachment of a C_{12}-carbon chain with δ-lactone at C-4′. The ^{1}H NMR spectrum of **2** exhibited two deshielded one-proton doublets at δ 6.46 ($J = 1.7$ Hz) and 6.55 ($J = 1.7$ Hz) assigned to *meta*-coupled aromatic ring A protons H-6 and H-8, respectively. Two one-proton doublets at δ 7.97 ($J = 3.0$ Hz) and 6.17 ($J = 9.6$ Hz) were ascribed correspondingly to *meta*-coupled H-2′ and *ortho*-coupled H-5′. A one-proton double doublet at δ 7.94 (9.6, 3.0 Hz) was attributed to *ortho*-, *meta*-coupled H-6′. A set of AA′, BB′-type coupling system in ring B of limonflavonyl lactone A was replaced with an AMX-type coupling system of **2**. Hence, the structure of **2** was assumed to be the 3′-methoxy derivatives of limonflavonyl lactone A. This assumption was supported by the spectroscopic data reported in Table 1. A one-proton broad signal at δ 6.48 was accounted to flavonyl H-3 proton. Four one-proton double doublets at δ 5.44 ($J = 6.3$, 6.3 Hz) and 5.04 ($J = 9.2$, 9.2 Hz) and at δ 4.67 ($J = 6.3$ Hz) and 4.65 ($J = 6.3$ Hz) were associated with the vinylic H-10′ and H-15′ and oxygenated methylene protons H_2-11a, H_2-11b, respectively. Five broad signals at δ 3.88, 3.84, 3.83 and at δ 1.60 and 1.54, all integrated for three-protons each, were accommodated correspondingly to C-5, C-7 and C-3′ methoxy

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of limonflavonyl lactone A (**1**) and limonflavonyl lactone B (**2**).

	^{1}H NMR		^{13}C NMR	
Position	**1**	**2**	**1**	**2**
2	–	–	163.6	163.5
3	6.25 brs	6.48 brs	105.1	103.4
4	–	–	177.6	178.1
5	–	–	161.7	160.3
6	6.28 d (1.8)	6.46 d (1.7)	92.7	94.9
7	–	–	156.8	156.7
8	6.40 d (1.8)	6.55 d (1.7)	95.8	95.9
9	–	–	156.3	156.2
10	–	–	104.3	103.1
1′	–	–	132.0	131.1
2′	8.01 dd (9.6, 1.3)	7.97 d (3.0)	110.6	110.8
3′	6.13 dd (9.6, 1.5)	–	118.5	155.9
4′	–	–	142.1	141.2
5′	6.10 dd (9.6, 1.5)	6.17 d (9.6)	118.5	118.9
6′	7.98 dd (9.6, 1.3)	7.94 dd (9.6, 3.0)	110.6	110.6
7′	–	–	173.5	174.1
8′	2.17 s	2.50 brs	25.7	25.8
9′	–	–	144.9	145.6
10′	5.49 dd (6.6, 2.3)	5.44 dd (6.3, 6.3)	139.1	138.7
11′	4.61 d (6.6), 4.58 d (6.6)	4.67 d (6.3), 4.65 d (6.3)	65.7	65.7
12′	1.74 brs	1.71 brs	39.5	39.5
13′	1.25 brs	1.19 brs	26.3	26.5
14′	2.14 d (9.3), 2.11 d (9.3)	2.05 brs 2.11 d (9.3)	29.7	29.01
15′	5.08 dd (9.3, 9.3)	5.04 dd (9.2, 9.2)	123.6	123.7
16′	–	–	142.1	144.2
17′	1.67 brs	1.60 brs	17.7	17.6
18′	1.61 brs	1.54 brs	16.7	16.8
Me	3.84 brs, 3.81 brs	3.88 brs, 3.84 brs, 3.83 brs	55.8, 50.8	56.3, 56.0

Coupling constants in Hertz (Hz) are provided in parenthesis.

and C-17′, C-18′ methyl protons. The remaining methylene and methine protons resonated between δ 2.50–1.19. The ^{13}C NMR spectrum of **2** showed important signals for C-4 carbonyl carbon at δ 178.1, lactone C-7′ carbon at δ 174.1, flavone carbons between δ 163.5–94.9, vinylic carbons at δ 145.6 (C-9′), 138.7 (C-10′), 123.7 (C-15′) and 144.2 (C-16′), oxygenated methylene carbon at δ 65.7 (C-11′), methoxy carbons at δ 56.3, 56.0 and the remaining methine, methylene and methyl carbons between δ 39.50–16.37. The DEPT spectrum of **2** indicated the existence of 5 methyl, 4 methylene, 10 methine and 12 quaternary carbons. The ^{13}C NMR values of **2** were compared with the related flavones [15,16]. The aromatic signals H-6 showed HMBC correlations with C-7, C-8, C-5, and C-10. There were correlations of H-8′ with C-3′, C-4′, C-5′, C-9′, and C-10′; H_2-11′ with C-10′; and H-15′ with C-16′, C-17′, C-18′, and C-14′. Based on the data described above, the structure of **2** was elucidated as 4′-(9′-ethylene-16′-methyl-non-9′,15′-dien-7′,11′-oate)-5,7,3′-trimethoxy-flavone (Figure 1).

