Spectral and Photophysical Behaviors of Curcumin and Curcuminoids

Pill-Hoon Bong

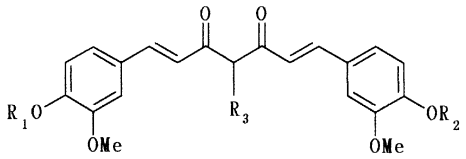
Department of Chemistry and Advanced Materials, Jeonju University, Jeonju 560-759, Korea
Received October 7, 1999

In order to obtain detailed information on ground and excited states of curcumin and curcuminoids, as well as to understand the photobiological characteristics of them, their spectral and photophysical behaviors are investigated in various conditions. Various curcuminoids were obtained and their structures were determined by spectroscopic methods. In *n*-hexane, the absorption and fluorescence spectra of these compounds contain some structure, which disappear in more polar solvent such as methanol. The fluorescence intensities of curcumin and dimethylated curcumin decrease as the concentration of water increases. The intensities also decrease as the solvent varies from neutral to extremely acidic (lower than pH 1.5) or to basic (higher than pH 8.0) condition. These results indicate that the spectral and photophysical properties of both of curcumin and curcuminoids are strongly influenced by solvent, water, and pH.

Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (**1**) is a natural yellow-orange dye extracted from the rhizomes of the plant *Curcuma longa L.* (*Zingiberaceae*).[1] A variety of pharmacological properties such as antitumour[2] and anticancer[3] activities were reported. It has also been used as a photodynamic agent useful for the destruction of bacteria[4] and tumor cells.[5] From the toxicological studies, however, curcumin is non-toxic even at high dosage.[6] Attempts to use it as an antioxidant additive for lubricants and motor oils,[7] photoresists,[8] and sunscreen compounds[9] have also been made.

To our knowledge, no systematic photochemical and photophysical investigation has been published concerning the comparative analyses of curcumin and curcuminoids up to now. Preparation and characterization of several curcuminoids, therefore, are investigated. This paper also describes spectral and photophysical studies of curcumin and curcuminoids. The effects of water concentration and protonation on the fluorescence are also investigated, aiming at obtaining detailed information on the ground and excited states as well as having a better understanding on the photobiology of these compounds.



1 : $R_1=R_2=R_3=H$ (Curcumin)
2 : $R_1=CH_3$, $R_2=R_3=H$
3 : $R_1=R_2=CH_3$, $R_3=H$
4 : $R_1=R_2=R_3=CH_3$
5 : $R_1=R_2=C_6H_5CH_2$, $R_3=H$
6 : $R_1=R_2=R_3=C_6H_5CH_2$
7 : $R_1=R_2=CH_3CO$, $R_3=H$
8 : $R_1=R_2=C_6H_5CO$, $R_3=H$
9 : $R_1=R_2=R_3=C_6H_5CO$

Results and Discussion

Curcumin (**1**) and curcuminoids (**2-9**) are practically insoluble in water at neutral and medium acidic pH, while soluble in both polar and nonpolar organic solvents. These compounds are, however, more soluble in alkaline solvents and in extremely acidic solvents, presumably due to the ionization of the phenolic or enolic groups and/or due to their degradation or change in their dissociation forms.

Preparation of Curcuminoids. There are two kinds of acidic hydrogens in curcumin (**1**). One is a phenolic hydrogen, the other is an active methylene hydrogen of β-diketones. The pKa values for the dissociation of these acidic protons in curcumin were reported to be 7.80, 8.5 and 9.0, respectively.[10] Since the difference of acidity between these two kinds of hydrogens is not large enough, the alkylation or acylation reaction with alkyl halides or acyl halides yields several products such as mono-O-substituted (**2**), di-O-substituted (**3**, **5**, **7**, **8**), and tri-substituted (mono-4-C and di-O) compounds (**4**, **6**, **9**).

In order to increase the yield and stability of phenoxide ion and/or enolate anion during the reaction, a catalytic amount of $NaHSO_4$ and $(n\text{-}Bu)_4N^{\oplus}I^{\ominus}$ were added along with base. It seems that metal halide or metal sulfate salt was formed to accelerate the anion formation. At the early stage of the reaction, the mono-substituted products were detected by TLC, which could not be isolated. In fact the reaction proceeded to yield the di-substituted one. When excess methyl iodide was used and the reaction was carefully controlled by acidification of reaction mixture, mono-O-methylated product (**2**) could be obtained. Tri-substituted products were obtained when the reaction mixture were refluxed for a sufficiently long time. Tetra-substituted product was not formed because the basicity of the base was not strong enough to make the enolate anion.

All products were separated by LC chromatography followed by recrystallization, which were characterized by NMR, IR, UV, and mass spectroscopy. Especially, the compound **3** obtained in my procedure is identical to that from

Badu and Rajasekharan.[11]

Absorption and Fluorescence. The absorption parameters of curcumin in various organic solvents were reported in a early paper by Jasim and Ali.[12] The spectral and photophysical properties of curcumin (**1**) and curcuminoids (**2-9**) were systematically investigated in *n*-hexane, a prototypic nonpolar aprotic solvent, as well as in methanol, a polar protic solvent (see Table 1). The primary purpose of this study is (i) to examine the influence of solvent polarity and protocity on spectral properties and (ii) to explain difference in photoreactivity between curcumin (**1**) and curcuminoids (**2-9**).

