Specific Deuteration in Patuletin and Related Flavonoids *via* Keto–Enol Tautomerism: Solvent- and Temperature-Dependent ^{1}H-NMR Studies

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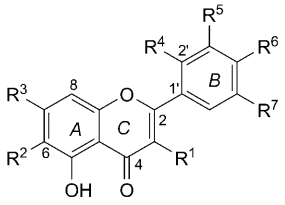
Dedicated to the fond memory of Prof. *Salimuzzman Siddiqui FRS* (1897–1994), the founding director of H.E.J. Research Institute of Chemistry, University of Karachi

An H/D exchange process in patuletin (**1**) and its derivatives in D-donor solvents (*e.g.*, CF_3COOD), which occurs regioselectively at C(8) was observed for the first time during NMR studies. The effect of substituents and temperature on the deuteration of various flavonoids (see *Fig. 1*) which include apigenin, chrysin, galangin, kaempferol, luteolin, morin, myricetin, patuletin, patulitrin, and quercetin, as well as derivatives of patuletin was examined extensively under NMR conditions. The rate constant of deuteration at C(8) of patuletin (**1**) and two flavones, luteolin (**3**) and apigenin (**12**), was also determined in CF_3COOD. The D-atom was introduced into the flavonoids *via* a keto–enol tautomerism (*Scheme 1*). During these studies, monodeuterated patuletin was also obtained as a new compound. The examined flavonoids have been reported to possess significant pharmacological activities, and their deuterated derivatives would be of importance for the identification and quantification of these compounds in biological matrices.

Introduction. – Flavonoids are polyphenol compounds that are diverse in both chemical structure and chemical properties. Since flavonoids are naturally present in fruits, vegetables, and tea, they are an integral part of the human diet. It is generally accepted that dietary flavonoids have important biological effects such as decreasing the risk of death from coronary heart disease and cancer [1a][1b][2]. Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. The antioxidant activity of flavonoids and their metabolites *in vitro* depends upon the arrangement of functional groups at the molecular-skeleton parent [1–5]. Increasingly, the flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including antiallergic, anti-HIV integrase, anti-inflammatory, antimicrobial, antioxidant, antitumor, cytotoxic, enzyme-inhibition, and oestrogenic activities [1–6].

Plants belonging to the genus *Tagetes* have been found to possess various biological properties, *e.g.*, antimicrobial, anti-inflammatory, antioxidant, and antiviral activities [7–10]. Two species of the genus, *Tagetes erecta* and *Tagetes patula*, are highly reputed in traditional medicine for their therapeutic uses. They are also the source of pesticidal agents [8–10]. In pursuance of our endeavor [11–13] for isolation of bioactive chemical constituents from *Tagetes* plants, we have recently obtained patuletin (=2-

(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-6-methoxy-4*H*-1-benzopyran-4-one; **1**), a rare 6-methoxy flavonol, and its 7-glucoside patulitrin (=2-(3,4-dihydroxyphenyl)-7-(*β*-D-glucopyranosyloxy)-3,5-dihydroxy-6-methoxy-4*H*-1-benzopyran-4-one; **2**) [14–17] in large quantity from the flowers of *Tagetes patula*, along with luteolin (=2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one; **3**), a flavonoid which has earlier been isolated from *Tagetes rupestris* [17] (*Fig. 1*). Bioassay-guided isolation studies revealed that patuletin (**1**) is the active antibacterial and antioxidant principle of the plant, while **2** is less active in both assays [18a][18b]. The tetra-*O*-benzoyl, tetra-*O*-cinnamoyl, and tetra-*O*-methyl derivatives **4**–**6** of **1** have also been prepared for the establishment of structure–activity relationships [18a][18b]. During these studies, it has been observed that the ^{1}H-NMR spectrum of **1** in CF_3COOD has a very weak signal for H–C(8), due to H/D exchange at C(8) of ring *A*, revealing the keto–enol tautomerism between OH–C(7) and H–C(8).



		R^1	R^2	R^3	R^4	R^5	R^6	R^7
Patuletin	(**1**)	OH	MeO	OH	H	OH	OH	H
Patulitrin	(**2**)	OH	MeO	GlcO	H	OH	OH	H
Luteolin	(**3**)	H	H	OH	H	OH	OH	H
Tetra-*O*-benzoylpatuletin	(**4**)	BzO	MeO	BzO	H	BzO	BzO	H
Tetra-*O*-cinnamoylpatuletin	(**5**)	CinnO	MeO	CinnO	H	CinnO	CinnO	H
Tetra-*O*-methylpatuletin	(**6**)	MeO	MeO	MeO	H	MeO	MeO	H
Galangin	(**7**)	OH	H	OH	H	H	H	H
Kaempferol	(**8**)	OH	H	OH	H	H	OH	H
Morin	(**9**)	OH	H	OH	OH	H	OH	H
Myricetin	(**10**)	OH	H	OH	H	OH	OH	OH
Quercetin	(**11**)	OH	H	OH	H	OH	OH	H
Apigenin	(**12**)	H	H	OH	H	H	OH	H
Chrysin	(**13**)	H	H	OH	H	H	H	H

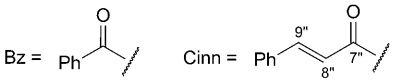


Fig. 1. *Flavonoids used in this study*

For a more comprehensive study and to establish the positional and substitutional effect of OH groups on H/D-exchange processes in different flavonoids, five flavonols (see **7**–**11**), three flavones (see **3**, **12**, and **13**), one flavonol glucoside (see **2**), as well as the benzoyl, cinnamoyl, and methyl derivatives **4**–**6** of **1** were now examined by means of solvent- and temperature-dependent ^{1}H-NMR experiments. The 8-deuterated patuletin, (8-D)patuletin (**1a**), was isolated as a new compound during these investigations. Moreover, flavonols which are more active as antioxidant agents than flavones [3][4][19] showed more reactivity towards H/D exchange as compared to flavones. Rate constants of the H/D exchange of selected flavonols and flavones were

also determined by the ^{1}H-NMR studies. This is the first time that H/D exchange during NMR studies was observed in patuletin (**1**) and patulitrin (**2**); previously this phenomenon was witnessed in the case of mangiferin (=2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9*H*-xanthen-9-one), a xanthone *C*-glucoside, which is more related to flavonols than to xanthones [20]. Furthermore, the preparation of polydeuterated flavonoids and isoflavonoids was documented to allow identification and quantitation of these compounds in biological matrices [21a][21b]; moreover, deuterated flavonoids have also been used for biosynthetic and metabolic study of flavonoids [21c].

Results and Discussion. – The ^{1}H-NMR spectra of patuletin (**1**) recorded in (D_6)acetone, (D_6)DMSO, and (D_5)pyridine (*Table 1*) exhibited no abnormal integration of H-atoms, whereas the spectrum run in (D_5)pyridine + D_2O showed the low intensity of H–C(8) as compared to other methine H-atoms at ring *B*. This revealed an H/D exchange possibly due to the formation of the conjugate acid (D-donor) of (D_5)pyridine and D_2O. On the other hand, the ^{1}H-NMR spectrum of **1** measured in CF_3COOD exhibited a very low intensity for H–C(8) (δ 7.95, 0.29 H; *Table 2*), revealing the higher reactivity of H–C(8) towards H/D exchange in a D-donor acidic solvent.

