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Stereoselective Photochemistry of Methoxy Chalcones in Solution and Their Radical Scavenging Activity

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The photochemical dimerization of 3 known methoxy derivatives of chalcones, (2*E*)-1-(2-methoxy)phenyl-3-phenylpropen-1-one (**1**), (2*E*)-1-(3-methoxy)phenyl-3-phenyl-propen-1-one (**2**), and (2*E*)-1-(4-methoxy) phenyl-3-phenylpropen-1-one (**3**), yielded 3 new δ-truxinic type dimers in solution: rel-(1β,2α)-di-(2-methoxy)-benzoyl-rel-(3β,4α)-diphenylcyclobutane (**4**), rel-(1β,2α)-di-(3-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane (**5**), and rel-(1β,2α)-di-(4-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane (**6**), stereoselectively. Precursor chalcones showed high superoxide radical scavenging activity although the dimers were inactive.

Key Words: Chalcone, Photodimerizations, Solution, Dimers, Radical Scavenging Activity.

Introduction

The photodimerizations of various chalcones and their derivatives in solution, solid and molten state have been studied[1−4], although the need is still great for unstudied stereoselective photodimerizations of chalcones. Intermolecular 4n(2+2) photocycloaddition of chalcones to give cyclobutane has proven to be a fast and simple method to shrink a cyclophane ring to a tricyclic system. A fast method to obtain cyclobutane rings is the photochemical dimerization of α,β-unsaturated carbonyl compounds (chalcones)[1−4]. These reactions can be carried out in solid state, molten state and solution by UV-vis irradiation, with variable results in terms of yield and product composition[1−4].

The cycloaddition of trans-chalcones may give 4 possible stereoisomers: *anti*, *syn*, head-to-head, and head-to-tail (Scheme 1). These reactions are stereospecific, and this stereospecificity has been explained by means of the Woodward-Hoffmann selection rules[5]. The formation of different stereoisomers in the dimerization of chalcones and related compounds may be dependent on the physical state of the substrate (solution, solid, or molten state) and the conditions, such as the use of glassware[1−4], which may absorb the light. In the literature, various cyclobutane containing chalcones have been reported to be synthesized[1−4] and

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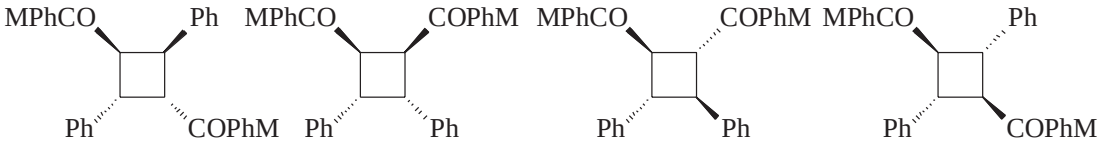
isolated from various plants[6−8], and many showed antimicrobial activities[6−8]. Analogous to these isolated and synthesized dimers of chalcones, 3 new chiral dimers of chalcones were synthesized stereoselectively in the current study.



α-truxillic *syn* head-to-tail | β-truxinic *syn* head-to-head | δ-truxinic *anti* head-to-head | ε-truxillic *anti* head-to-tail

MPh = o-MeOPh, m-MeOPh, p-MeOPh

Scheme 1.