Absorption spectra of curcumin and curcuminoids consists of broad a structureless band in methanol but contains some structures in *n*-hexane as in toluene and in Triton X-100 micelles.[13] The absorption maximum of curcumin (**1**), mono- and di-substituted curcuminoids (**2**, **3**, **8**, **7**) is red-shifted by *ca*. 50~70 nm in contrast to those of tri-substituted curcuminoids (**4**, **6**, **9**) and compound **5**. The nature of the solvent affects the absorption spectra of curcumin and curcuminoids only slightly, producing a small red-shift (*ca*. 0~20 nm) after going from *n*-hexane to methanol.

Curcumin, in an analogy to other β-diketones, is expected to exist in the enol configuration. Using nuclear magnetic resonance data, these unirradiated curcumin and curcuminoids are found to exist entirely in the enol form. If the two ends of the chromophore can communicate *via* resonance structures in the enol forms, conjugation of the π-electrons may results in the coupling of the two feruloyl chromophores into one extended π-electron system. Upon excitation, enol form of β-diketones can undergo reketonization.[14] Again, these two chromophores can act independently or can be coupled.

Thus similarity in spectral properties of curcumin and curcuminoids may be attributed either to the *meta*-methoxyphenyl groups or to the independent action of the side of the curcuminoid molecule that retains the ketone group. Because of the difference in planar keto-enol configuration and/or the non-existence of linear extended conjugation in the curcuminoid molecule originating from steric hindrance by 4-substitution, however, absorption spectral properties of tri-substituted curcuminoids (**4**, **6**, **9**) and compound **5** are somewhat different from those of curcumin (**1**), and mono- and di-substituted curcuminoids (**2**, **3**, **7**, **8**, except **5**). Especially, the absorption maximum of compound **5** shows up at 346 nm, signalling large attribution of di-*para*-benzoxy groups.

Figures 1 and 2 depict the excitation and fluorescence spectra of curcumin (**1**) and compound **7** in *n*-hexane and in methanol, respectively. They show that the spectra are highly symmetric, revealing the symmetrical nature of the fluorescent curcumin-solvent complex.[15]

Chignell *et al*. reported that curcumin fluoresced strongly in toluene.[13] They also showed that the fluorescence intensity as well as the position of the most intense band of curcumin was very sensitive to the nature of the solvent, unlike its absorption maximum. A large red-shift in the fluorescence maximum was observed in curcumin and most of curcuminoids (**1-9**, except **6**) after going from *n*-hexane to methanol ($\Delta\lambda$ = 77 nm), which indicates that the exited singlet state must be very polar.

When the fluorescence maxima of curcumin (**1**) and alkylated curcuminoids (**2-5**) are compared with those of acylated curcuminoids (**7-9**) after changing the solvent from *n*-

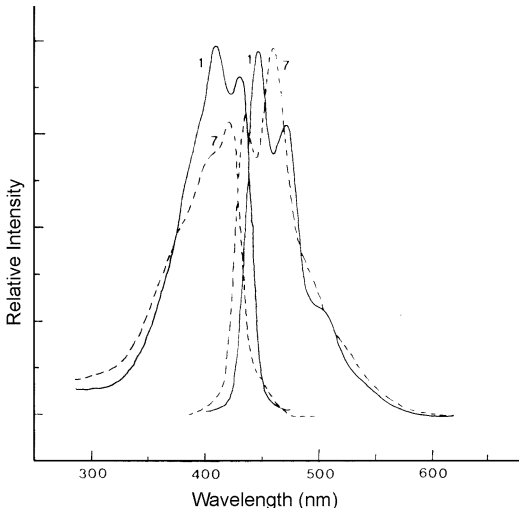


Figure 1. Excitation and fluorescence spectra of curcumin (**1**) (–) and compound **7** (--) in *n*-hexane.

Table 1. Spectral and photophysical properties of curcumin (**1**) and curcuminoids (**2~9**) in n-hexane and in methanol

Compound	Substitution type	in hexane			in methanol		
		λ_{Abs}^{max} (nm)	$\varepsilon \times 10^4$ ($M^{-1}cm^{-1}$)	λ_{fl}^{max} (nm)	λ_{Abs}^{max} (nm)	$\varepsilon \times 10^4$ ($M^{-1}cm^{-1}$)	λ_{fl}^{max} (nm)
1	-	409	4.7	444, 469, 500*	428	4.8	546
2	mono-O-methylated	409	4.1	444, 469, 500*	417	3.7	532
3	di-O-methylated	413	4.0	448, 472, 504*	420	4.6	538
4	tri(mono-4-C and di-O)-methylated	358	1.4	452, 500*	362	0.9	469, 503
5	di-O-benzylated	346	2.4	367, 385, 405*	349	2.5	433, 464, 510*
6	tri(mono-4-C and di-O)-benzylated	350	1.8	470, 505	350	1.6	466, 500*
7	di-O-acetylated	395	5.0	433, 458	399	5.0	470, 500*
8	di-O-benzoylated	400	4.7	437, 459, 500*	400	4.9	470, 500*
9	tri(mono-4-C and di-O)-benzoylated	366	3.3	461, 500*	367	3.3	467, 500*