Table 1. *^{1}H-NMR Data of Patuletin* (**1**) *in Different Solvents and of (8-D)Patuletin* (**1a**) *in (D_6)Acetone.* δ in ppm, J in Hz.

	1[a])			**1a**[b])
	(D_6)acetone	(D_5)pyridine	(D_6)DMSO	(D_6)acetone
H–C(8)	6.59 (*s*)	6.82 (*s*)[c])	6.50 (*s*)	–
H–C(2′)	7.82 (*d*, *J* = 2.08)	8.59 (*d*, *J* = 2.00)	7.65 (*d*, *J* = 2.10)	7.82 (*d*, *J* = 1.96)
H–C(5′)	6.98 (*d*, *J* = 8.47)	7.38 (*d*, *J* = 8.42)	6.88 (*d*, *J* = 8.46)	6.98 (*d*, *J* = 8.48)
H–C(6′)	7.69 (*dd*, *J* = 8.47, 2.08)	8.11 (*dd*, *J* = 8.42, 2.00)	7.52 (*dd*, *J* = 8.46, 2.10)	7.70 (*dd*, *J* = 8.48, 1.96)
MeO–C(6)	3.87 (*s*)	3.94 (*s*)	3.74 (*s*)	3.87 (*s*)
OH–C(5)	12.31 (*s*)[d])	13.39 (*s*)[d])	12.55 (*s*)[d])	12.30 (*s*)[d])
Ar–OH	9.12 (*s*)[d])	11.61–12.08 (hump)[d])	9.20–9.41 (*s*, hump)[d])	7.92 (*s*)[d])
	8.48 (*s*)[d])			8.30 (*s*)[d])
	8.27 (*s*)[d])			8.52 (*s*)[d])
	7.94 (*s*)[d])			9.13 (*s*)[d])

[a]) 400 MHz. [b]) 500 MHz. [c]) Integration 0.85 H in (D_5)pyridine + D_2O. [d]) Disappeared on shaking with D_2O.

To investigate this phenomenon further, the ^{1}H-NMR spectra of **1** were taken in different mixtures (D_6)DMSO/CF_3COOD (*Table 3*). The spectral data revealed that in pure (D_6)DMSO and up to (D_6)DMSO/CF_3COOD 1:1.5 the integration of H–C(8) was the same as those of other H-atoms of the molecule; however, at the ratio of 1:2 the integration of H–C(8) decreased due to the initiation of the H/D exchange. Moreover, the effect of temperature on the rate of the H/D exchange in **1** was also studied by variable-temperature ^{1}H-NMR spectroscopy in CF_3COOD (*Table 4*) over the range of 30° (303 K) to 70° (343 K). At 30°, 29% of the patuletin molecules were undeuterated at C(8). On increasing the temperature, as expected, the H/D-exchange

Table 2. *^{1}H-NMR Data of Patuletin* (**1**), *Patulitrin* (**2**), *and 3,3′,4′,7-Tetra*-O-*methylpatuletin* (**6**) *in CF_3COOD.* δ in ppm, J in Hz.

	1[a])	**2**[b])	**6**[b])
H–C(8)	7.95 (*s*, 0.29 H)	8.20 (*s*, 0.88 H)	7.92 (*s*, 0.75 H)
H–C(2′)	8.99 (br. *s*, 1.00 H)	9.07 (br. *s*, 1.00 H)	8.99 (br. *s*, 1.00 H)
H–C(5′)	8.04 (*d*, *J* = 8.59, 1.00 H)	8.12 (*d*, *J* = 8.10, 1.00 H)	8.04 (*d*, *J* = 8.64, 1.00 H)
H–C(6′)	8.93 (br. *d*, *J* = 8.59, 1.00 H)	8.98 (br. *d*, *J* = 8.10, 1.00 H)	8.97 (br. *d*, *J* = 8.64, 1.00 H)
MeO–C(6)	4.96 (*s*, 3.00 H)	5.09 (*s*, 3.00 H)	4.88 (*s*, 3.00 H)
MeO–C(3)[c])	–	–	4.97 (*s*, 3.00 H)
MeO–C(3′)[c])	–	–	4.90 (*s*, 3.00 H)
MeO–C(4′)[c])	–	–	4.88 (*s*, 3.00 H)
MeO–C(7)[c])	–	–	4.83 (*s*, 3.00 H)
H–C(1″)	–	6.54 (*d*, *J* = 6.71, 1.00 H)	–
H–C(2″) to CH_2(6″)	–	4.81–5.23 (*m*, 6.00 H)	–

[a]) 300 MHz. [b]) 400 MHz. [c]) ^{1}H-NMR Values are interchangeable.

rate increased, and then, at 60°, 100% of the molecules of **1** were deuterated only at C(8). It is known that the bond-dissociation energy of C–D is higher than that of C–H [22]; thus, the H/D exchange led to the formation of a stable 8-deuterated patuletin *i.e.*, (8-D)patuletin (**1a**). Interestingly, when **1** was heated with CF_3COOD in a boiling water bath, almost complete H/D exchange took place, and after evaporation of the CF_3COOD at room temp., the ^{1}H-NMR spectrum in (D_6)acetone (*Table 1*) revealed the exclusive formation of **1a**, as well as its stability under the above experimental conditions. The ^{1}H- and ^{13}C-NMR spectra in (D_6)acetone (*Tables 1* and *5*, resp.) showed that **1** and **1a** were present in a proportion of 1:4. This regioselectivity and reactivity at C(8) towards deuteration is in agreement with the fact that the nucleophilicity of C(8) is much higher than that of other CH moieties, *i.e.*, H–C(2′), H–C(5′), and H–C(6′) in patuletin (**1**), as supported by its upfield ^{13}C-NMR chemical-shift value (δ 94.61) compared with those of other C-atoms (CH) of the molecule ((D_5)pyridine, *Table 5*). Moreover, the ^{13}C-NMR spectra of **1a** did not contain the resonance for the deuterated C(8), due to its increased spin–lattice relaxation time (T_1) and also increased complexity and decreased intensity from ^{13}C,D splitting and a decreased nuclear *Overhauser* effect [23]. The δ(C) of all the undeuterated C-atoms of **1a** are similar to those of **1**, except for the C-atoms adjacent to C(8), *i.e.*, C(7) and C(8a) which appeared upfield [23] as compared to those of **1** due to deuteration at C(8). In the EI-MS, the molecular-ion peak of **1a** was observed at *m*/*z* 333.

For the H/D-exchange process in **1**, two plausible mechanisms (*Scheme 1*) are suggested, where mechanism *A* is a keto–enol tautomerism and would be the predominant one provided that there is a free OH group at C(7). It is known that aromatic compounds including flavonoids carrying OH groups react like enols with electrophiles, except that the final product is also an enol because of the aromaticity of the compound [24]. On the other hand, mechanism *B* is a simple electrophilic-substitution reaction. The effect of substitution at C(7) on the reactivity of C(8) was nicely shown by patulitrin (**2**) and the patuletin derivatives, the *O*-benzoyl (see **4**), *O*-

Table 3. *^{1}H-NMR Data of Patuletin* (**1**), *Quercetin* (**11**), *and Apigenin* (**12**) *in (D_6)DMSO to (D_6)DMSO/CF_3COOD 1.0 : 2.0.* δ in ppm, J in Hz.

