In-Solution and On-Plate Light-Catalyzed *E*/*Z* Isomerization of Cyclic Chalcone Analogues. Lipophilicity of *E*- and *Z*-2-(*X*-Benzylidene)-1-Benzosuberones

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Abstract

Some cyclic chalcone analogues [*E*-2-(*X*-benzylidene)-1-indanones, -tetralones, and -benzosuberones], on-plate UV light-catalyzed formation of new chromatographic spots, can be observed during thin-layer chromatography (TLC). Gas chromatographic (GC) analysis of selected derivatives indicates the formation of one new substance in each case. GC coupled with mass spectrometry and ^{1}H NMR analysis of the samples reveals that the compounds formed are the respective *Z*-2-(*X*-benzylidene)-1-indanones, -tetralones, and -benzosuberones. Two-dimensional TLC shows that the *E*/*Z* isomerization is a reversible process. By means of the RP-TLC, the logarithm of *n*-octanol–water partition coefficient (log *P*) values of *E*- and *Z*-isomeric pairs of selected 2-(*X*-benzylidene)-1-benzosuberones is determined. The *Z*-isomers are less lipophilic than the *E*-isomers.

Introduction

Recently, the literature has reported on synthesis, regarding in vitro antifungal (1) and antitumor (cytotoxic) (2,3) activity of a series of *E*-2-arylidene-1-benzocyclanones, such as *E*-2-(X-benzylidene)-1-indanones (**1**), -tetralones (**2**), and -benzosuberones (**3**) (Figure 1). Structurally these compounds can be regarded as cyclic analogues of chalcones, a class of flavonoids with a broad range of biological effects (4). Previous studies have revealed that *E*-2-(*X*-benzylidene)-1-benzosuberones (**3**) are a group of useful cytotoxic agents, and some derivatives can serve as prototypic (lead) molecules for subsequent structural modifications (2,3). To better understand the relationship between the steric and electronic characters and the observed biological effects of the compounds, IR and nuclear magnetic resonance (NMR) studies (5,6) have been performed. In addition, to characterize the lipophilic nature of the compounds with different ring size and substitution, the logarithm of *n*-octanol–water partition coefficient (log *P*) values (7) of selected derivatives were determined by an optimized reverse-phase thin-layer chromatography (RP-TLC) (8). The log *P* is the most widely accepted physicochemical parameter in medicinal chemistry to measure the lipophilicity of chemicals (7). During the RP-TLC investigation, an unexpected minor spot beside the major one could also be observed on the chromatograms (8). Similar observations were made in normal-phase TLC as well. The size and UV intensity of these latter (minor) spots were found to increase during illumination of the plates with UV light after applying the starting spots but prior to development of the chromatograms. Developing the chromatograms in the dark, immediately after applying the starting spots eliminated the appearance of the minor spots.

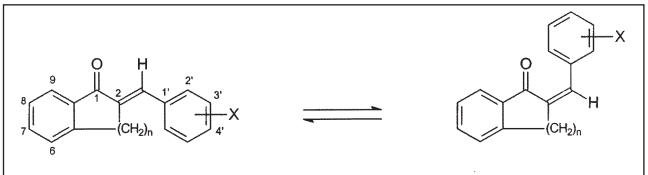

Figure 1. Structure of compounds **1a-g** to **4a-g, 2a-g** to **5a-g,** and **3a-n** to **6a-n**.

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Theoretically, *E*-(**1**-**3**) and *Z*-(**4**-**6**) isomers of the investigated 2-(*X*-benzylidene)-1-benzocyclanones can be equally formed in the reactions used for the synthesis of the compounds. The *Z*-configuration, however, is unfavorable because of a strong steric interaction between the aryl and the carbonyl groups (9). Accordingly, synthesis of the compounds yielded the energetically more favored *E*-isomers (2,3). On the other hand, photoisomerization and photodimerization of alkenes and α,β-unsaturated ketones, including chalcones, are well-known phenomena in the literature (10). UV light-induced *E/Z* isomerization of chalcones (11–13) and *E*-2-arylidene-indanones (14–16) and -tetralones (15,16) has been published, and the respective *Z*-isomers were structurally characterized. Based on these earlier observations, it is supposed that UV light-catalyzed, on-plate *E/Z* isomerization of the investigated compounds took place during their RP-TLC investigations (8).