*Shoulder

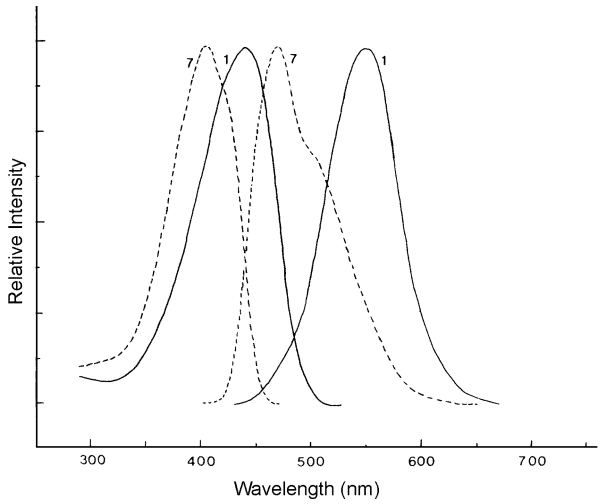


Figure 2. Excitation and fluorescence spectra of curcumin (**1**) (–) and compound **7** (--) in methanol.

hexane to methanol, the maxima for the former (51-77 nm) is shifted more severely than those for the latter (6-12 nm). Since the latter has more carbonyl groups than the former, the (n, π^*) character of these carbonyl groups can affect the shift of the fluorescence maxima. Especially, because of large 4-benzyl group substitution, compound **6** may have no planar enol conformation. Contrary to the others, therefore, the fluorescence maximum of compound **6** is blue-shifted (39 nm) after changing the solvent from *n*-hexane to methanol.

In *n*-hexane the fluorescence spectra of most compounds, except for compounds **4** and **9** (tri-substituted) contained some structures and could be resolved into several Gaussian bands. On the other hand, in methanol, the spectra of most compounds except for compounds **4** and **5** were broad and structureless. This structured band can be ascribable to vibronic transitions or can originate from various electronic transitions related to the various functionalities present in both curcumin and curcuminoids. These transitions, which may originate from the contribution of the β-diketone groups (which is important for the photochemical activity of curcuminoids), are strongly influenced by solvent protocity.[13]

Even though all curcuminoids may exist in several *cis-trans* isomeric forms of enol configuration, the fluorescence spectra exhibits no wavelength dependence. The spectra recorded at different wavelengths were identical with the original one. The excitation spectra recorded at different emission wavelengths were also identical. These results indicate that all the isomers have the same fluorescent excited state or similar photophysical properties in this steady-state experiments if curcuminoids exist as mixture of isomers in solution.

In conclusion, these spectral results indicate that the chromophore moieties (such as β-diketone, diene, phenyl rings, and substituents, *etc)* probably do not act independently but interact strongly with each other, due to the conjugation of their π-electrons in the enolic configurations as well as due to the electronic properties of substituents.

Table 2. Effect of various concentrations for water (%, v/v) on the fluorescence of curcumin (**1**) and compound **3** in methanol[a]

[H_2O] %(v/v)	η^0/η^b	Curcumin (**1**)		Compound **3**	
		I_{fl}^0/I_{fl}^c	$I^0/I\times\eta^0/\eta$	I_{fl}^0/I_{fl}^c	$I^0/I\times\eta^0/\eta$
0	1.00	1.00	1.00	1.00	1.00
3	0.99	1.21	1.20	1.03	1.03
5	0.96	1.40	1.35	1.09	1.05
7	0.95	1.58	1.50	1.13	1.07
10	0.92	1.92	1.77	1.20	1.10
20	0.85	2.93	2.49	-	-
30	0.79	4.20	3.32	-	-

[a]Concentration of curcumin (**1**) and compound **3** is 2×10^{-5} M. [b]From ref. 14. [c]I_{fl}^0 is the fluorescence intensity in anhydrous methanol.

Effect of Water. It has been observed that water quenches the fluorescence of curcumin (**1**) and dimethylated curcumin (compound **3**) as shown in Table 2 and Figure 3. A very small amount of compound **3** is also extracted from *Curcuma longa L.* together with curcumin (**1**).[1] For comparative investigation, therefore, curcumin (**1**) and compound **3** are chosen specifically. Jasim and Ali[16] reported that water quenching of curcumin can be attributed to a reaction between H_2O (electron donor, D) and the fluorescence curcumin (C^*), resulting in the formation of a nonfluorescent[17] and more stable complex ($C^{*-}D^+$) with lower S_0 vibrational energy levels (thereby with lower energy contents and longer λ_{fl}). However, their analysis is not quantitative.

$$C^* + D \rightarrow C^{*-}D^+$$

Water quenching of the fluorescence of curcumin (**1**) and compound **3** presents a convenient way of measuring the fluorescence lifetime of the excited singlet state. Assuming that the quenching of the excited singlet state by water occurs selectively and further using the steady-state approximation on the excited species, the following Stern-Volmer relation-

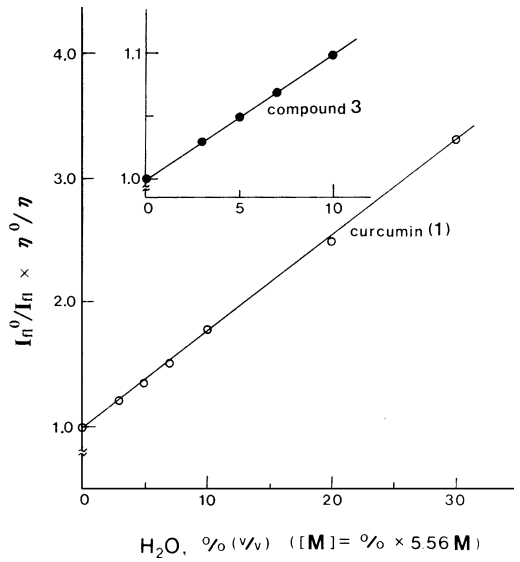


Figure 3. Effect of water (Stern-Volmer plot for the fluorescence quenching by water) of curcumin (**1**) and compound **3** in methanol.

ship can be derived

$$I_{fl}^{o} / I_{fl} = 1 + k_q \cdot \tau \cdot [H_2O]$$

where k_q is the rate constant for quenching which is assumed to be the same as that for the diffusion-controlled reaction, τ is the lifetime of the excited singlet state, and I_{fl}^{o} is the fluorescence intensity in the absence of water.