(D_6)DMSO/CF_3COOD	**1**[a])	**11**[a])		**12**[b])	
	H–C(8)	H–C(6)	H–C(8)	H–C(6)	H–C(8)
1.0 : 0.0	6.50 (*s*, 1.00 H)	6.17 (*d*, J = 1.80, 1.00 H)	6.39 (*d*, J = 1.80, 1.00 H)	6.17 (*d*, J = 1.79, 1.00 H)	6.46 (*d*, J = 1.79, 1.00 H)
1.0 : 0.2	6.49 (*s*, 1.00 H)	6.15 (*d*, J = 1.80, 1.00 H)	6.37 (*d*, J = 1.80, 1.00 H)	6.16 (*d*, J = 1.80, 1.00 H)	6.42 (*d*, J = 1.80, 1.00 H)
1.0 : 0.4	6.48 (*s*, 1.00 H)	6.12 (*d*, J = 1.79, 1.00 H)	6.32 (*d*, J = 1.79, 1.00 H)	6.13 (*d*, J = 1.31, 1.00 H)	6.37 (*d*, J = 1.31, 1.00 H)
1.0 : 0.6	6.47 (*s*, 1.00 H)	6.08 (*d*, J = 1.50, 1.00 H)	6.28 (*d*, J = 1.50, 1.00 H)	6.10 (*d*, J = 1.70, 1.00 H)	6.34 (*d*, J = 1.70, 1.00 H)
1.0 : 0.8	6.43 (*s*, 1.00 H)	6.05 (br. *s*, 0.99 H)	6.25 (br. *s*, 0.50 H)	6.10 (br. *s*, 1.00 H)	6.33 (br. *s*, 1.00 H)
1.0 : 1.0	6.41 (*s*, 1.00 H)	6.05 (br. *s*, 0.99 H)	6.26 (br. *s*, 0.25 H)	6.13 (br. *s*, 0.98 H)	6.37 (br. *s*, 0.50 H)
1.0 : 1.5	6.35 (*s*, 1.00 H)	6.06 (br. *s*, 0.60 H)	6.26 (br. *s*, 0.20 H)	6.16 (br. *s*, 0.51 H)	6.40 (br. *s*, 0.17 H)
1.0 : 2.0	6.33 (*s*, 0.66 H)	6.09 (br. *s*, 0.60 H)	6.28 (br. *s*, 0.20 H)	6.22 (br. *s*, 0.48 H)	6.45 (br. *s*, 0.16 H)

[a]) 400 MHz. [b]) 500 MHz.

Table 4. *^{1}H-NMR Data of Patuletin* (**1**), *Quercetin* (**11**), *and Apigenin* (**12**) *at Different Temperatures from 30° to 70° in CF_3COOD at 400 MHz.* δ in ppm.

T	**1**	**11**		**12**	
	H–C(8)	H–C(6)	H–C(8)	H–C(6)	H–C(8)
30° (303 K)	7.79 (*s*, 0.29 H)	7.60 (*s*, 0.66 H)	disappeared	7.58 (*s*, 0.60 H)	7.62 (*s*, 0.28 H)
40° (313 K)	7.92 (*s*, 0.16 H)	7.68 (*s*, 0.60 H)	disappeared	7.67 (*s*, 0.60 H)	7.90 (*s*, 0.14 H)
50° (323 K)	8.03 (*s*, 0.10 H)	7.78 (*s*, 0.40 H)	disappeared	7.80 (*s*, 0.45 H)	disappeared
60° (333 K)	disappeared	7.85 (*s*, negligible)	disappeared	7.90 (*s*, 0.40 H)	disappeared
70° (343 K)	disappeared	disappeared	disappeared	8.01 (*s*, 0.20 H)	disappeared

Table 5. *^{13}C-NMR Data of Patuletin* (**1**), *(8-D)Palutetin* (**1a**), *and Quercetin* (**11**). δ in ppm.

	1		**1a**		**11**
	(D_5)pyridine[a])	(D_6)acetone[a])	(D_5)pyridine[b])	(D_6)acetone[a])	(D_6)acetone[c])
C(2)	147.92	147.14	147.89	147.14	146.94
C(3)	137.53	136.40	137.53	136.40	136.73
C(4)	177.51	176.80	177.49	176.80	176.55
C(4a)	104.89	104.58	104.86	104.58	104.12
C(5)	153.07	153.12	153.04	153.12	162.31
C(6)	132.01	131.72	132.02	131.72	99.15
C(7)	158.61	157.79	158.57	157.76	164.98
C(8)	94.61	94.53	–	–	94.44
C(8a)	152.82	152.39	152.75	152.34	157.76
C(1′)	121.11	123.86	121.08	123.86	123.77
C(2′)	116.65	115.82	116.63	115.82	115.76
C(3′)	147.09	145.75	147.08	145.75	145.81
C(4′)	149.35	148.30	149.37	148.30	148.33
C(5′)	116.65	116.22	116.63	116.22	116.19
C(6′)	121.11	121.55	121.13	121.55	121.46
6-MeO	60.34	60.79	60.33	60.79	–

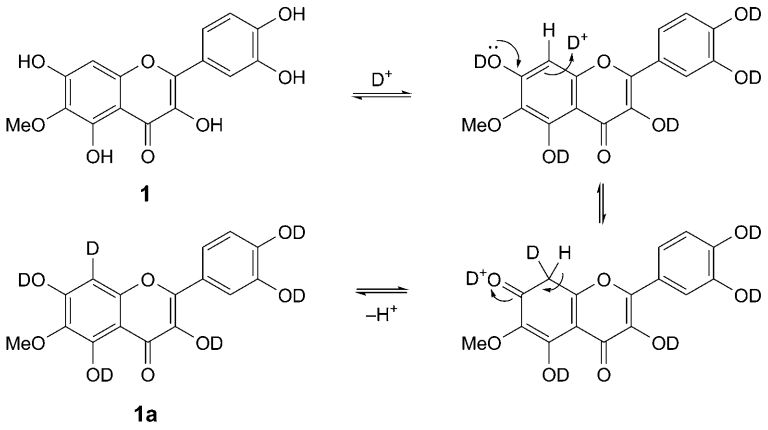
[a]) 100 MHz. [b]) 75 MHz. c) 125 MHz.

cinnamoyl (see **5**), and *O*-methyl derivatives (see **6**) [25], of which **4** and **5** are new synthetic compounds [18a]. The structures of all these compounds were determined through spectral studies including 1D- and 2D-NMR spectroscopy (*Exper. Part*, *Table 2*). In the ^{1}H-NMR spectra (CF_3COOD, *Exper. Part*, *Table 2*) of patulitrin (**2**) and **4**–**6**, very minor differences in the intensity of H–C(8) relative to that of other H-atoms of the respective molecules were observed; this might be due to the absence of a free OH group at C(7) preventing the keto–enol tautomerism, and thus the exchange reaction proceeds through a simple electrophilic-substitution reaction as outlined in *Scheme 1* (*Mechanism B*). The above observations suggested that the contribution of keto–enol tautomerism (*Mechanism A*) to the exchange process is prevailing over the electrophilic substitution (*Mechanism B*) in molecules having a free OH group at C(7).