The main goal of the present work was to develop optimized chromatographic methods that can be used for separation and structure elucidation of the new compounds formed during the previously mentioned TLC investigations of the *E*-2-(X-benzylidene)-1-benzocyclanones **1**–**3**. Because RP-TLC is the method of choice for chromatographic determination of log *P* values of compounds difficult to isolate or handle (or both) in a pure state (7), a determination of the log *P* values of *E/Z* isomeric pairs of selected 2-(X-benzylidene)-1-benzosuberones by means of a previously published validated RP-TLC method (8) is also planned.

Experimental

Materials

Synthesis of the *E*-2-(X-benzylidene)-1-benzocyclanones (**1-3**) was performed by base-catalyzed Claisen–Schmidt condensation as described previously (1–3,5). All compounds were purified by column chromatography over Kieselgel 60 (0.2–0.5 mm) (Reanal, Budpest, Hungary) using toluene or toluene–methanol (99:1, v/v) as mobile phases. The compounds had melting points in accordance with their literature values. The structures of the investigated samples were verified by IR (Nicolet Impact 400 Fourier transform IR spectrometer) and ^{1}H NMR (Varian ^{Unity}Inova, 400 MHz spectrometer) (Palo Alto, CA). IR spectra were recorded in KBr pellets. ^{1}H NMR spectra were run in $CDCl_3$ solutions using tetramethyl silane as internal standard. Structure verifications were accomplished by 2D nuclear Overhauser effect (NOE) experiments using standard Varian software.

E-2-(X-benzylidene)-1-benzocyclanones (**1**–**3**) were subjected to UV light-induced isomerization in 2 mg/mL dichloromethane or benzene solutions by allowing the solutions to be exposed by scattered laboratory light for one week, or in 3.5mM benzene solutions by illuminating them with 3- × 125-W mercury lamps for 4 h. The solutions were analyzed by TLC and gas chromatography (GC) coupled with flame ionization (FID) detection as well as mass spectrometry (MS), as described later. Evaporation of the solutions illuminated by the mercury lamps resulted in yellowish solids, which were analyzed by GC and ^{1}H NMR (Varian ^{Unity}Inova, 400 MHz spectrometer).

Chromatographic investigations

GC and GC–MS investigations for in-solution isomerization

GC and GC–MS investigations were performed on HP 5890 Series II GCs equipped with FID and an HP-5971A mass selective detector (MSD), respectively. Separation of the corresponding isomers was accomplished on (1) HP-5 (25-m × 0.32-mm i.d., 0.17-µm film thickness), (2) HP-5 MS (30-m × 0.25-mm i.d., 0.25-µm film thickness), and (3) RTX-5 (30-m × 0.32-mm i.d., 1.0-µm film thickness) capillary columns using different temperature programs. The HP-5971A MSD was operated in scan mode (50–650 *m/z*).

The GC–FID conditions were as follows: column, HP-5 capillary column (25-m × 0.32-mm i.d., 0.17-µm film thickness); injection temperature, 280°C; detection temperature, 280°C; oven temperature program, 100°C (1 min) to 200°C at 5°C/min (0.5 min) to 250°C at 15°C/min (1 min); carrier, helium, 1.8 mL/min; injection, 1 µL, split 40 mL/min.

GC–MS investigations were performed under two GC conditions. Method A: column, HP-5 MS capillary column (30-m × 0.25-mm i.d., 0.25 µm film thickness); injection temperature, 280°C; detection temperature, 300°C; oven temperature program, 100°C (1 min) to 200°C at 15°C/min (0.5 min) to 280°C at 15°C/min (5 min); carrier, helium, 1.0 mL/min, constant flow; injection, 1 µL, splitless; purge time, 0.5 min. Method B: column, RTX-5 capillary column (30-m × 0.32-mm i.d., 1.00-µm film thickness); injection temperature, 280°C; detection temperature, 280°C; Oven temperature program, 50°C (1 min) to 300°C at 20°C/min (16.5 min), carrier, helium, 1.0 mL/min, constant flow; injection, 1 µL, split 30 mL/min. Pure *E*-isomers and *E/Z* mixtures of **1a,f,g**, **2a,f,g**, **3a,f,g, 4a,f,g**, **5a,f,g**, and **6a,f,g** were analyzed by method A, and the respective samples of the rest of the compounds (Figure 1) were analyzed by method B.