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. IR spectra were recorded on KBr pellets using Jasco FT/IR-5000 instrument. UV spectra scanned in methanol on Lambda Bio 20

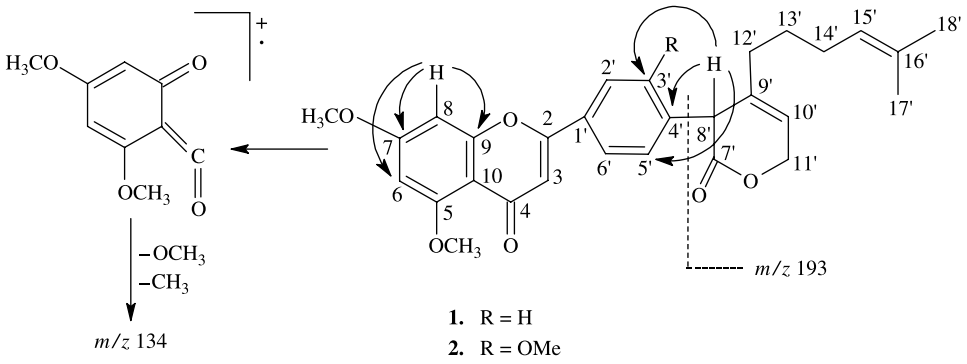

Figure 1. The structures, MS fragmentation pattern and key HMBC correlations of **1** and **2**.

Spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (75 Hz) spectra were recorded on an Advance DRY 400, Bruker Spectrospin in $CDCl_3$. The MS were measured in FAB ionization mode with a JEOL-JMS-DX 303. Silica gel G (Qualigens, 60–120 mesh, Mumbai, India) was used for column chromatography. Silica gel G (Qualigens) was used for analytical TLC. Spots were visualized by exposure to iodine vapors, UV radiation and by spraying reagents.

3.2 Plant material

The fresh fruits of *C. limon* were procured from the Mehaurali market of Delhi and identified by Dr M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen No. JH/PRL/FP-15 is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

3.3 Extraction and isolation

The fruit peels (1 kg) dried at 45°C in an electric oven were coarsely powdered and exhaustively extracted with methanol in a Soxhlet apparatus for 40 h. The combined extracts were concentrated and dried on a steam-bath under reduced pressure to get 200 g of dark brown mass. It was dissolved in 250 ml methanol and adsorbed on silica gel (60–120 mesh) for the preparation of slurry. The slurry was dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, petroleum ether–chloroform (9:1, 3:1, 1:1, 1:3 v/v), chloroform, chloroform–methanol (99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3 v/v) and methanol, successively, in order of increasing polarity.

3.3.1 *Limonflavonyl lactone A (**1**)*

Elution of the column with chloroform: methanol (99:1) mixture yielded colorless powder of **1**, recrystallized from acetone, 1.11 g (0.11% yield); R_f: 0.46 ($CHCl_3$: EtOAc: MeOH; 6:1:2); m.p.: 81–81°C; UV λ_{max} (MeOH): 257, 330 nm (log ϵ 3.6, 3.8); UV λ_{max} (MeOH + MeONa): 257, 330 nm; UV λ_{max} (MeOH + NaOAc): 257, 328 nm; UV λ_{max} (MeOH + boric acid): 258, 328 nm; UV λ_{max} (MeOH + $AlCl_3$): 249, 329 nm; UV λ_{max} (MeOH + $AlCl_3$ + HCl): 257, 326 nm; IR ν_{max} (KBr): 2930, 2850, 1736, 1690, 1613, 1498, 1307, 1228, 1204, 1159, 1058, 951, 897, 816, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR spectral data: Table 1; HREIMS *m/z*: 474.5561 (calcd for $C_{29}H_{30}O_6$, 474.5567); MS *m/z* (rel. int.): 474 $[M]^+$ ($C_{29}H_{30}O_6$) (3.1), 294 (5.3), 193 (93.6), 180 (12.7), 136 (11.6).

3.3.2 *Limonflavonyl lactone B (**2**)*

Further elution of the column with chloroform: methanol (99:1) furnished pale yellow powder of **2**, recrystallized from chloroform–methanol

(1:1), 881.9 mg (0.088% yield); R_f: 0.38 ($CHCl_3$: EtOAc: MeOH; 6:1:2); m.p.: 120–121°C; UV λ_{max} (MeOH): 273, 335 nm (log ϵ 5.2, 2.8); UV λ_{max} (MeOH + MeONa): 273, 335 nm; UV λ_{max} (MeOH + NaOAc): 271, 337 nm; UV λ_{max} (MeOH + boric acid): 275, 333 nm; UV λ_{max} (MeOH + $AlCl_3$): 269, 332 nm; UV λ_{max} (MeOH + $AlCl_3$): 272, 330 nm; IR ν_{max} (KBr): 2923, 2850, 1725, 1690, 1611, 1495, 1314, 1241, 1222, 1204, 1156, 1121, 1098, 940, 896, 815 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR spectral data: Table 1; HREIMS *m/z*: 504.5823 (calcd for $C_{30}H_{32}O_7$, 504.5831); MS *m/z* (rel. int.): 504 $[M]^+$ ($C_{30}H_{32}O_7$) (3.1), 294 (7.5), 205 (12.7), 193 (91.7), 180 (14.1), 136 (23.2).

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