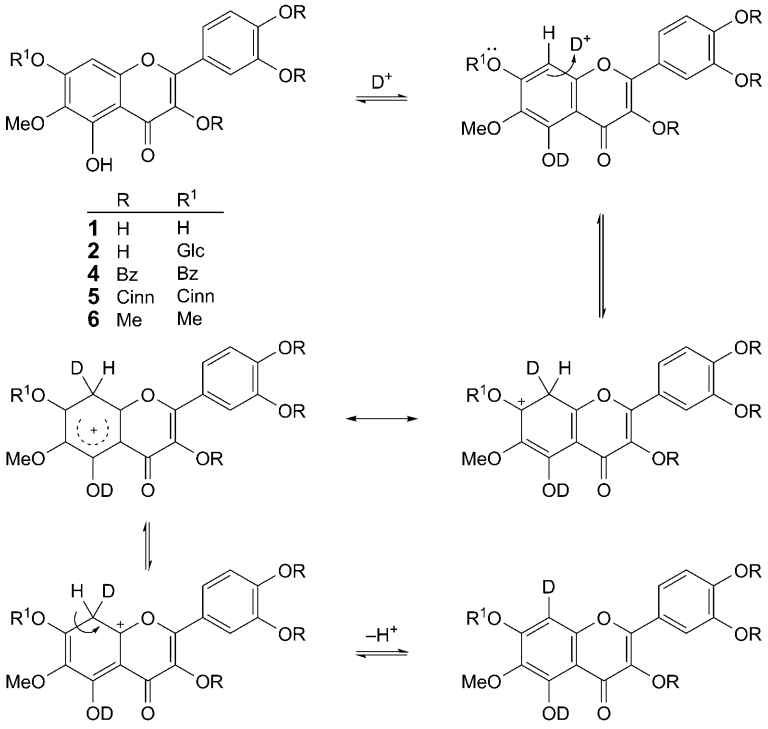
To study the positional effect of OH groups on deuteration of flavonoids, the ^{1}H-NMR spectra in CF_3COOD of five flavonols, *i.e.*, galangin (**7**), kaempferol (**8**),

Scheme 1. Mechanism A: *Keto–enol Tautomerism for Deuteration at C(8) of Patuletin* (**1**) *and Formation of* **1a**; Mechanism B: *Electrophilic-Substitution Reaction at C(8) of Patuletin* (**1**), *Patulitrin* (**2**), *and Compounds* **4** *to* **6**

Mechanism A



Mechanism B



	R	R^1
1	H	H
2	H	Glc
4	Bz	Bz
5	Cinn	Cinn
6	Me	Me

	R	R^1
1	D	D
2	D	Glc
4	Bz	Bz
5	Cinn	Cinn
6	Me	Me

morin (**9**), myricetin (**10**), and quercetin (**11**), and of three flavones, *i.e.*, apigenin (**12**), chrysin (**13**), and luteolin (**3**) (*Fig. 1*), were also examined (*Table 6*). These data revealed that the signal of H–C(8) vanished in flavonols **7**–**11**, while in flavones **3**, **12**, and **13** it appeared but with reduced intensity.

Table 6. *^{1}H-NMR Data of* **3** *and* **7**–**13** *in CF_3COOD.* δ in ppm.

	H–C(6)	H–C(8)
Galangin (**7**)	7.58 (br. *s*, 0.31 H)	disappeared
Kaempferol (**8**)	7.53 (br. *s*, 0.83 H)	disappeared
Morin (**9**)	7.55 (br. *s*)[a])	disappeared
Myricetin (**10**)[b])	7.53 (br. *s*, 0.80 H)	disappeared
Quercetin (**11**)	7.61 (br. *s*, 0.12 H)	disappeared
Luteolin (**3**)	7.63 (br. *s*, 0.75 H)	7.80 (br. *s*, 0.40 H)
Apigenin (**12**)	7.68 (br. *s*, 0.75 H)	7.87 (br. *s*, 0.27 H)
Chrysin (**13**)	7.54 (br. *s*, 0.91 H)	7.74 (br. *s*, 0.64 H)

[a]) Integration could not be measured due to overlapping. [b]) In myricetin (**10**), the integration of H–C(2′) and H–C(6′) also decreases in CF_3COOD.

The rate constant of deuteration at C(8) were calculated for patuletin (**1**) and the two flavones luteolin (**3**) and apigenin (**12**) in CF_3COOD. The progress of the reaction was monitored by ^{1}H-NMR spectroscopy, and the concentration of deuterated and nondeuterated flavonoids was determined by integrating the residual signal of H–C(8) relative to the nonexchangable H-atom signals in the spectra obtained at time intervals of *ca.* 5–10 min. The change in the peak integration with time in CF_3COOD appeared at δ 7.92, 7.87, and 7.86 in **1**, **3**, and **12**, respectively (*Table 7*). The deuteration rate of these flavonoids follows the general rate expression given by rate $= -$ d[flavonoid]/d$t =$ k [flavonoid], where k is the rate constant. The deuteration kinetics of **1**, **3**, and **12** were those of first-order reactions, as obtained by the least-squares fitting method, with the slope values of $6 \cdot 10^{-4}$ s^{-1}, $3.3 \cdot 10^{-4}$ s^{-1}, and $3.3 \cdot 10^{-4}$ s^{-1}, respectively, as shown in *Fig. 2*; a plot of ln [flavonoid] *vs.* time for **1** and **3** gave linear fits over most of the range for first-order reactions. The k value for quercetin (**11**) could not be determined since the rate of H/D exchange at C(8) was very fast under the experimental conditions. The order of reactivity of these compounds is **11** > **1** > **3** = **12**.

On comparing the integration of H–C(8) in the ^{1}H-NMR spectra of patuletin (**1**), quercetin (**11**), and apigenin (**12**) in CF_3COOD at variable temperatures (+ 30° to + 70°; *Table 4*) and at different ratios (D_6)DMSO/CF_3COOD (*Table 3*), the reactivity order observed was **1** < **11** > **12**. In quercetin (**11**), the rate of H/D exchange at C(8) was very high, and H–C(8) had almost disappeared even at 30°; however, in the case of the MeO–C(6) derivative patuletin (**1**), the complete exchange occurred at relatively higher temperature (60°). It might be due to the negative inductive effect of MeO–C(6) of **1** which decreases the electron density at C(8), as also revealed by the downfield ^{13}C-NMR chemical-shift value (δ 94.53) of C(8) in contrast to the value for quercetin (δ 94.44) (in (D_6)acetone; *Table 5*). Thus, the absence of MeO–C(6) is necessary for a greater nucleophilicity at C(8) or for faster keto–enol tautomerism. On the other hand, the presence of an OH–C(3) enhances the nucleophilicity at C(8) as

Table 7. *Integration of ^{1}H-NMR* (300 MHz) *Peaks during Deuteration of Flavonoids* **1**, **11**, **3**, *and* **12** *with CF_3COOD at Different Time Intervals*