Chalcones belong to the largest class of plant secondary metabolites, which, in many cases, serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects and herbivores[9]. They are known to possess an antioxidant character at various extents[10−11]. The antioxidant activity of natural compounds like chalconoids is related to a number of different mechanisms such as free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxide[12]. Because oxidative stress is known to cause many diseases, scientists have become more interested in natural sources to fight it, looking for active components of plants in this respect in recent years. Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore appear to be very important in the prevention of these diseases. Several methods have been developed in recent years to evaluate the total antioxidant capacity of biological samples. The basis of most of these methods relies on a substrate that is oxidized in the procedures, and oxygen consumption, oxidation products, or substrate loss is monitored in different manners by various methods[13]. In the literature, there are reports about the antiviral and antimicrobial activities of various chalcones[14−19]. However, the radical scavenging activities of the hetero chalcones **1**, **2** and **3** have not been reported. Thus, the superoxide radical scavenging activities of chalcones **1**, **2** and **3** and of their dimerization products **4**, **5** and **6** were measured according to a widely used method[20] that utilizes xanthine as the substrate of xanthine oxidase for the production of superoxide radicals that are then, in the presence of antioxidants, scavenged. The remaining superoxide radicals are determined by the reaction with nitroblue tetrazolium salt (NBT) spectrophotometrically.

Experimental

General and Instrumentation

NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$. The mass spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrophotometer. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000-400 cm^{-1}) spectrometer. Melting points were obtained using a Gallenkamp apparatus and are uncorrected. UV-vis spectral analyses were carried out on a Unicam UV2-100, at 25 °C. Thin-layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminum plates. PTLC was carried out on Merck precoated 60 Kieselgel F_{254} (20 x 20 0.5 mm) silica gel plates.

Materials and Methods

2-Methoxyacetophenone, 3-methoxyacetophenone, 4-methoxyacetophenone and benzaldehyde were purchased from Aldrich and used without further purification. The solvents (chloroform, n-hexane, diethyl ether and ethyl alcohol) used were either of analytical grade or bulk solvents distilled before use.

Superoxide Radical Scavenging Activity: Superoxide radicals generated by the xanthine–xanthine oxidase system were determined by monitoring the product of the reaction with NBT spectrophotometrically[20]. The methanolic solution of the samples (0.1 mL, 1.0 mg/mL) was added to the reaction mixture containing 100 μM xanthine, 600 μM NBT, 0.05 U/mL xanthine oxidase and 0.1 M phosphate buffer (pH 7.4), making up a final volume of 2.0 mL. Following incubation at 25 °C for 10 min, the absorbance was read at 560 nm, and compared with that of the control in which the enzyme, xanthine oxidase, was absent. The results are expressed as the concentration of the test sample giving 50% reduction in the absorbance of control and are compared with those of butylated hydroxytolouene (BHT).

Photodimerization of 1 in solution: A solution of compound **1** (110 mg, 0.462 mmol) in 30 mL of chloroform, kept in a Pyrex flask, was exposed to UV light (400 W high-pressure Hg lamp). The progress of the reaction was followed by silica gel TLC (n-hexane-diethyl ether, 1:1). The reaction was stopped after ~20 h. The solution was evaporated and the residue purified by PTLC (0.5 mm, 20 x 20, 2 plates) to give compound **4** (36 mg, 33% yield, R_f = 0.25, n-hexane-diethyl ether, 1:1).

Photodimerization of 2 in solution: A solution of compound **2** (167 mg, 0.7 mmol) was treated in the same way as compound **1** (42 mg, 25% yield, R_f= 0.5, reaction time ~6 h).

Photodimerization of 3 in solution: A solution of compound **3** (80 mg, 0.336 mmol) was treated in the same way as compound **1** (33 mg, 41% yield, R_f= 0.4).

The spectral data (^{1}H, ^{13}C, FT-IR, UV and MS) of compounds **1**, **2**, and **3** are the same as those in the literature[21–25].

rel-(1β,2α)-di-(2-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane, 4: Amorphous solid, mp 53-55 °C; UV λ_{max}^{CHCl3} nm: 253, 310; ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table; LC-MS/MS m/z (%); m/z = 499(95) $[M+Na]^+$, 476(18) $[M]^+$, 475(50) $[M-1]^+$, 238(6), 180(7), 135(100), 131(12), 104(48); FT-IR cm^{-1}: 3065, 2939, 2839, 1800-1680, 1660, 1597, 1485, 1463, 1436, 1248, 1021, 753, 698.