The simple Stern-Volmer plots of (I_{fl}^{o}/I_{fl}) *vs.* [H_2O] on the fluorescence of curcumin (**1**) and compound **3** are found to be non-linear, exhibiting positive curvature as was observed by others.[18,19] This deviation reveals the role of the static quenching process, where only a certain fraction W of the excited state is actually quenched by the collisional mechanism. Several models were employed to describe this process, all leading to the following modified form of the Stern-Volmer relationship.[20]

$$I_{fl}^{o}/I_{fl} = (1 + k_q \cdot \tau \cdot [H_2O])/W$$

The rate constant for diffusion (k_{diff}) and that for energy transfer (k_{et}) are sensitive to the viscosity (*i.e.* they are inversely proportional to the viscosity). Therefore, it can be assumed that $W = \eta^{o}/\eta$, where η^{o} is the viscosity of anhydrous methanol and η is that of methanol-water solvent mixture. The modified Stern-Volmer plots of $(I_{fl}^{o}/I_{fl}) \times (\eta^{o}/\eta)$ *vs.* [H_2O] on the fluorescence quenching for both curcumin (**1**) and compound **3** are linear as shown in Figure 3. This implies that water quenching is also affected by the viscosity of solvent. The slope is, therefore, dependent on the fluorescence lifetime of substrate as well as on the viscosity of the solvent. The slopes in the plots are 1.38×10^{-2} M^{-1} for curcumin (**1**) and 1.80×10^{-3} M^{-1} for compound **3**, respectively. It indicates that the effect of water quenching on the fluorescence of curcumin (**1**) is larger than that of compound **3**. Possible explanations for these results are (i) the fluorescence lifetimes of these compounds are different and (ii) intermolecular hydrogen bonding of phenolic group with water stabilize the nonfluorescent complex ($C^{*-}D^{+}$) in compound **3**. If the quenching rate-constant is assumed to be the same as that for the diffusion-controlled reaction,[21] the fluorescence lifetime (lifetime of the excited singlet state) τ is 1.44 and 0.19 psec for curcumin (**1**) and compound **3**, respectively.

Jasim and Ali[16] already reported that water quenches the fluorescence of curcumin very efficiently in acetone. Comparing their observation with the above result, it is possible that the fluorescence lifetime is larger in acetone than in methanol.

pH Effect. The potential use of curcumin as a coloring agent, a pharmaceutical prescription, or a drug is closely related to the stability of the compound. Curcumin is slightly soluble in water. The compound is, however, soluble in alkali or in glacial acetic acid. Its color is not constant in aqueous media or in an organic solvent due to its degradation or conversion to its dissociation form.[22] It is exposed to hydrolytic degradative reaction in aqueous solution.[22] Ferulic acid, feruloylmethane, vanilline, and acetone have been identified as the main degradation products. Tϕnnesen[23] reported that below pH 7 the degradation rate is lower than that at higher pH. A kinetic study of curcumin in ethanol/water as a function of temperature has also been reported.[24]

Table 3. pH effect on the fluorescence of curcumin (**1**) and compound **3** in CH_3OH-H_2O (2/8, v/v)[a]

Curcumin (**1**)				Compound **3**			
pH	I_{fl}^{0}/I_{fl}^{b}	pH	I_{fl}^{0}/I_{fl}	pH	I_{fl}^{0}/I_{fl}^{b}	pH	I_{fl}^{0}/I_{fl}
0.62	0.65	7.65	0.98	0.82	0.58	7.10	1.00
0.75	0.88	7.98	0.99	1.00	0.70	7.16	1.00
1.04	0.95	8.08	0.99	1.33	0.90	7.50	0.99
1.33	0.99	8.23	1.00	1.47	0.92	7.84	0.99
2.04	1.00	8.27	1.00	2.30	0.93	8.06	0.64
3.50	1.00	8.40	0.78	3.25	0.92	8.10	0.54
5.22	0.99	8.52	0.30	5.85	0.96	8.45	0.22
7.00	1.00	8.74	0.00	7.00	1.00	8.92	0.07
		11.80	0.00			11.80	0.05

[a]Concentration of curcumin (**1**) and compound **3** is 2×10^{-5} M. [b]I_{fl}^{0} is the fluorescence intensity at pH 7.00.

The pH effect on the fluorescence of curcumin (**1**) and compound **3** are summarized in Table 3. The fluorescence intensity of the former decreases with decrease in pH below 1.0 or with increase in pH above 8.3. The intensity of the latter compound also decreases with decrease in pH below 1.5 or with increase in pH above 8.0.

