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Isolation and synthesis of a dimeric dihydrochalcone from *Agapanthus africanus*

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Abstract

A new dimeric dihydrochalcone, *rel*-(1β,2α)-di-(2,4-dihydroxybenzoyl)-*rel*-(3β,4α)-di-(4-hydroxyphenyl)-cyclobutane, accompanied by its apparent precursor, the known chalcone isoliquiritigenin, have been isolated from the roots of *Agapanthus africanus* (Liliaceae). The structure is based on spectroscopic methods including extensive NMR analyses, mass spectrometry and circular dichroism. Conclusions regarding the structure and relative configuration are supported by synthesis of the dimeric dihydrochalcone via a pericyclic $[\pi 2_s + \pi 2_s]$ photocyclo-addition of the corresponding chalcone and consideration of the molecular symmetry involved.

Keywords: *Agapanthus africanus*; Liliaceae; Isoliquiritigenin; Chalcone; Substituted cyclobutane; Dimeric dihydrochalcone; Molecular symmetry; Synthesis

1. Introduction

Agapanthus africanus (Liliaceae) is one of the seven known *Agapanthus* species and is widely grown in South Africa as a popular ornamental garden plant, either indoors or outdoors. Its natural habitat is restricted to the upper slopes of Table Mountain and the southern and eastern regions of the Drakensberg Mountains in South Africa. *A. africanus* is used as a traditional medicine (prepared as an infusion or decoction) (Steenkamp, 2003) by local South African women during pregnancy to induce or augment labour and to treat constipation during pregnancy. In this context our study was aimed at the isolation and characterization of possible biologically active compounds from *A. africanus*.

Generally considered as minor flavonoids, chalcones belong to a class of yellow phenolic anthochloro pigments which constitute the most important intermediates in the biosynthesis of flavonoids (Roux and Ferreira, 1974; Ali and Kagan, 1974). Dihydrochalcones represent a less common variation of the chalcone skeleton which on rare occasions may occur as dimers based on a cyclobutane ring (e.g., **1**). Hitherto, structurally related dimeric dihydrochalcones have only been isolated from *Goniothalamus gardneri* and *Goniothalamus thwaitesii* (Seidel et al., 2000), *Combretum albopunctatum* (Katerere et al., 2004) and *Brakenridgea zanguebarica* (Drewes and Hudson, 1983). However, we now report on the isolation of a symmetrical dimeric dihydrochalcone (**1**) from *A. africanus* which is apparently composed of two known chalcone (isoliquiritigenin) units (**4**) with a head-to-head coupling between the two moieties. The structure is supported by synthesis.

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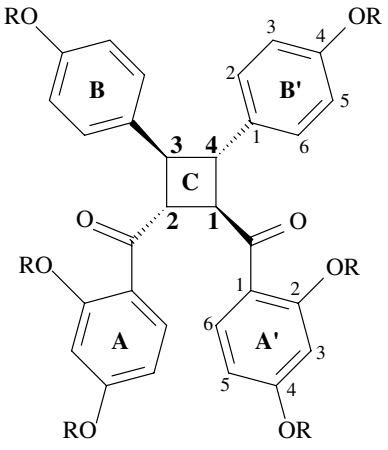
2. Results and discussion

The in-depth investigation into the polyphenolic content of the roots of *A. africanus* revealed the presence of the novel substituted cyclobutane (**1**), analogous to a dimeric dihydrochalcone, accompanied by the chalcone, isoliquiritigenin (**4**), which is believed to be its precursor. Due to its complexity the acetone extract of the roots was subjected to a series of chromatographic fractionations followed by derivatisation of the metabolites prior to final TLC separations in order to obtain an acceptable level of purity. Thus, compounds (**1** and **4**) were isolated as their peracetate derivatives (**2** and **5**). In order to prevent the formation of artifacts, extreme care was exercised to avoid exposure of the extracts at all stages to undue temperature or UV-irradiation. Although severe chromatographic overlap in the initial extract precludes verification of the presence of (**1**), its conspicuous optical activity (see below) leaves little doubt as to its natural origin (see Fig. 1).