rel-(1β,2α)-di-(3-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane, 5: Viscous oil; UV λ_{max}^{CHCl3} nm: 257, 313; ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table; LC-MS/MS m/z (%); m/z = 499(28) $[M+Na]^+$, 476(31) $[M]^+$, 475(100) $[M-1]^+$, 238(2), 135(15), 131(5), 104(8); FT-IR cm^{-1}: 3054, 2937, 2829, 1800-1680, 1668, 1597, 1583, 1487, 1430, 1263, 1035, 877, 797, 698.

rel-(1β,2α)-di-(4-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane, 6: Amorphous solid, mp 64-66 °C; UV λ_{max}^{CHCl3} nm: 285; ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table; LC-MS/MS m/z (%); m/z = 499(8) $[M+Na]^+$, 476(28) $[M]^+$, 475(100) $[M-1]^+$, 180(2), 135(4); FT-IR cm^{-1}: 3021, 2928, 2846, 1800-1680, 1659, 1599, 1510, 1456, 1260, 1170, 1027, 841, 772, 699.

Results and Discussion

Three known methoxyl derivatives of chalcones (**1-3**)[21−25] were prepared by the known procedure[26] according to the route indicated in Scheme 2. These chalcones, when exposed to UV light (400 W high-pressure Hg lamp) in solution, were converted to the 3 new respective δ-truxinic type (rel-*anti*-head-to-head dimers) cyclobutane containing compounds **4**, **5** and **6** with yields (chromatographed products, PTLC) of 33%, 25% and 41%, respectively. The yields of these types of reactions have been usually low, as in our case, or even lower[2−4]. The minor products of these reactions were less than $\sim$ 4% and were not characterized.

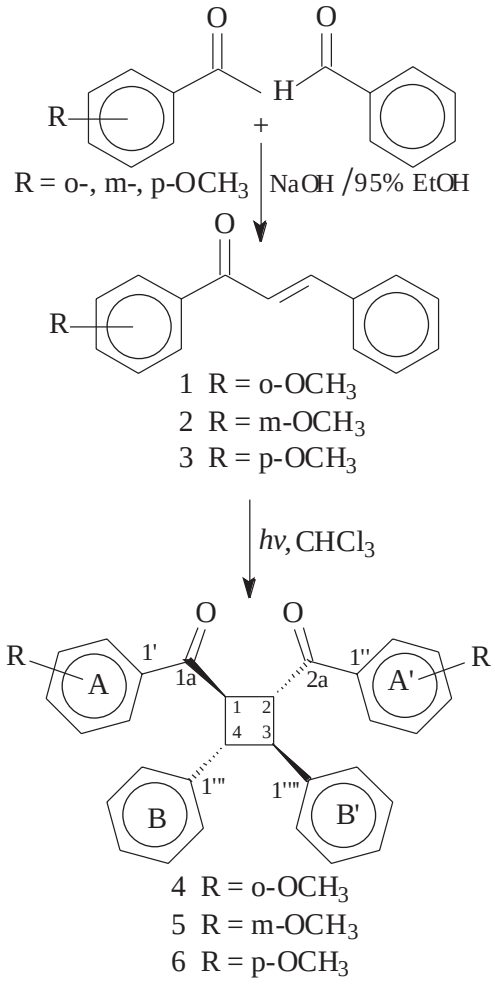
Table. NMR data of compounds 4-6 in $CDCl_3$.