If proton forms a complex with oxygen atoms in carbonyl group and hydroxide ion forms an enolate anion of β-diketone in curcumin (**1**) and compound **3**, the proton and hydroxide ion will raise the energy of the lowest $^1(n, \pi^*)$ state. Because the proton is not expected to affect the energy of $^1(\pi, \pi^*)$ state, the intensity of fluorescence originating from the $^1(\pi, \pi^*)$ state decreases as the pH increases above the pKa value. This result is similar to the case of 1,2-bispyrazylethylene which has small energy gap between $^1(n, \pi^*)$ state and the lowest $^1(\pi, \pi^*)$ state.[25]

These results lead me to conclude that the pH effect on the fluorescence of curcumin (**1**) and compound **3** is either due to its degradation or due to the change in its properties of first excited singlet state.

Experimental Section

Materials and Instruments. Commercial reagents were obtained from Aldrich Chemical Co. and were used without further purification. All solvents were carefully dried and distilled by standard methods prior to use. Pure curcumin was obtained by recrystallization from *n*-hexane-acetone solvent system. ^1H NMR and ^{13}C NMR spectra were recorded on JEOL-JML EX 400 MHz and Bruker AM-200 MHz spectrometers. Mass spectra were determined at 70 eV with V.G. Autospec UltimaE by the electron impact (EI) method. UV absorption and IR spectra were recorded on Shimadzu UV-160A and JASCO FT/IR-5300 spectrophotometer, respectively.

Spectroscopic Measurements. The apparatus and techniques for fluorescence measurement have been described

previously.[26] UV absorption spectra were recorded on Shimadzu UV-160A spectrophotometer. pH was recorded on Corning pH meter controlled by a buffer solution. The fluorescence spectra were measured on a JASCO FP 770 spectrofluorometer. The maximal optical density of the solution used for determination of the fluorescence intensities was less than 0.1 in a cell of 1 cm thick, and all the intensities were corrected for the differences in refractive indices and viscosity[27] of solvents.

General Procedure for Preparation of Curcumin Derivatives. Curcumin (1.9 g, 5.0 mmole) was dissolved in acetone (30 mL), and KOH (0.56 g, 0.01 mole), $NaHSO_4 \cdot H_2O$ (20 mg, catalytic amount), and $(n\text{-}Bu)_4N^{\oplus}I^{\oplus}$ (10 mg, catalytic amount) were added. The mixture was stirred for 6hr at room temp., and the substrate (methyl iodide, acetyl chloride, benzyl bromide, benzoyl bromide, 0.01 mole) was added. The reaction mixture was stirred for 12 hr and then poured into 1 N acetic acid (100 mL). The aqueous solution was extracted with ethyl ether (30 mL × 2), followed by the wash of the organic extract with saturated $NaHCO_3$ solution (30 mL × 2) and water (30 mL × 2). The solution was dried over anhydrous $MgSO_4$ and evaporated to dryness. The reaction products were separated by column chromatography (Kieselgel 60, Art 7731, 70-230 mesh, Merk) with *n*-hexane-dichloromethane-acetone (4 : 1 : 1, v/v) as eluting solvents. Each product was recrystallized in *n*-hexane-acetone solvent system. The purity of the materials was confirmed by the high performance liquid chromatography (HPLC).[28]

Spectral Data of Mono-O-methylated Curcumin: ([1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione], **$C_{22}H_{22}O_6$**, **2**); Yield 0.20 g (10%); IR (KBr) 3450 (broad, OH), 1635, 1610, 1500, 1250, 1130, 1010, 970 cm^{-1}; UV (in hexane) λ_{max} 409 nm, ε 4.1×10^4 M^{-1} cm^{-1}: (in MeOH) λ_{max} 417 nm, ε 3.7×10^4 M^{-1} cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.51 (2H, d, J = 16 Hz, HC=C, 1,7-H, alkene, *trans*), 7.10 (2H, m, 6-ArH), 6.88 (4H, m, 2.5-ArH), 6.49 (2H, d, J = 16, =CH-CO-, 2,6-H, alkene, *trans*), 5.80 (1H, s, -CH=C(OH)-, 4-H, enolate), 3.88 (6H, s, 3-$ArOCH_3$), 3.81 (3H, s, 4-$ArOCH_3$) ppm; ^{13}C NMR ($CDCl_3$) δ 183.7, 183.0, 161.5, 150.8, 149.3, 140.1, 130.6, 129.9, 128.1, 123.0, 122.0, 114.3, 111.5, 109.9, 101.0, 56.3, 55.3 ppm; MS m/z (EI) Data threshold 10.00% of normalizing intensity (8.758E+04); 382 (M^+, 18), 381 (M^+-H, 23), 380 (M^+-2H, 100): Data threshold 7.00% of normalizing intensity (1.395E +D6); 366 (M^+-H-CH_3, 12), 348 (M^+-3H-OCH_3, 22), 191 (M^+-$C_{11}H_{11}O_3 \equiv C_{11}H_{11}O_3$, 3,4-dimethoxycinnamyl, 100), 161 (M^+-$C_{13}H_{17}O_3$, 98).