Time [s] (min)	**1**	**11**		**3**	**12**
	H–C(8) (δ 7.92)	H–C(8) (δ 7.89)	H–C(6) (δ 7.70)	H–C(8) (δ 7.87)	H–C(8) (δ 7.86)
300 (5)	–	0.30 H	5.30 H	–	–
540 (9)	–	–	–	3.35 H	3.05 H
720 (12)	1.00 H	–	–	–	–
960 (16)	–	0.30 H	4.45 H	–	–
1020 (17)	–	–	–	2.85 H	2.05 H
1260 (21)	0.60 H	–	–	–	–
1440 (24)	–	0.30 H	3.80 H	2.35 H	–
1500 (25)	–	–	–	–	2.05 H
1800 (30)	0.45 H	–	–	–	–
1860 (31)	–	–	–	2.05 H	–
1980 (33)	–	0.30 H	3.10 H	–	–
2100 (35)	–	–	–	–	1.70 H
2280 (38)	–	–	–	1.75 H	–
2340 (39)	0.35 H	–	–	–	–
2520 (42)	–	–	–	–	1.55 H
2700 (45)	–	0.30 H	2.55 H	1.50 H	–
3000 (50)	–	–	–	–	1.35 H
3120 (52)	–	–	–	1.35 H	–
3180 (53)	–	0.30 H	2.30 H	–	–
3420 (57)	0.25 H	–		–	–
3480 (58)	–	–	–	–	1.15 H
3540 (59)	–	–	–	1.15 H	–
3840 (64)	–	0.25 H	1.95 H	–	–
3960 (66)	–	–	–	1.05 H	1.00 H
4380 (73)	–	–	–	0.90 H	–
4440 (74)	–	–	–	–	0.80 H
4800 (80)	–	0.25 H	1.50 H	–	–
4920 (82)	–	–	–	–	0.75 H
5520 (92)	–	0.25 H	1.20 H	–	–
6420 (107)	–	0.25 H	1.00 H	–	–

the 3-hydroxy derivative quercetin (**11**) is more reactive than the 3-deoxy derivative apigenin (**12**) towards C(8) deuteration.

Moreover, the H/D-exchange process was also observed at C(6) of compounds **3** and **7**–**13** (*Tables 3*, *4*, and *6*). However, in these compounds, the electron density at C(6) is lower than that at C(8) as revealed by the comparison of their ^{13}C-NMR data [5b][26a][26b]. In quercetin (**11**), C(6) resonated at δ 99.15, which is downfield with respect to the signal of C(8) (δ 94.44, *Table 5*). In the ^{1}H-NMR spectrum of **11** in CF_3COOD at 30°, the integration of H–C(6) was slightly reduced as compared to other H-atoms of the molecule, while H–C(8) had disappeared (*Table 4*). However, with the increase in temperature, the exchange process at C(6) was accelerated, and at 70°, H–C(6) was completely replaced by a D-atom. In the light of these observations, it may be concluded that C(8) is more nucleophilic than C(6), and therefore, deuteration

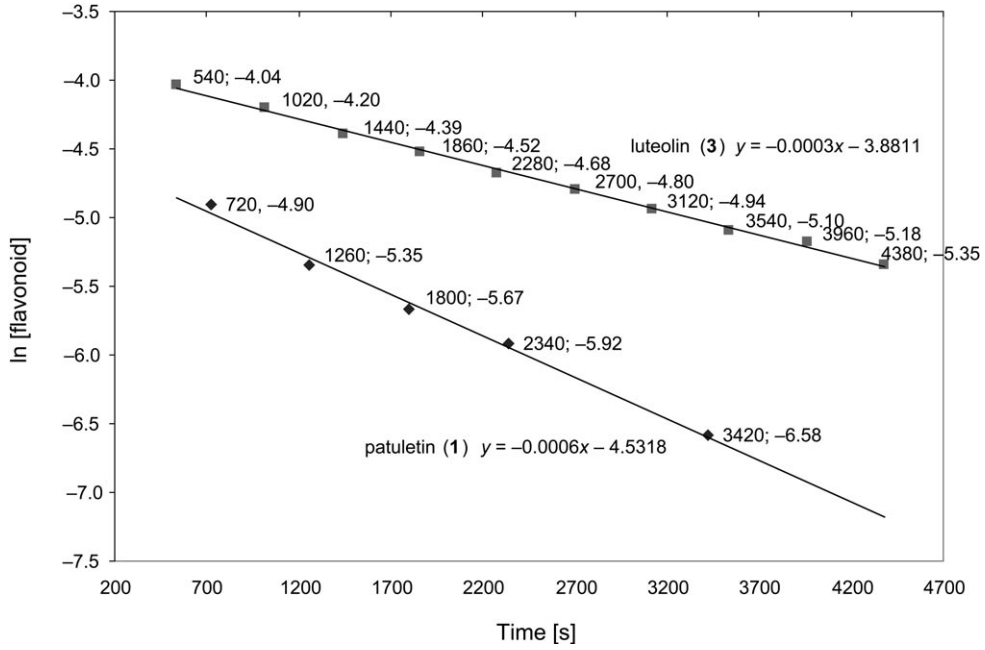
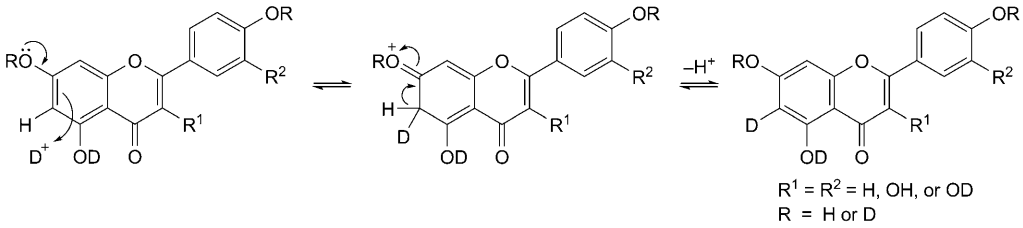


Fig. 2. *Plots of ln [flavonoid]* vs. *time for the first-order deuteration reaction of patuletin* (**1**) *and luteolin* (**3**) *with* CF_3COOD

at C(8) is much faster as compared to C(6). It is noteworthy that C(6) has two OH groups at the adjacent C(7) and C(5); however, the contribution of OH–C(5) in keto–enol tautomerism was not pronounced, which might be due to its chelation with the keto group at C(4) (*Scheme 2*).

Scheme 2. *Keto–Enol Tautomerism for Deuteration at C(6) of Flavonoids* **3** *and* **7**–**13**



On treatment of quercetin (**11**) with CF_3COOD at high temperatures, a mixture of two deuterated compounds (shown by NMR and MS) was obtained, in which the major one was deuterated both at C(8) and C(6), and the minor one was deuterated only at C(8). The 5′-hydroxy derivative myricetin (**10**) of quercetin exhibited deuteration also at ring *B* as revealed by its ^{1}H-NMR spectrum in CF_3COOD which contained only two resonances of very low integration, one for H–C(6) and the other for both H–C(2′) and H–C(6′), while that of H–C(8) had vanished.

It is interesting to note that during spectral studies in CF_3COOD, morin (**9**), a regioisomer of querectin with an OH group at C(2′), converted into the 2′-deoxy derivative kaempferol (**8**). Moreover, the authentic sample of **9** also contained trace amounts of **8** as an impurity, as shown by comparison of its TLC and ^{1}H-NMR data with that of an authentic sample of **8**. It may be suggested that OH–C(3) at ring *C* is responsible for the removal of OH–C(2′) in morin (**9**) by a photochemical reaction.