	4^a		5^a		6^a	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1, 2	4.41, AA′BB′, J=9.2, 5.6, nd, 9.2Hz	53.86	4.63, AA′BB′, J=8.8, 5.4, nd, 8.8Hz	47.47	4.54, AA′BB′, J=8.8, 5.4, nd, 8.8Hz	47.40
3, 4	4.16, AA′BB′, J=9.2, 5.6, nd, 9.2Hz	45.20	3.92, AA′BB′, J=8.8, 5.4, nd, 8.8Hz	48.29	3.97, AA′BB′, J=8.8, 5.8, nd, 8.8Hz	47.59
1a, 2a	-	201.22	-	198.67	-	197.48
1′/1″	-	127.52	-	136.70	-	132.10
2′/2″	-	158.82	7.42, m	112.09	7.81, AX, J=8.8Hz	131.16
3′/3″	6.68, d, J=8.4Hz	130.51	-	159.57	6.77, AX, J=8.8Hz	113.63
4′/4″	7.34, m	133.68	7.02, ddd, J=8.2, 2.6, 1.2Hz	120.90	-	163.69
5′/5″	6.93, dt, J=7.6, 1.0Hz	120.26	7.30, m	129.52	6.77, AX, J=8.8Hz	113.63
6′/6″	7.23, dd, J=7.6, 1.8Hz	111.12	7.44, dt, J=7.6, 1.4Hz	121.42	7.81, AX, J=8.8Hz	131.16
2xOCH_3	3.31, s	54.33	3.59, s	55.05	3.78, s	55.36
1‴/1⁗	-	142.63	-	141.45	-	141.60
2‴/2⁗	7.31, m	127.15	7.31, m	127.46	7.30, m	127.39
3‴/3⁗	7.31, m	128.26	7.31, m	128.75	7.30, m	128.59
4‴/4⁗	7.31, m	126.41	7.31, m	127.24	7.30, m	127.02
5‴/5⁗	7.31, m	128.26	7.31, m	128.75	7.30, m	128.59
6‴/6⁗	7.31, m	127.15	7.31, m	127.46	7.30, m	127.39

[a)] Assignment based on ^{1}H, APT, ^{1}H-^{1}H COSY, NOESY, and HETCOR.; nd: AB' Coupling constant was not detected.

The structures of the cyclobutyl rings of products **4**, **5** and **6** were elucidated from their ^{1}H NMR spectra, which show highly shielded CH proton signals at δ_H 4.41 / 4.16, δ_H 4.63 / 3.92, and δ_H 4.54 / 3.97, respectively.

The stereochemistry of compounds **4**, **5**, and **6** was determined from NMR spectrometry information and literature data[1−4]. Two symmetrical multiplets (AA′BB′) at δ_H 4.41 (δ_C 53.86) / δ_H 4.16 (δ_C 45.20) for compound **4**, at δ_H 4.63 (δ_C 47.47) / δ_H 3.92 (δ_C 48.29) for compound **5** and at δ_H 4.54 (δ_C 47.40) / δ_H 3.97 (δ_C 47.59) for compound **6** were observed for the cyclobutyl protons in ^{1}H NMR spectrum. NMR patterns allowed the calculation of the coupling constants of the cyclobutyl protons ($J_{AA'}$ = 9.2/8.8 Hz, J_{AB}= 5.4/5.6/5.8 Hz, $J_{AB'}$ = not detected, $J_{BB'}$ = 9.2/8.8 Hz)[1−4]. The values of these coupling constants suggest that **4**, **5** and **6** were formed by head-to-head coupling, but they do not allow a certain assignment with respect to *syn*/*anti* stereochemistry. A more accurate structural determination was attained by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and NOESY spectra and literature data[1−4]. The close similarity of the ^{1}H and ^{13}C NMR patterns of the cyclobutyl moieties with a δ-truxinic structure strongly suggests that the formation of cyclobutane ring occurs by *anti* head-to-head junction in compounds **4**, **5** and **6**[1−4].



Scheme 2.

The structural connectivities of compounds **4**, **5** and **6** were established as individual parts from ^{1}H-^{1}H COSY. It was found that for the most down field for the cyclobutyl ring, methine designated H-1/H-2 at δ_H 4.41 (δ_C 53.86) was connected to H-3/H-4 at δ_H 4.16 (δ_C 45.20) for **4**, H-1/H-2 at δ_H 4.63 (δ_C 47.47), to H-3/H-4 at δ_H 3.92 (δ_C 48.29) for **5** and H-1/H-2 at δ_H 4.54 (δ_C 47.40) and to H-3/H-4 at δ_H 3.97 (δ_C 47.59) for **6**. The important NOESY interactions in compounds **4**, **5** and **6** were seen between H-1 and H-3 and between H-2 and H-4. Thus the presence of a cyclobutane ring was established. The chemical shifts of compounds **4**, **5** and **6** are in total agreement with those of similar structures in the literature with δ-truxinic type structures[1−4].