Comparison of the ^{1}H NMR data of chalcone (**5**) with data in the literature confirmed it to be isoliquiritigenin (Seshadri, 1972). The spectrum clearly displays an ABX- and AA′BB′-spin system for the aromatic A- and B-rings, respectively, (in agreement with the three concomitant acetoxy groups) with H-α and H-β resonating as an AB-spin system (2 × d; δ_H 7.30 and 7.75; ^3J = 15.8 Hz), characteristic of chalcones. The *O*-acetyl derivative of the dimer (**2**) yields a similar spectrum with the same aromatic ABX- and AA′BB′-spin systems (accompanied by the relevant acetoxy groups) but notably lacking H-α and H-β. Instead two distinct multiplets, apparently integrating for one proton each, resonate at δ_H 3.91 and 4.34, and an HMQC experiment shows individual coupling ($^1J_{CH}$) of each of these resonances with that of a specific carbon (δ_C 47.3 and 50.8, respectively). Collectively these chemical shifts, thus, endorse the replacement of the α,β-unsaturated system of the chalcone with a saturated moiety incorporating methine protons. The remainder of the ^{13}C NMR spectrum in conjunction with HMQC and HMBC data is consistent with structural features displayed by the analogous chalcone (**5**). Following deacetylation by hydrolysis of the peracetate (**2**), FAB-MS of the free phenol (**1**) gives [M$^+$] at *m*/*z* = 512 ($C_{30}H_{24}O_8$), twice the value expected from the corresponding chalcone ($C_{15}H_{12}O_4$). Considering the nature of the NMR data this suggested that compound (**1**) is a symmetric dimer composed of two moles of the chalcone (**4**) probably fused via a cyclobutyl ring at C-α and C-β. It implies that each NMR resonance represents two equivalent nuclei or groups originating from a symmetrical arrangement of the moieties about the four-membered ring. The conclusion would consequently also account for the chemical shifts and spin–spin coupling of the multiplets displayed by the ^{1}H NMR spectrum of the derivative (**2**), each of which represents two of the four mutually coupled methine protons, typical of an AA′BB′ spin system associated with a cyclobutyl moiety (Cibin et al., 2003a). Contrary to the chalcone derivative (**5**), the dimer peracetate (**2**) shows prominent optical activity, yielding a complex CD-curve (cf. Section 3.3.1).



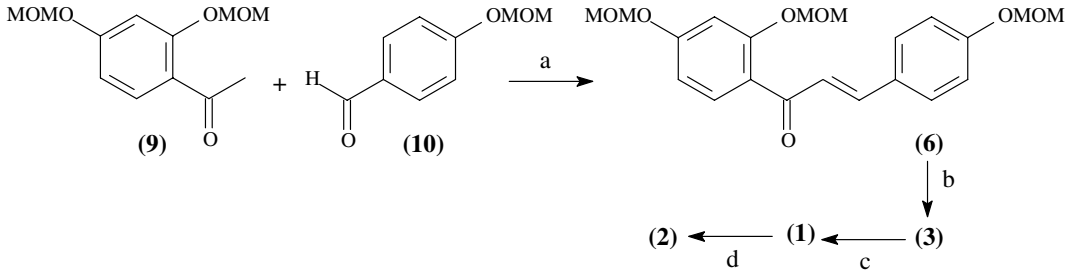
1, R = H

2, R = Ac

3, R = MOM

Fig. 1. *rel*-(1β,2α)-di-(2,4-Dihydroxybenzoyl)-*rel*-(3β,4α)-di-(4-hydroxyphenyl)-cyclobutane (**1**), *rel*-(1β,2α)-di-(2,4-diacetoxybenzoyl)-*rel*-(3β,4α)-di-(4-acetoxyphenyl)-cyclobutane (**2**), *rel*-(1β,2α)-di-(2,4-dimethoxymethoxybenzoyl)-*rel*-(3β,4α)-di-(4-methoxymethoxyphenyl)-cyclobutane (**3**).