In conclusion, the ^{1}H-NMR studies revealed that patuletin (**1**) and related flavonoids underwent H/D-exchange processes. Extensive NMR investigation demonstrated the regioselective deuteration at C(8) of **1** while other flavonoids which are devoid of a substituent at C(6) showed deuteration at C(8) as well as at C(6). Flavonols were more reactive than flavones towards H/D exchange. The kinetic study established the reactivity order of flavonols **1** and **11** and flavones **3** and **12** as **11** > **1** > **3** = **12**. The reactivity order of H/D exchange at ring-*A* C-atoms in these compound was C(8) > C(6). It is important to note that there are a number of reports in the literature dealing with the deuteration studies of various natural products, *e.g.*, estrogens [27a], flavanones [21c], isoflavones [21a], isoflavanones [21a], lignans [27b], and resorcinols [27c]; however, there is very little previous work on the controlled synthesis of polydeuterated polyhydroxy-substituted flavones and flavonols [21a][21b]. It has been reported that H/D exchange occurred at highly activated sites *ortho* to two OH or MeO groups in flavonoids. No exchange occurred at the aromatic ring containing only one OH or two adjacent MeO groups [21a]. Our results which correlate to the flavonoid behavior described in [21a][21b] are as follows: *a*) owing to the highest nucleophilicity of C(8) in the flavonols examined, this position is regioselectively deuterated, and the presence of RO (R = Me, Glc) at C(6) and C(7) greatly reduces the reactivity of H–C(8) in ring *A*; *b*) the presence of an OH–C(3) enhances the nucleophilicity of C(8) and C(6), *cf.* quercetin (**11**) with apigenin (**12**) or luteolin (**3**); *c*) H-atoms of aromatic rings containing one OH or two adjacent OH groups at the ring do not exchange, *e.g.*, H-atoms in ring *B* of apigenin (**12**), luteolin (**3**), patuletin (**1**), and quercetin (**11**), *etc.*; *d*) *meta*-oriented OH groups activated the H-atoms *ortho* and *para* to them for exchange such as in ring *A* of all the above flavonoids, and in rings *A* and *B* of morin (**9**) and myricetin (**10**) in which two OH groups are *meta*-oriented; *e*) the rate of the exchange process increases as the temperature increases resulting in the complete replacement of H–C(8); *f*) the free OH group at C(7) facilitates the keto–enol tautomerism and thus the H/D exchange.

Various biological activities attributed to flavonoids, *e.g.*, antimicrobial [2][6a][18a][25], anticancer [28], anti-HIV-1 integrase [6b], as well as platelet-aggregation inhibitory [29a][29b][30], and UV-protection properties [2][31] depend on the structure of these compounds, particularly on the number of free OH groups at the molecular-skeleton parent. There are also reports of flavonoids inhibiting the activities of an array of enzymes [29b]. These biological effects are believed to come from the antioxidant properties of the related flavonoids [2–4][19][29a][29b][32a][32b], which are powerful antioxidants against free radicals because they act as 'radical scavengers'. This activity is attributed to their H-donating ability, which depends on the position and degree of hydroxylation. It has been suggested that the OH–C(3) group is a significant contributor to high antiradical activity as flavonols are more potent antioxidants than the corresponding flavones. Glycosylation of this group,

as in rutin, reduces the radical-scavenging capacity. In general, aglycones are more potent antioxidant than their corresponding glycosides. An *A*-ring sugar results in a greater activity decrease than glycosylation at C(3) of the heterocycle moiety. Methylation of OH groups also suppresses the antioxidant activity [3][4][19][29a][29b].

The keto–enol tautomerism has been suggested as necessary for the 'scavenger' activity of the flavonoids [29a]. Moreover, in case of flavanones, it has been reported that D-atoms are incorporated into these compounds *via* enolization [21c]. The present studies also showed similar trends between the process of deuteration and antioxidant activity of the flavonoids. Thus, flavonols (see **1** and **11**) which exhibited more reactivity towards H/D exchange as compared to flavones (see **3** and **12**) were also more potent antioxidants [4][16a][18b][19]. Furthermore, a free OH group at C(7) enhances the H/D exchange as observed in **1** and **11**, while in case of compound **2** the reaction was slower due to the substitution of OH–C(7) OH by a sugar moiety. Interestingly, **1** was a more powerful antioxidant than its 7-glucoside **2** [16a][18b], and **11** was also more active than its 7-glucoside [4][29b]. Substitution at both OH–C(3) and OH–C(7) has a greater influence in the decrease of the H/D exchange as well as of the antioxidant activity, as observed in the cases of **4**–**6** [18b]. On the other hand, quercetin (**11**) is itself a more powerful antioxidant than patuletin (**1**) [18b], and its H/D exchange at C(8) was also very efficient as compared to that of **1** which contained a MeO group at C(6).

Moreover, in the current studies, (8-D)patuletin (**1a**) was also prepared as a new compound which could be used as a reference compound to understand diverse pharmacological properties of patuletin, as labeled rutin has been used for drug-metabolism studies [21a]. This investigation described the significance of H/D exchange for obtaining deuterated compounds by a simple method. H/D Exchange is considered as a powerful technique for gaining structural information about stereo-isomers, reaction mechanistic studies, structure elucidation, and analytical purposes. It has also been used for the preparation of polydeuterated polyphenol isoflavonoids as internal standard in quantifying these compounds in biological fluids, and for profiling estrogens [21a][27a]. More recently, synthesis of deuterated oxidative metabolites of acrylamide and acrylonitrile has been reported for the quantification of their toxicities in humans [33].

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Experimental Part

1. *General.* All the chemicals and solvents used were of anal. grade. The flavonoids **7**–**13** were from *Merck* (Hohenbrunn, Germany). TLC: precoated silica-gel plates (SiO_2; *Merck KGaA*, 64271 Darmstadt, Germany; *HF60*$_{254}$). Flash column chromatography (FC): SiO_2 *9385*. UV Spectra: *U-3200* (*Hitachi*, Japan); λ_{max} (log ε) in nm. IR Spectra: *FT-IR-8900* (*Shimadzu*, Japan); in $CHCl_3$ or KBr disc; $\tilde{\nu}$ in cm^{-1}. EI- and HR-EI-MS: *MAT-312* (*Finnigan*, Germany) and *JMS-HX-110* spectrometer (*Jeol*, Japan); in *m*/*z* (rel. %).

2. *^{1}H- and ^{13}C-NMR Measurements.* 2.1. *General Procedure.* NMR Spectra: *Bruker Aspect AV 300*, *AV 400*, and *AV 500*; at 300, 400, and 500 MHz for ^{1}H; in (D_6)DMSO, (D_6)acetone, (D_5)pyridine, $CDCl_3$,

and CF_3COOD; the ^{1}H-NMR of **1**, **11**, and **12** at different temperatures ($+30°$ to $+70°$) in CF_3COOD at 400 MHz. For the individual measurement, the following standard parameters were used: ^{1}H-NMR, KFID (**1**–**13**) spectral width 5–7 kHz (**1**), 7 kHz (**1a**, **2**, and **4**), 5.6 kHz (**5**), 5.7 kHz (**6**), 8–10 kHz (**11**), 6 kHz (**9** and **10**), 5 kHz (**8**, **7**, **3**, and **13**), 6–10 kHz (**12**).