TLC method for investigation of the reversibility of E/Z isomerization

Normal-phase TLC investigation of the pure *E*-isomers (**1**-**3**) and the isomeric mixtures obtained by UV illuminations were performed on 20- × 20-cm silica gel 60 F_{254} aluminum sheets (No. 5554, Merck, Darmstadt Germany), using dichloromethane as developing solvent. RP-TLC investigations were performed on 20- × 20-cm chromatographic glass plates precoated with 0.25-mm silanized silica gel 60 F_{254} layer (No. 5747, Merck), using methanol–water mixtures as mobile phase. For visualization, the chromatograms were illuminated by λ = 254 nm UV light and subsequently subjected to iodine vapor. High-performance (HP) TLC-densitometry was performed on 10- × 10-cm silica gel 60 F_{254} HPTLC plates (No. 5629, Merck). These latter plates were also used to study the on-plate *E/Z* reversibility. In each case, the chromatographic chamber was presaturated with the mobile phase for 60 min. On-plate spectra were recorded by means of a Shimadzu CS 9301 (Kyoto, Japan) scanner.

Before applying the spots, the RP-TLC plates had been washed with methanol (ascending chromatography), dried, and heated at 110°C for 1 h. Standard solutions (2 mg/mL) of the compounds or the isomeric mixtures were prepared in dichloromethane (for normal-phase TLC) or in a methanol–chloroform (1:1, v/v) mixture (for RP-TLC) and 2 µL of the solutions (4 µg of substance) were spotted onto the plates. After development of the chro-

matograms (150 mm), the plates were dried, and the spots were detected as mentioned previously. At least two parallel TLC determinations were carried out for each sample.

RP-TLC method for log P determination

Details of the RP-TLC log *P* determinations were the same as in the previously published method (8). The optimized system used in this work consisted of precoated silanized silica gel 60 F_{254} (Merck, No. 5747) as stationary phase and methanol–water (70:30, v/v) mixture as mobile phase. After application of the starting spots [2 µL of 2 mg/mL methanol–chloroform (1:1, v/v) solutions], the plates were left to stand in diffuse daylight for 2 h, then developed and detected by densitometry. The calibration set (8 compounds of known octanol–water log *P* values) was the same as used previously (8). The calibration equation obtained is:

$$\log P = 7.599\ R_M + 2.497 \qquad \text{Eq. 1}$$

where n is 8, r is 0.983, s = 0.05, and R_M is $\log(1/R_f - 1)$. The average standard deviation of log *P* values determined by the RP-TLC method was less than 0.1 log unit. Such a precision corresponds with previous RP-TLC log *P* determinations (17).

Results and Discussion

Isomerization in solution

For comparative structural and chromatographic investigations, *E*-2-benzylidene-1-indanone (**1a**)–tetralone (**2a**) and –benzosuberone (**3a**), as well as some of their substituted derivatives (**1b-g**, **2b-g**, and **3b-n**) were synthesized (Figure 1). The isomeric purity of the compounds was checked by ^{1}H NMR and GC–FID. All investigated compounds were found to be stereohomogeneous *E*-isomer based on the results of the two analyses.

In order to obtain the products with a formation that was observed during the previous TLC investigations, dichloromethane solutions of the compounds were exposed to scattered laboratory light, and the composition of the solutions was checked by GC–FID and normal-phase TLC through a 1-week period. GC analysis showed the appearance of a new peak with a shorter retention time than that of the starting *E*-2-(X-benzylidene)-1-benzocyclanone (**1-3**) in each chromatogram, even after 1 day of standing on shelve (Table I and II). Normal-phase TLC analysis of the samples using dichloromethane as eluent also confirmed the formation of a new compound in the solutions (Table III and IV). GC–MS investigations of the solutions showed that the two compounds corresponding to the two peaks in each solution gave identical mole peaks and fragmentations. The fragmentation patterns are in accordance with those previously found for some **1-3** (18). As an example, GC–MS spectra of *E*- and *Z*-isomers of 2-(4'-cyanobenzylidene)-1-indanone **1f** [retention time (t_R) = 21.40 min] and **4f** (t_R = 20.63 min) are shown in Figure 2. These observations supported the hypothesis that the newly formed compounds were the respective *Z*-isomers (Figure 1). The GC retention characteristics (shorter retention time for the least planar *Z*-isomers) are in accordance with the results from similar investigations of the related *E*- and *Z*-dypnones (12).