Spectral Data of Di-O-methylated Curcumin: ([1,7-bis-(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione], **$C_{23}H_{24}O_6$**, **3**); Yield 1.05 g (53%); IR (KBr) 1620, 1595, 1510, 1255, 1130, 1020 cm^{-1}; UV (in hexane) λ_{max} 413 nm, ε 4.0×10^4 M^{-1} cm^{-1}: (in MeOH) λ_{max} 420 nm, ε 4.6×10^4 M^{-1} cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.59 (2H, d, J = 16 Hz, HC=C, 1,7-H, alkene, *trans*), 7.12 (2H, d of d, J = 8 Hz, J = 2 Hz, 6-ArH), 7.06 (2H, d, J = 2 Hz, 2-ArH), 6.86 (2H, d, J = 8 Hz, 5-ArH), 6.47 (2H, d, J = 16 Hz, =CH-CO-, 2,6-H, alkene, *trans*), 5.80 (1H, s, -CH=C(OH)-, 4-H, enolate), 3.91 (6H, s, 3-$ArOCH_3$), 3.98 (6H, s, 4-$ArOCH_3$) ppm; ^{13}C NMR ($CDCl_3$) δ 183.2, 151.1, 149.3, 140,4, 128,1, 122.6, 122.1, 111.2, 109.8, 101.2, 56.0, 55.9 ppm; MS m/z (EI) 396 (M^+, 18), 378 (M^+-H_2O, 24), 300 (13), 191 (M^+-$C_{12}H_{13}O_3C_{11} \equiv H_{11}O_3$, 3,4-dimethoxycinnamyl, 100), 163 (M^+-$C_{13}H_{13}O_4 \equiv C_{10}H_{11}O_2$, 3,4-dimethoxystyryl, 15).

Spectral Data of Tri(mono-4-C and di-O)-methylated Curcumin: ([1,7-bis(3,4-dimethoxyphenyl)-4-methyl-1,6-heptadiene-3,5-dione], **$C_{24}H_{26}O_6$**, **4**); Yield 0.45 g (22%); IR (KBr) 3500 (broad, OH) 1650, 1620, 1595, 1525, 1400, 1275, 1140, 1020 cm^{-1}; UV (in hexane) λ_{max} 358 nm, ε 1.4×10^4 M^{-1} cm^{-1}: (in MeOH) λ_{max} 362 nm, ε 9.1×10^3 M^{-1} cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.32 (2H, d, J = 16 Hz, HC=C, 1,7-H, alkene, *trans*), 7.11(2H, d of d, J = 8 Hz, J = 2 Hz, 6-ArH), 7.02 (2H, d, J = 2 Hz, 2-ArH), 6.94 (2H, d, J = 8 Hz, 5-ArH), 6.82 (2H, d, J = 16, =CH-CO-, 2,6-H, alkene, *trans*), 5.35 and 5.33 (1H, s, -CO-CH(CH_3)-CO-, 4-H, Z and E enolate), 3.94 (6H, s, 3-$ArOCH_3$), 3.91 (6H, s, 4-$ArOCH_3$), 1.98 (3H, s, 4-CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 193.3, 190.7, 163.8, 150.8, 149.1, 137.1, 131.4, 129.1, 121.3, 118.8, 116.3, 111.5, 109.6, 55.6, 43.2 ppm; MS m/z (EI) Data threshold 5.00% of normalizing intensity (3.11E+06); 412 (M^+, 9), 411 (M^+-H, 26), 410 (M^+-2H, 100): Data threshold 10.00% of normalizing intensity (1.320E+07); 300 (47), 191 ($C_{11}H_{11}O_3$, 3,4-dimethoxycinnamyl, 45), 164($C_{10}H_{12}O_2$, 3,4-dimethoxystyrene, 100).

Spectral Data of Di-O-benzylated Curcumin: ([1,7-bis(4-benzyloxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], **$C_{35}H_{32}O_6$**, **5**); Yield 2.37 g (86%); IR (KBr) 3450 (broad, OH) 1670, 1590, 1520, 1260, 1130, 990 cm^{-1}; UV (in hexane) λ_{max} 346 nm, ε 2.4×10^4 M^{-1} cm^{-1}: (in MeOH) λ_{max} 349 nm, ε 2.5×10^4 M^{-1} cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.64 (2H, d, J = 16, HC=C, 1,7-H, alkene, *trans*), 7.38-6.81 (16H, m, 2,5,6-ArH and phenyl), 6.52 (2H, d, J = 16 Hz, =CH-CO-, 2,6-H, alkene, *trans*), 3.86 (6H, s, 3-$ArOCH_3$), 3.36 (4H, s, 4-$ArOCH_2$-) ppm; ^{13}C NMR ($CDCl_3$) δ 196.7, 150.8, 149.7, 143.7, 136.6, 136.4, 130.4, 128.0, 127.6, 127.1, 126.6, 123.5, 121.2, 113.3, 110.8, 56.1, 37.7 ppm; MS m/z (EI) Data threshold 20.00% of normalizing intensity (2.845E+04); 548 (M^+, 35), 547 (M^+-H, 44), 361 (100): Data threshold 10.00% of normalizing intensity (9.982E+05); 267 ($C_{17}H_{15}O_3$, 4-benzyloxy-3-methoxycinnamyl, 8), 91 (C_7H_7, benzyl, 100).