^{13}C-NMR Measurement in (D_5)pyridine (**1** and **1a**), (D_6)acetone (**1a** and **4**), and $CDCl_3$ (**5**) at 75, 100, and 125 MHz, resp. 2D-COSY, HMQC, and HMBC with usual pulse programs and acquisition parameter. The chemical shifts were referenced to the central peak of (D_6)DMSO (δ(H) 2.50 and δ(C) 39.50), $CDCl_3$ (δ(H) 7.25 and δ(C) 77.00), CF_3COOD (δ(H) 11.30 and δ(C) 113.18 and 161.66), (D_5)pyridine (δ(H) 7.19, 7.55, and 8.71 and δ(C) 123.50, 135.50, and 149.90), and (D_6)acetone (δ(H) 2.05 and δ(C) 30.0 and 206.29).

2.2. *Reaction Kinetics.* Kinetic measurements of the flavonoids **1**, **3**, and **12** were carried out in CF_3COOD, and progress of the reaction was monitored with the *Avance-AV-300* spectrometer. The NMR spectra were recorded after time intervals of *ca.* 5–10 min until no changes in peak heights were observed over the defined time periods. The peak integration of nonexchangable H-atoms in the flavonoid molecules were used to calculate the concentration of the deuterated products, and the first-order rate constant was obtained by the least-squares fitting method.

3. *Plant Material.* Flowers of *Tagetes patula* were collected in 2000 and 2003 on the Karachi University Campus. *T. patula* was identified by Dr. *Rubina Dawar* of the Department of Botany, University of Karachi, and the voucher specimen (No. 67280) was deposited with the herbarium of the same department.

4. *Extraction and Isolation*. The dried orange red flowers of *Tagetes patula* (181 g) were extracted twice with MeOH. The combined MeOH extract was concentrated *in vacuo* and kept overnight at r.t. when brownish insoluble matter (=*JFR*) settled down which was filtered. It turned black on exposure to light and air. The filtrate on evaporation of the solvent furnished a residue *JF* (17 g) that was partitioned between dist. H_2O and petroleum ether. The aq. phase was extracted successively with $CHCl_3$ (3×), AcOEt (6×), and BuOH (3×), and each phase (petroleum ether, $CHCl_3$, AcOEt, and BuOH) was washed with H_2O, dried (anh. Na_2SO_4), and concentrated providing respective residues, *JF-P*, *JF-C*, *JF-EA*, and *JF-BuOH*. Since the first three AcOEt phases (*JFEA*, *1–3*) showed a single spot on TLC, they were pooled in a fraction *JF-EA1* (1.2 g) which was found to be patuletin (**1**, 0.55%). Since the remaining three AcOEt phases and H_2O washings of all the six AcOEt phases had the same TLC profile showing a single spot, they were combined to yield the fraction *JF-EA2* (0.27 g), spectral studies of which elucidated its structure as patulitrin (**2**; 0.11%). The TLC of the first BuOH phase (*JF-BuOH1*) also showed the presence of **2**, while BuOH phases *2* and *3* (*JF-BuOH2*) demonstrated different TLC profile.

In another work, the fresh, undried, and uncrushed red flowers (1160 g) of *T. patula* were extracted with petroleum ether (3 × 10 l) followed by MeOH at r.t. The extracts were combined separately and the solvents evaporated to give a concentrate (*JFP*) and a gummy material (*JFM*), resp. The methanol extract *JFM* was divided into a MeOH-soluble and -insoluble (*JFMI*) portion, and the former was concentrated to give a residue (*JFMM*). A portion (6.9 g) of *JFMM* was subjected to FC (SiO_2 *9385*, petroleum ether, AcOEt, MeOH, and H_2O of increasing polarity): *Fractions 1–142*. *Fr. 20b* was a pure compound (single spot on TLC (petroleum ether/AcOEt 7.5:2.5)) and identified as luteolin (**3**) by spectral studies.

5. *(8-D)Patuletin* (=*2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-6-methoxy-(8-^{2}H)-4*H*-1-benzopyran-4-one*; **1a**). Patuletin (**1**; 0.030 g) was converted into **1a** when treated with CF_3COOD (10 ml) on a water bath for 2 h. UV (MeOH): 257.4, 371.0. IR (KBr): 3408.1, 3285.8, 2924.6, 1662.0, 1597.1. ^{1}H-and ^{13}C-NMR ((D_6)acetone): *Tables 1* and *5*. EI-MS: 333.07 (70.6), 332.07 (100.0), 289.05 (66.5).

6. *3,3′,4′,7-Tetra*-O-*benzoylpatuletin* (=*3,7-Bis(benzoyloxy)-2-[3,4-bis(benzoyloxy)phenyl]-5-hydroxy-6-methoxy-4*H*-1-benzopyran-4-one*; **4**). Patuletin (**1**; 0.1 g) in pyridine (0.5 ml) was treated with benzoic anhydride (0.2078 g), and the mixture was kept at r.t. After 3 d, it was freed of pyridine and divided into petroleum ether-soluble and -insoluble portions. The latter was kept for crystallization with $CHCl_3$/MeOH 1:1 furnishing yellow shiny crystals of **4** (85 mg; m.p. 204°) producing a single spot on TLC (SiO_2, petroleum ether/AcOEt 6.7:3.3, R_f 0.54). Yield 0.085 g (37.8%) of **4** [18a]. UV (MeOH): 330, 273–232, (MeONa) 445, 272, 228. IR ($CHCl_3$): 3662.1, 3460.6, 2924.6, 1604.06, 1748.9, 1251.1. ^{1}H-NMR (300 MHz, CF_3COOD): 8.09 (*s*, 0.66 H, H–C(8)); 8.95 (*d*, *J* = 1.89, 1.00 H, H–C(2′)); 8.38 (*d*,

$J = 7.44$, 1.00 H, H–C(5′)); 8.92 (*dd*, $J = 1.90$, 8.63, H–C(6′)); 4.87 (*s*, MeO–C(6)); 8.76, 8.81, 9.02, 9.06 (br. *d*, $J = 7.75$, 7.72, 7.89, 7.72, H–C(2″,6″) of 4 Bz); 8.17, 8.17 (br. *t*, $J = 7.50$, H–C(3″,5″) of 2 Bz); 8.39, 8.39 (*m*, H–C(3″,5″) of 2 Bz); 8.30, 8.37, 8.49, 8.54 (br. *t*, $J = 7.24$, 7.65, 7.46, 7.52, H–C(4″) of 4 Bz). ^{1}H-NMR (300 MHz, (D_6)acetone): 7.32 (*s*, H–C(8)); 8.28 (*d*, $J = 2.10$, H–C(2′)); 7.74 (*d*, $J = 8.60$, H–C(5′)); 8.17 (*dd*, $J = 2.02$, 8.60, H–C(6′); 3.91 (*s*, MeO–C(6)); 12.30 (*s*, OH–C(5)); 7.98, 8.00, 8.21, 8.21 (*dd*, $J = 1.51$, 8.50; 1.30, 8.41; 1.50, 8.91; 1.51, 8.91, H–C(2″,6″) of 4 Bz); 7.46, 7.46, 7.61, 7.61 (*td*, $J =$ 8.02, 1.51; 8.00, 1.51; 7.50, 1.51; 7.51, 1.50, H–C(3″,5″) of 4 Bz); 7.64, 7.64 (br. *t*, $J = 7.50$, H–C(4″) of 2 Bz); 7.76, 7.76 (*m*, H–C(4″) of 2 Bz). ^{13}C-NMR (125 MHz, (D_6)acetone): 151.29 (C(2)); 137.07 (C(3)); 177.64 (C(4)); 154.16 (C(5)); 132.80 (C(6)); 156.94 (C(7)); 103.32 (C(8)); 152.03 (C(8a)); 110.52 (C(4a)); 129.57 (C(1′)); 125.08 (C(2′)); 143.98 (C(3′)); 146.44 (C(4′)); 125.39 (C(5′)); 128.03 (C(6′)); 60.83 (*Me*O–C(6)); 128.74, 129.08, 129.24, 129.25 (C(1″) of Bz); 130.72, 130.74, 130.97, 131.19 (C(2″,6″) of Bz); 128.03, 129.57, 129.57, 129.84 (C(3″,5″) of Bz); 134.96, 135.00, 135.07, 135.24 (C(4″) of Bz); 164.12, 164.21, 164.36, 164.64 (CO of Bz). EI-MS: 748 (2.3), 644 (7.2), 540 (5.0), 105 (100), 183.1 (21). HR-EI-MS: 748.1561 (M^+, $C_{44}H_{28}O_{12}^+$; calc. 748.1581).