For a more complete structural characterization of the newly formed compounds, 3.5mM benzene solution of the pure *E*-isomers of **1a-g**, **2a-g**, and **3a-n** were illuminated with 3- × 125-W mercury lamps for 4-h. GC–FID investigation in each solution showed the appearance of a new peak with identical t_R with that seen in the chromatogram of the respective solution exposed to scattered laboratory light. Furthermore, the solutions illuminated by the mercury lamps were evaporated and the solid residues were analyzed by TLC and ^{1}H NMR. In the ^{1}H NMR spectra of the investigated residues, the chemical shift values for the benzylic protons in the *Z*-isomers (**4a**,**b**,**f**,**g**; **5a**,**b**,**f**,**g**; and **6a**,**b**,**f**,**g**) are shifted upfield by 0.63–1.17 ppm as compared with the chemical shift values of the benzylic protons in the corresponding *E*-isomers (**1a**,**b**,**f**,**g**; **2a**,**b**,**f**,**g**; and **3a**,**b**,**f**,**g**). This effect is likely to be attributed to the shielding anisotropy contribution arising from the C-1 carbonyl group in all the *E*-isomers. In the corresponding *Z*-isomers, however, the anisotropy effect of the C-1 carbonyl group is stronger in the series **4** (where the enone moiety is planar) than it is in the series **5** and **6**, where the enone moiety is deviated from planarity (5). Because of this effect, the chemical shift values of the H-2',6' aromatic protons in **4** are shifted downfield by 0.29–0.46 ppm in comparison to the respective values in the corresponding **1** isomers (Table V).

Table I. GC Retention Time Data of *E*- and *Z*-2-(4'-*X*-Benzylidene)-1-Benzocyclanones 1a-g to 3a-g and 4a-g to 6a-g*

Compound/ series	1	4	2	5	3	6
a	9.67	9.06	10.13	9.37	10.27	9.48
b	10.85	10.23	11.39	10.57	11.65	10.66
c	9.42	8.90	9.80	9.22	10.10	9.34
d	11.65	11.07	12.15	11.50	12.44	11.57
e	12.96	12.37	13.49	12.79	13.80	12.84
f	13.29	12.64	14.14	13.19	14.30	13.30
g	14.92	14.42	15.59	14.94	16.00	15.06

* GC investigations were performed on a HP 5890 Series II GC equipped with FID using: an HP-5 capillary column (25-m × 0.32-mm i.d., 0.17-µm film thickness); injection temperature, 280°C; detection temperature, 280°C; oven temperature program, 100°C (1 min) to 200°C at 15°C/min (0.5 min) to 280°C at 15°C/min (5 min); carrier, helium, 1.8 mL/min; and injection, 1 µL split 40 mL/min.

Table II. GC Retention Time Data of *E*- and *Z*-2-(*X*-Benzylidene)-1-Benzosuberones 3h-n and 6h-n*

Compound/ series	3	6	Compound/ series	3	6
h	11,17	10,21	l	13,67	9,87
i	9.88	9.33	m	11.62	10.63
j	12.10	11.34	n	12.68	11.55
k	13.36	12.49			

* GC investigations were performed on a HP 5890 Series II GC equipped with FID using: an HP-5 capillary column (25-m × 0.32-mm i.d., 0.17-µm film thickness); injection temperature, 280°C; detection temperature, 280°C; oven temperature program, 100°C (1 min) to 200°C at 15°C/min (0.5 min) to 280°C at 15°C/min (5 min); carrier, helium, 1.8 mL/min; and injection, 1 µL split 40 mL/min.

For selected members of each series (**1f**, **4f**, **2f**, **5f** and **3g**, **6g**) the *E*/*Z* configuration of the isomeric pairs was also proven by two-dimensional NOE experiments. In the case of **1f**, **2f**, and **3g**, the strong NOE cross peaks between the H-2',6' aromatic and the H-3 methylene protons confirmed the *E*-configuration of the compounds. On the other hand, the strong NOEs between the benzylic (= CH–) and the H-3 methylene protons correspond to the *Z*-configuration of **4f**, **5f**, and **6g**.