The ^{1}H NMR spectrum of compound **6** also shows an AX spin system for the A/A' *p*-methoxyphenyl group at δ_H 7.81 (H-2‴/6‴, J = 8.8 Hz) and δ_H 6.77 (H-3‴/5‴, J = 8.8 Hz). These data clearly show the *p*-methoxyphenyl part of compound **6**. Similar correlations were also seen in the ^{1}H-^{1}H COSY NMR spectra of compounds **4** and **5**. However, the B/B' phenyl parts of compounds **4**, **5** and **6** were observed as multiplets at $\sim \delta_H$ 7.30 ppm (superimposed) in the ^{1}H NMR spectra.

The LC-MS/MS of compounds **4**, **5** and **6** gave $[M-1]^+$ at m/z 475 (50%, 100%, 100%), $[M]^+$ at m/z 476 (18%, 31%, 28%) and $[M+Na]^+$ at m/z 499 (95%, 28%, 8%), which were consistent with the molecular formulae to be $C_{32}H_{28}O_4$ requiring dimeric structures.

Based upon the above observations, the complete chemical shift assignments for **4**, **5** and **6** were deduced and are listed in the Table. Compounds **4**, **5** and **6** were thus shown to be rel-(1β,2α)-di-(2-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane, rel-(1β,2α)-di-(3-methoxy)benzoyl-rel-(3β,4α)-diphenylcyclobutane, and rel-(1β,2α)-di-(4-methoxy) benzoyl-rel-(3β,4α)-diphenylcyclobutane, respectively. These 3 chiral compounds were synthesized and identified for the first time in this work.

Superoxide radicals were generated by the xanthine-xanthine oxidase and NBT systems in the tests[20]. The decrease in absorbance at 560 nm with the presence of antioxidants indicates the consumption of superoxide anions in the reaction mixture. The superoxide radical scavenging activities of chalcones **2** and **3** compare well with those of BHT (Figure). Compound **1** showed even higher activity, which makes it a good candidate as a general antioxidant, analogous to naturally occurring ones.

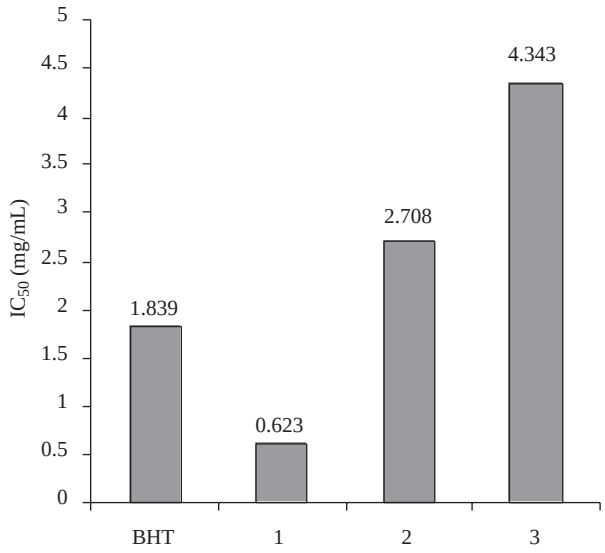


Figure. Superoxide radical scavenging activity of chalcones **1**, **2** and **3**. IC_{50} represents the mg/mL concentration providing 50% inhibition of radical formation or scavenging of available radicals. The results are of duplicate measurements and are compared with butylated hydroxytoluene (BHT).

Acknowledgments

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