Spectral Data of Tri(mono-4-C and di-O)-benzylated Curcumin: ([1,7-bis(4-benzyloxy-3-methoxyphenyl)-4-benzyl-1,6-heptadiene-3,5-dione], **$C_{42}H_{38}O_6$**, **6**); Yield 0.16 g (5 %); IR (KBr) 3450 (broad, OH), 1715, 1670, 1590, 1520, 1270, 1130, 1020 cm^{-1}; UV λ_{max} 350 nm, ε 1.8×10^4 (in hexane), 1.6×10^4 (in MeOH) M^{-1} cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.65 (2H, d, J = 16 Hz, HC=C, 1,7-H, alkene, *trans*), 7.39-6.67 (21H, m, 2,5,6-ArH and phenyl), 6.55 (2H, d, J = 16, =CH-CO-, 2,6-H, alkene, *trans*), 3.94 (6H, s, 3-$ArOCH_3$), 3.77 (4H, s, 4-$ArOCH_2$-), 1.94 (2H, s, 4-CH_2-) ppm; MS m/z (EI) Data threshold 20.00% of normalizing intensity (1.140E +04); 639 (M^++1, 21), 638 (M^+, 45), 637 (M^+-H, 100): Data threshold 4.00% of normalizing intensity (4.121E+06); 267 ($C_{17}H_{15}O_3$, 4-benzyloxy-3-methoxycinnamyl, 16), 91 (C_7H_7, benzyl, 100).

Spectral Data of Di-O-acetylated Curcumin: ([1,7-bis(4-acetoxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], **$C_{25}H_{24}O_8$**, **7**); Yield 2.07 g (91%); IR (KBr) 3450 (broad, OH) 1755, 1635, 1600, 1510, 1300, 1250, 1200, 1120, 1030, 970 cm^{-1}; UV λ_{max} 395 nm (in hexane), 399 nm (in MeOH) ε 5.0×10^4 M^{-1} cm^{-1}: ^1H NMR ($CDCl_3$) δ 7.62 (2H, d, J = 16, HC=C, 1,7-H, alkene, *trans*), 7.17-7.05 (6H, m, 2,5,6-ArH), 6.57 (2H, d, J = 16, =CH-CO-, 2,6-H, alkene, *trans*), 5.86 (1H, s, -CH=C(OH)- 4-H, enolate), 3.88 (6H, s, 3-$ArOCH_3$), 2.33 (6H, s, 4-$ArOCOCH_3$) ppm; ^{13}C NMR ($CDCl_3$) δ 183.1, 168.7, 151.4, 141.3, 139.9, 133.9, 124.2, 123.3, 121.0, 111.5, 101.7, 55.9, 20.8 ppm; MS m/z (EI) 453 (M^++1, 4), 452 (M^+, 15), 410 (M^+-$COCH_2$, 21), 368 (31), 350 (M^+-$OCOCH_3$-$COCH_3$, 62), 191 ($C_{11}H_{11}O_3$, 4-acetoxy-3-methoxystyryl, 48), 190 ($C_{11}H_{10}O_3$, 4-oxy-3-methoxycinnamyl methylene, 51), 177 ($C_{10}H_9O_3$, 4-hydroxy-3-methoxy cinnamyl, 100).

Spectral Data of Di-O-benzoylated Curcumin: ([1,7-bis(4-benzoxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], **$C_{35}H_{28}O_8$**, **8**); Yield 2.46 g (85%); IR (KBr) 3450 (broad, OH) 1735, 1625, 1600, 1510, 1300, 1250, 1200, 1120, 1070, 1030, 970 cm^{-1}; UV λ_{max} 400 nm, ε 4.7×10^4 (in hexane), 4.9×10^4 (in MeOH) M^{-1} cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.23-7.15 (18H, m, phenyl and HC=C, 1,7-H, alkene), 6.58 (2H, d, J = 16 Hz, =CH-CO-, 2,6-H, alkene, *trans*), 5.87 (1H, s, -CH=C(OH)-, 4-H, enolate), 3.85(6H, s, 3-$ArOCH_3$) ppm; ^{13}C NMR ($CDCl_3$) δ 182.2, 163.5, 150.8, 140.7, 139.0, 133.1, 132.9, 129.3, 128.1, 127.8, 123.6, 122.6, 120.3, 110.7, 101.1, 55.2 ppm; MS m/z (EI) Data threshold 10.00% of normalizing intensity (1.149E+04); 577 (M^++1, 12), 576 (M^+, 75), 546 (M^+-OCH_3+H, 17), 368 (M^+-2 × benzoyl+2H, 53), 350 (M^+-2 × benzoyl-OH+H, 100): Data threshold 10.00% of normalizing intensity (8.285E+05); 285 (17), 239 (22), 105 (C_7H_5O, benzoyl, 100).

Spectral Data of Tri(mono-4-C and di-O)-benzoylated Curcumin: ([1,7-bis(4-benzoxy-3-methoxyphenyl)-4-benzoyl-1,6-heptadiene-3,5-dione], **$C_{42}H_{32}O_9$**, **9**); Yield 0.17 g (5%); IR (KBr) 3450 (broad, OH), 1750, 1670, 1500, 1260, 1170, 1120 cm^{-1}; UV λ_{max} 367 nm, ε 3.3×10^4 M^{-1} cm^{-1} (in hexane or in MeOH); ^1H NMR ($CDCl_3$) δ 8.3-6.7 (26H, m, phenyl and HC=C, 1,7-H, alkene), 6.60 (2H, d, J = 16 Hz, =CH-CO-, 2,6-H, alkene, *trans*), 3.77 (6H, s, 3-$ArOCH_3$) ppm; ^{13}C NMR ($CDCl_3$) δ 207.0, 194.1, 164.4, 151.5, 137.7, 133.3, 130.2, 129.2, 128.7, 123.3, 112.0, 55.8 ppm; MS m/z (EI) Data threshold 15.00% of normalizing intensity (1.100E+04); 680 (M^+, 27), 679 (M^+-H, 47), 664 (M^+-OH+H, 58), 480 (M^+-benzoyl-phenyl-H_2O, 100), 281 ($C_{17}H_{13}O_4$, 4-benzoxy-3-methoxycinnamyl, 37); Data threshold 10.00% of normalizing intensity (5.092E+05); 122 ($C_7H_6O_2$, benzoic acid, 16), 105 (C_7H_5O, benzoyl, 100).