On-plate isomerization

Comparative TLC investigation (silica gel and dichloromethane) of the compounds formed during illumination of the *E*-2-(*X*-benzylidene)-1-benzocyclanones **1a-g**, **2a-g**, and **3a-n** applied onto the chromatographic plates was performed with those of the respective *Z*-2-(*X*-benzylidene)-1-benzocyclanones (**4a-g**, **5a-g**, and **6a-n**) formed in dichloromethane solutions of **1a-g**, **2a-g**, and **3a-n** on laboratory light illumination. It was found that the R_f values of **4a-g**, **5a-g**, and **6a-n** were identical to those of the respective spots which appeared on the chromatograms that were developed after two days of illumination by scattered laboratory light of the **1a-g**, **2a-g**, and **3a-n** starting spots. This observation strongly supported the hypothesis that the same UV light-induced *E*/*Z* isomerization took place both on the plates and in solution. It is worth mentioning, however, that the in-solution *E*/*Z* isomerization of the 5-membered compounds (**4a-g**) was found to be much less favored than that of the respective 6- and 7-membered analogues, which was indicated by the integrals of the respective *E* and *Z* peaks in the GC chromatograms. In addition, the on-plate *E*/*Z* isomerization of the 5-membered compounds (**4a-g**) on the silica surface was generally accompanied by other type(s) of light-catalyzed reactions, which was indicated by the appearance of other (minor) spots in the chromatograms than those corresponding to the respective *Z*-isomers. This latter phenomenon needs further experiments to clear the nature of the accompanying reactions.

In order to get further support for the role of light in the on-plate *E*/*Z* isomerization process, experiments were performed in which the plates with the applied *E*-2-(*X*-benzylidene)-1-benzocyclanone (**1a-g**, **2a-g**, and **3a-n**) starting spots were stored in the dark for two days before developing the TLC chromatograms. Under such experimental conditions, appearance of the second spots was not observed, giving further evidence for the role of light in the isomerization process.

Table III. Normal-Phase TLC R_f Values of *E*- and *Z*-2-(4'-*X*-Benzylidene)-1-Benzocyclanones 1a-g to 3a-g and 4a-g to 6a-g*

Compound/ series	1	4	2	5	3	6
a	0.19†	0.19†	0.34	0.41	0.28	0.34
b	0.13	0.32	0.27	0.40	0.28	0.38
c	0.15	0.34	0.27	0.36	0.31	0.39
d	0.16	0.38	0.34	0.43	0.32	0.43
e	0.18	0.39	0.32	0.44	0.32	0.44
f	0.12	0.16	0.19†	0.19†	0.21	0.18
g	0.13	0.21	0.28†	0.28†	0.29†	0.29†

* TLC investigations were performed on 20- × 20-cm silica gel 60 F_{254} aluminum sheets using dichloromethane as developing solvent.
† Separation could only be achieved when the chromatograms were repeatedly developed by dichloromethane.

Table IV. Normal-Phase TLC R_f Values of *E*- and *Z*-2-(*X*-Benzylidene)-1-Benzosuberones 3h-n and 6h-n*

Compound/ series	3	6	Compound/ series	3	6
h	0.29	0.37	l	0.35†	0.35†
i	0.33	0.40	m	0.38	0.43
j	0.36	0.45	n	0.35	0.40
k	0.37	0.45			

* TLC investigations were performed on 20- × 20-cm silica gel 60 F_{254} aluminum sheets using dichloromethane as developing solvent.
† Separation could only be achived when the chromatograms were repeatedly developed by dichloromethane.

Reversibility of *E*/*Z* isomerization

The on-plate *E*/*Z* isomerization was supposed to be a reversible process. This was proven by a two-dimensional TLC experiment performed with the *E*-2-(3'-methylbenzylidene)-1-benzosuberone (**3h**) on silica gel HPTLC plate using dichloromethane as eluent. In the first experiment, one of the two spots of **3h** applied on the HPTLC plate was illuminated by 365 nm UV light (analytical UV lamp) for 30 min (Figure 3, direction 1, left spot). On developing the chromatogram, the expected two spots because of the formation of the respective *Z*-isomer (**6h**) was