7. *3,3′,4′,7-Tetra*-O-*cinnamoylpatuletin* (*=(2*E*)-3-Phenylprop-2-enoic Acid 1,1′-{2-{3,4-bis{[(2*E*)-1-oxo-3-phenylprop-2-en-1-yl]oxy}phenyl}-5-hydroxy-6-methoxy-4-oxo-4*H*-1-benzopyran-3,7-diyl} Ester*; **5**). Patuletin (**1**; 0.1 g) in pyridine (0.95 ml) was treated with cinnamoyl chloride (0.508 g), and the mixture was kept for 10 d at r.t. The residue obtained on concentration of the mixture was treated with MeOH yielding a MeOH-soluble and -insoluble off-white powdery compound (18.2 mg) demonstrating a single spot on TLC (SiO_2, petroleum ether/AcOEt 6.7:3.3, R_f 0.44). Yield 0.125 g (57.6%) of **5** [18a]. UV (MeOH): 286, 219 (MeONa), 405.4, 390, 275. IR ($CHCl_3$): 3661.6, 3062.9, 1738.2, 163.91, 1601.27, 1120.5. ^{1}H-NMR (300 MHz, CF_3COOD): 7.99 (*s*, 1.00 H, H–C(8)); 8.81 (*d*, $J = 2.30$, 1.00 H, H–C(2′)); 8.38 (*d*, $J = 6.93$, 1.00 H, H–C(5′)); 8.88 (*m*, 1.00 H, H–C(6′)); 4.85 (*s*, MeO–C(6)); 8.21, 8.21, 8.36, 8.36 (br. *d*, $J = 9.21$, 9.21, 7.89, 7.89, H–C(2″,6″) of 4 Cinn); 8.03, 8.03 (*td*, $J = 7.82$, 2.63, H–C(3″,5″) of 2 Cinn); 8.12, 8.12 (*m*, H–C(3″,5″) of 2 Cinn); 8.15, 8.15 (*m*, H–C(4″) of 2 Cinn); 8.31, 8.31 (br. *t*, $J =$ 9.21, H–C(4″) of 2 Cinn); 7.29, 7.38, 7.51, 7.53 (*d*, $J = 15.98$, H–C(8″) of 4 Cinn); 8.57, 8.73, 8.85, 8.87 (*d*, $J = 15.99$, H–C(9″) of 4 Cinn). ^{1}H-NMR (400 MHz, $CDCl_3$): 6.92 (*s*, H–C(8)); 7.89 (*d*, $J = 1.87$, H–C(2′)); 7.46 (*d*, $J = 7.00$, H–C(5′)); 7.83 (*dd*, $J = 1.91$, 8.71, H–C(6′)); 3.95 (*s*, MeO–C(6)); 7.47, 7.47 (br. *d*, $J = 6.50$, H–C(2″,6″) of 2 Cinn); 7.60, 7.60 (*m*, H–C(2″,6″) of 2 Cinn); 7.32, 7.32 (br. *t*, $J = 6.81$, H–C(3″,5″) of 2 Cinn); 7.40, 7.40 (*m*, H–C(3″,5″) of 2 Cinn); 7.35, 7.35 (br. *t*, $J = 6.91$, H–C(4″) of 2 Cinn); 7.43, 7.43 (*m*, H–C(4″) of 2 Cinn); 6.53, 6.55, 6.67, 6.70 (*d*, $J = 15.91$, H–C(8″) of 4 Cinn); 7.77, 7.83, 7.92, 7.94 (*d*, $J = 15.91$, H–C(9″) of 4 Cinn). ^{13}C-NMR (100 MHz, $CDCl_3$): 149.93 (C(2)); 136.25 (C(3)); 176.78 (C(4)); 153.97 (C(5)); 131.87 (C(6)); 155.64 (C(7)); 101.74 (C(8)), 150.97 (C(8a)); 109.86 (C(4a)); 127.71 (C(1′)); 123.98 (C(2′)); 142.74 (C(3′)); 145.16 (C(4′)); 124.10 (C(5′)), 126.68 (C(6′)); 60.73 (*Me*O–C(6)); 133.80, 133.81, 133.99, 134.03 (C(1″) of Cinn); 128.42, 128.42, 128.42, 128.48 (C(2″,6″) of Cinn); 128.95, 128.97, 128.98, 129.05 (C(3″,5″) of Cinn); 130.95, 130.95, 131.00, 131.00 (C(4″) of Cinn); 163.75, 163.75, 163.83, 164.26 (C(7″) of Cinn); 115.61, 115.96, 115.96, 116.15 (C(8″) of Cinn); 147.80, 147.80, 147.92, 148.57 (C(9″) of Cinn). EI-MS: 852.3 (3), 592.1 (4), 332.0 (5), 289.2 (50). HR-EI-MS: 852.21868 (M^+, $C_{52}H_{36}O_{12}^+$; calc. 852.22068).

8. *3,3′,4′,7-Tetra*-O-*methylpatuletin* (*=2-(3,4-Dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy-4*H*-1-benzopyran-4-one*; **6**). Patuletin (**1**; 0.1 g) was treated with K_2CO_3 (1 g) and dimethyl sulfate (0.14 ml) in dried acetone at r.t. After 4 d (TLC monitoring), the mixture was concentrated and the residue treated with petroleum ether, petroleum ether/$CHCl_3$ 4:1, 1:1, and 1:4, $CHCl_3$, and $CHCl_3$/MeOH 1:1, and finally with MeOH. Since all the petroleum ether and petroleum ether/$CHCl_3$ fractions had the same TLC profile, they were combined and subjected to prep. TLC (SiO_2, petroleum ether/$CHCl_3$ 9.8:0.2) affording two bands, a pink and a blue one. The pink band was extracted to yield **6** (20 mg). TLC (SiO_2, petroleum ether/$CHCl_3$ 9.8:0.2): R_f 0.47. UV, IR, ^{1}H- and 2D-NMR, and EI-MS: in agreement with structure. ^{1}H-NMR (CF_3COOD): *Table 2*.

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