References

1. Jasim, F.; Ali, F. *Microchem. J.* **1988**, *38*, 106.
2. Kuttan, R.; Bhanumathy, P.; Nirmala, K.; George, M. C. *Cancer Lett.* **1985**, *29*, 197.
3. Soudamini, K. K.; Kuttan, R. *J. Ethnopharmacol.* **1989**, *27*, 227.
4. Dahl, T. A.; McGowan, W. M.; Shand, M. A.; Srinivasan, V. S. *Arch. Microbiol.* **1989**, *151*, 183.
5. Pervaiz, S.; Skiles, H.; Rajasekharan, K. N.; Gulliya, K. S. *Abstract of 81st Annual Meeting of Amer. Assoc. Cancer Research*; Washington, 1990.
6. Tϕnnesen, H. H. *Thesis*; Oslo, 1986.
7. Sharma, O. P. *Biochem. Pharmacol.* **1976**, *25*, 1811.
8. Martin, R. L.; Rajaratnam, M. M.; Turci, P. **Eur. Pat. Appl. EP 287,750** (Cl. G03F7/08), 1988.
9. Nambudiry, M. E.; Natraj, C. V. **Can. Pat. Appl. CA 2,029,263** (Cl. A61K7/42), 1991.
10. Tonnesen, H. H.; Karlsen, J. *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 402.
11. Badu, K. V. D.; Rajasekharan, K. N. *Organic Preparations and Procedures International Brief* **1994**, *26*, 674.
12. Jasim, F.; Ali, F. *Microchem. J.* **1989**, *39*, 156.
13. Chignell, C. F.; Bilski, P.; Reszka, K. J.; Motten, A. G.; Sik, R. H.; Dahl, T. A. *Photochem. Photobiol.* **1994**, *59*, 295.
14. (a) Weedon, A. C. *The Chemistry of Enols*; Rapport, Z., Ed.; Wiley & Sons: Chichester, 1992; pp 591-638. (b) Emsley, J. *Struct. Bond.* **1984**, *57*, 147.
15. (a) Guibault, G. G. In *Comprehensive Analytical Chemistry*; Svehla, G., Ed.; 1977; Vol. VIII. (b) Guibault, G. G. *Practical Fluorescence: Theory, Methods, and Technigues*; Dekker: New York, 1973.
16. Jasim, F.; Ali, F. *Microchem. J.* **1992**, *46*, 209.
17. (a) Chandross, E. A.; Thomas, H. T. *Chem. Phys. Lett.* **1971**, *9*, 397. (b) Beens, H.; Knibbe, H.; Weller, A. *J. Chem. Phys.* **1967**, *47*, 1183.
18. Behera, P. K.; Mishra, A. K. *J. Photochem. Photobiol. A: Chem.* **1993**, *71*, 115 and references cited therein.
19. Roy, R.; Mukherjee, *Chem. Phys. Lett.* **1987**, *140*, 210.
20. Moriya, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1723.
21. (a) Smoluchowski, M. *Phys. Chem.* **1917**, *92*, 129. (b) Debye, P. *Trans. Electrochem. Soc.* **1942**, *82*, 265. (c) Wagner, P. J.; Kochevar, I. *J. Am. Chem. Soc.* **1969**, *90*, 2232. $k_q \cong k_{diff} = 2 \times 10^5$ T/η (then, T is absolute temperature and η (Poise) is the viscosity of solvent): in methanol (η = 0.623 centipoise) at 25 °C, $k_{diff} = 9.6 \times 10^9$ $M^{-1}sec^{-1}$.
22. Tϕnnesen, H. H.; Karlsen, J. *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 132.
23. Tϕnnesen, H. H. *ACS Symposium Series* **1992**, *506*, 143.
24. Racz, I.; Spiegl, P. *Sci. Pharm.* **1972**, *40*, 251.
25. Shim, S. C.; Bong, P.-H. *Bull. Korean Chem. Soc.* **1986**, *7*, 53.
26. (a) Shim, S. C.; Lee, K. T.; Bong, P.-H. *J. Photochem. Photobiol., A: Chem.* **1987**, *40*, 381. (b) Bong, P.-H.; Shim, S. C.; Shizuka, H. *J. Chem. Soc. Perkin Trans. 2* **1990**, 1227.
27. Weast, R. C. *CRC Handbook of Chemistry and Physics*, 67th Ed.; CRC Press. Inc.: 1986; p F-35.
28. HPLC conditions: column, Shodex SIL-5B normal phase column and μ-Bondapak C18 reversed phase column; detector, Shimadzu LC-10AD UV detector at 350 nm and 400 nm; eluting solvents and flow rate, *n*-hexane/diethyl-ether (4/1, v/v) and 2 mL/min for normal phase column and water/methanol/tetrahydrofuran (30/70/3, v/v) and 1 mL/min for reversed phase column.