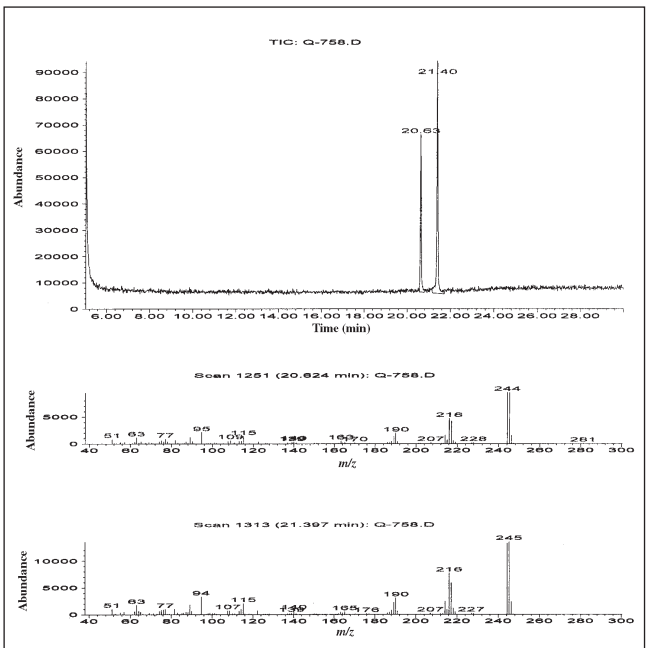

Figure 2. GC–MS spectrum of of E (**1f**; t_R = 21.40 min) and Z (**4f**; t_R = 20.63 min) isomers of 2-(4'-cyanobenzylidene)-1-indanone. (GC investigation was performed on a HP 5890 Series II GC coupled with an HP 5971A MSD using: an HP-5 MS capillary column (30-m × 0.25-mm i.d., 0.25-µm film thickness); injection temperature, 280°C; detection temperature, 300°C; oven temperature program, 100°C (1 min) to 200°C at 15°C/min (0.5 min) to 280°C at 15°C/min (5 min); carrier, helium; 1.0 mL/min; injection, 1 µL splitless; purge time, 0.5 min; and MSD, scan mode.

obtained. The recorded UV spectra of the spots showed differences according to the different spectroscopic characteristics [splitting of band III of the *E*-isomer (**3h**) into two bands (bands II and III) in the case of the respective *Z*-isomer (**6h**)] expected for the *E*- and *Z*-chalcones (10,11,13) (Figures 4C and 4D). Similar spectra could be obtained on HPTLC investigation of a 2-mg/mL benzene solution of **3h** illuminated by 365 nm UV light for 30 min (Figures 4A and 4B), providing further evidence for the *E/Z* isomerization under both conditions.

After development of the TLC chromatogram in the first direction, the plate was allowed to dry and the two separated spots were reilluminated as before. Under such experimental conditions, it was investigated whether the formed *Z*-isomer (Figure 3, spot with the higher R_f in direction 1) could reisomerize to the starting *E*-isomer or not. Developing the plate in the second dimension (eluent: dichloromethane) yielded the appearance of two spots that could be observed in both lanes (Figure 3, direction 2). Based on the R_f value of the reference (nonilluminated) *E*-isomer (Figure 3, the top spot in direction 1) and the recorded UV spectra of the spots formed in the second development, it could be determined that some *E*-isomer (**3h**) (Figure 3, spot with the lower R_f in direction 1) underwent isomerization to form the *Z*-isomer (**6h**), although a smaller amount of *Z*-isomer **(6h)** (Figure 3, spot with the higher R_f in direction 1) reisomerized to the *E*-isomer (**3h**) (Figure 3, direction 2). Accordingly, the isomerization proved to be reversible.

RP-TLC investigation of dichloromethane solutions of the above isomeric mixtures (solutions of **1a**-**g**, **2a**-**g**, and **3a**-**n** illuminated by scattered laboratory light) also proved the on-plate formation of the respected *Z*-isomers. The *Z*-isomers formed in

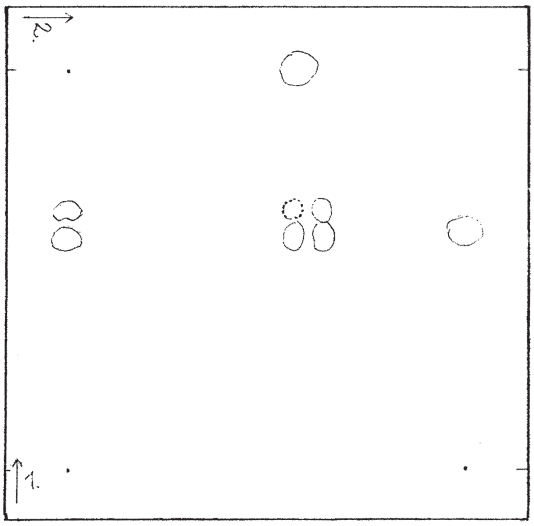

Figure 3. HPTLC (eluent: dichloromethane) investigation of **3h** and **6h**. Direction 1: chromatogram of UV-illuminated (left spot) and nonilluminated (right spot) **3h**. Direction 2: chromatogram of UV-illuminated **3h** (the lower left spot in direction 1), UV-illuminated **6h** (the higher left spot in direction 1), and nonilluminated **3h** (the top spot in direction 1).

Table V. ^{1}H NMR Chemical Shifts* of Selected *E*- and *Z*- 2-(4'-*X*-Benzylidene)-1-Benzocyclanones 1-3 and 4-6†

Compound	H-3	H-4	H-5	H-6	H-7	H-8	H-9	= CH–	H-2',6'	H-3',5'	H-4'
1a	4.04	-	-	7.54	7.60	7.39	7.90	7.67	7.66	7.45	7.43
4a	3.90	-	-	7.48	7.58	7.39	7.83	6.99	8.07	7.41	7.38
1b	4.00	-	-	7.53	7.59	7.40	7.89	7.64	7.56	7.25	-
4b	3.88	-	-	7.47	7.57	7.39	7.83	6.97	8.02	7.21	-
1f	4.05	-	-	7.56	7.64	7.44	7.90	7.62	7.73	7.73	-
4f	3.92	-	-	7.49	7.62	7.41	7.82	6.99	8.06‡	7.67‡	-
1g	4.09	-	-	7.58	7.66	7.46	7.93	7.69	7.81	8.31	-
4g	3.95	-	-	7.50	7.63	7.48	7.82	7.04	8.10	8.23	-
2a	3.14	2.95	-	7.25	7.49	7.37	8.14	7.88	7.45	7.42	7.36
5a	3.15	2.93	-	7.27	7.48	7.34	8.12	6.84	7.52	7.32	7.29
2b	3.14	2.94	-	7.24	7.48	7.36	8.14	7.86	7.36	7.24	-
5b	3.14	2.91	-	7.27	7.47	7.34	8.13	6.81	7.46	7.14	-
2f	3.07	2.96	-	7.26	7.51	7.37	8.12	7.79	7.50	7.69	-
5f	3.16	2.94	-	7.28	7.50	7.34	8.05	6.81	7.53	7.58	-
2g	3.10	2.98	-	7.27	7.52	7.38	8.13	7.83	7.56	8.26	-
5g	3.18	2.97	-	7.29	7.51	7.35	8.05	6.85	7.58	8.16	-
3a	2.61	2.08	2.90	7.20	7.47	7.36	7.78	7.84	7.51	7.42	7.36
6a	2.47	2.02	3.03	7.24	7.48	7.35	7.99	6.69	7.40	7.26	7.28
3b	2.61	2.08	2.89	7.19	7.46	7.35	7.78	7.82	7.42	7.23	-
6b	2.44	2.00	3.01	7.23	7.47	7.35	7.96	6.65	7.31	7.08	-
3f	2.55	2.05	2.89	7.20	7.48	7.36	7.77	7.76	7.55	7.69	-
6f	2.47	2.01	3.00	7.24	7.48	7.34	7.89	6.65	7.42	7.52	-
3g	2.56	2.06	2.91	7.21	7.49	7.36	7.77	7.80	7.61	8.26	-
6g	2.51	2.04	3.02	7.25	7.49	7.35	7.90	6.70	7.47	8.10	-

* In ppm, δTMS = 0 ppm.
† ^{1}H NMR spectra were run on a Varian UnityInova, 400 MHz spectrometer in $CDCl_3$ solutions using TMS as internal standard.
‡ Interchangeable assignments.

the solutions were found to have the same chromatographic characteristics as those formed on the chromatographic plates when the applied spots of **1a-g**, **2a-g**, and **3a-n** (pure *E*-isomers) were illuminated by 365 nm UV light for 30 min or by scattered laboratory light for 2 days.

Determination of log *P* of isomers

The structure and function of any biological system are closely related to the lipophilic properties of its component molecules. Thus, lipophilicity of xenobiotics plays an important role regarding the transport, distribution, and ligand-receptor interactions of the compounds (7). Accordingly, knowledge of log *P* values of the respective *E*/*Z* isomers of 2-(*X*-benzylidene)-1-benzosuberones (**3**) might be useful in understanding the biological effects of this class of cyclic chalcone analogues, of which several derivatives have been found to possess promising tumor cytotoxic activities (2,3). Reversible *E*/*Z* isomerization of the cyclic chalcone analogues **1-3**, however, makes isolation of pure *Z*-isomers time-consuming, and performing experiments with the pure *Z*-isomers requires protection from light and acids (19). Because log *P* determination of compounds by the RP-TLC method does not require isolation of the pure substances (20), application of RP-TLC is a method of choice for log *P* determination of chalcones and related compounds. Taking advantage of the RP-TLC method, log *P* determination of the *Z*-isomers of selected representatives of *E*-2-(*X*-benzylidene)-1-benzosuberones (**3**) was performed. The same RP-TLC method was applied that had been developed, optimized, and validated recently for log *P* measurements of some *E*-2-(*X*-benzylidene)-1-benzosuberones **1**-**3** (8).

Data in Table VI show a significant difference between the log *P* values of the corresponding *E*- and *Z*-isomers, primarily, that the *Z*-isomer is less lipophilic in the case of all the 12 examined derivatives. The average of differences of log *P* values of the isomeric pairs was found to be 0.65, which is higher than the average contribution of a methylene group (0.5 units) to lipophilicity (21). The relatively high difference of lipophilicity of the *E*- and *Z*-isomers is a sign of the importance of steric effects in partitioning. Further investigations are required to have a better understanding of the phenomena.

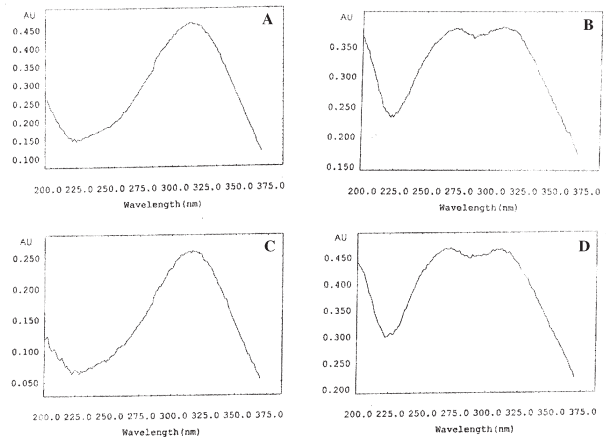

Figure 4. Onplate UV–vis spectra of **3h** and **6h**. Pure **3h** (λ_{max} = 314.2 nm) (A). **6h** formed on illumination of benzene solution of **3h** (λ_{max} = 272.9 nm and 309.0 nm) (B). Pure **3h** (λ_{max} = 313.7 nm) (C). **6h** formed on the HPTLC plate on illumination of the **3h** chromatographic starting spot (λ_{max} = 272.1 nm and 308.1 nm) (D).

Table VI. The log *P* Values of *E*- (3) and *Z*- (6) Isomers of Some *E*- and *Z*-2-(*X*-Benzylidene)-1-Benzosuberones Determined by RP-TLC Method*

Compounds	log P_{TLC} *E*-isomer (3)	log P_{TLC} *Z*-isomer (6)	Δlog *P*
3a/6a	4.48	3.34	1.14
3b/6b	5.10	4.60	0.50
3c/6c	4.45	3.91	0.54
3d/6d	5.10	4.65	0.45
3e/6e	5.22	4.70	0.52
3h/6h	4.98	4.28	0.70
3i/6i	4.60	4.02	0.58
3j/6j	5.22	4.52	0.70
3k/6k	5.35	4.71	0.64
3l/6l	4.55	3.66	0.89
3m/6m	5.04	4.43	0.61
3n/6n	5.04	4.48	0.56
			Average: 0.65

* RP-TLC investigations were performed on 20- × 20-cm precoated silanized silica gel 60 F_{254} plates using methanol–water (70:30, v/v) mixture as mobile phase.

Conclusion

Through a combination of TLC, ^{1}H NMR, and GC–MS techniques, it has been demonstrated that the cyclic chalcone analogue *E*-2-(*X*-benzylidene)-1-benzosuberones **1a-g**, **2a-g**, and **3a-n** underwent on plate *E*/*Z* isomerization to yield the respective **4a-g**, **5a-g**, and **6a-n**. The isomerization was found to be UV light-catalyzed and occur in solution and both on normal-phase and RP silica plates. Reversibility of the isomerization was confirmed by normal-phase TLC experiments. Log *P* values of 12 pairs of *E*- and *Z*-2-(*X*-benzylidene)-1-benzosuberones (**3** and **6**) have been determined by application of the previously published RP-TLC method (8). This study showed that the RP-TLC method is a useful tool for investigation of lipophilicity of *E*- and *Z*-isomers of compounds without the need for time-consuming isolation of the pure isomers.

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