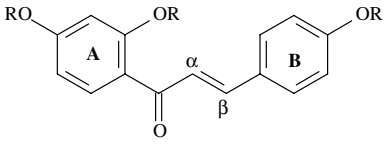
In order to resolve the structure we conceived that dimeric dihydrochalcones are synthetically accessible via a pericyclic [$_\pi 2_s$ + $_\pi 2_s$] cyclo-addition between two molecules of the corresponding chalcone. Geometric restrictions imposed by the four-membered ring, however, allows only a suprafacial addition which necessitates photolytic conditions to render the reaction symmetry-allowed. Thus, the 4,2′,4′-trimethoxymethoxychalcone (**6**) was prepared via the classic base-catalyzed (50% KOH/MeOH) aldol-type condensation of 2,4-dihydroxyacetophenone with 4-hydroxybenzaldehyde, following initial protection of both substrates by methoxymethylation (**9** and **10** respectively) (Scheme 1). Irradiation (λ = 354 nm) of the chalcone (**6**) in MeOH for 4 days afforded a dimeric dihydrochalcone (**3**), which, following deprotection by mild acid (3 N HCl) gave the free phenol (**1**). Acetylation of the latter yielded a peracetate derivative (**2**), albeit in a modest overall yield (29%), with NMR data identical to that of the peracetate derivative of the natural product. The major product (**3**) from the photodimerization is accompanied by a minor product which displays similar but not identical NMR data (cf. Section 3.4.4) to that of (**3**). The compound which most likely represents one of the various possible isomers expected from the reaction, as



Scheme 1. Synthesis of the dimeric dihydrochalcone and derivatives (**1**, **2** and **3**). *Reagents and conditions*: (a) 50% KOH/EtOH at 20 °C; (b) hv, 354 nm, 4 days; (c) 3 M HCl/MeOH reflux 3 h; (d) Ac_2O/pyr.

discussed below, was not at present further investigated (see Fig. 2).

Considering that the suprafacial [$_\pi 2_s + _\pi 2_s$] cycloaddition between two molecules of the *E*-chalcone could occur head-to-head or head-to-tail (leading to truxinic or truxillic type dimers, respectively), with an alignment which may be staggered or eclipsed, and that photolytic *E*/*Z*-isomerisation may yield the *Z*-chalcone which could also participate in the addition, a total of fifteen different photo dimers theoretically become possible as products (Moriarty, 1974; Green and Reito, 1974). Five of these are diastereomeric truxillic-type dimers and the remaining ten are comprised of six truxinic diastereomers, four of which may occur as enantiomeric pairs. Four of the truxillic-type diastereomers belong to either C_s or C_{2v} symmetry point groups and the fifth to the C_i group, all achiral and optically inactive. This also applies to the two truxinic-type diastereomers with C_s symmetry. The remaining four truxinic-type diastereomers, however, belong to the C_1 or C_2 point groups which are chiral and give rise to optically active enantiomers. Considering that the derivative (**2**) of the natural product is optically active (cf. CD data, Section 3.3.1) it implies that its structure and configuration must coincide with one of the optically active enantiomers and that it, therefore, cannot be of the truxillic-type which are all achiral. The latter observation is confirmed by the fragment ions arising from cleavage of the cyclobutane ring in the FAB-MS spectrum of (**3**) (M^+, *m*/*z* 776, $C_{42}H_{48}O_{14}$), showing a fragment at *m*/*z* 388 (the chalcone, $C_{21}H_{24}O_7$) (Mabry and Markham, 1975) as well as two fragments at *m*/*z* 300 and 476 (Scheme 2). Collectively, these fragments signify a truxinic structure for this dimer, since a truxillic structure, on the contrary could only produce the one fragment at *m*/*z* 388.



4, R = H

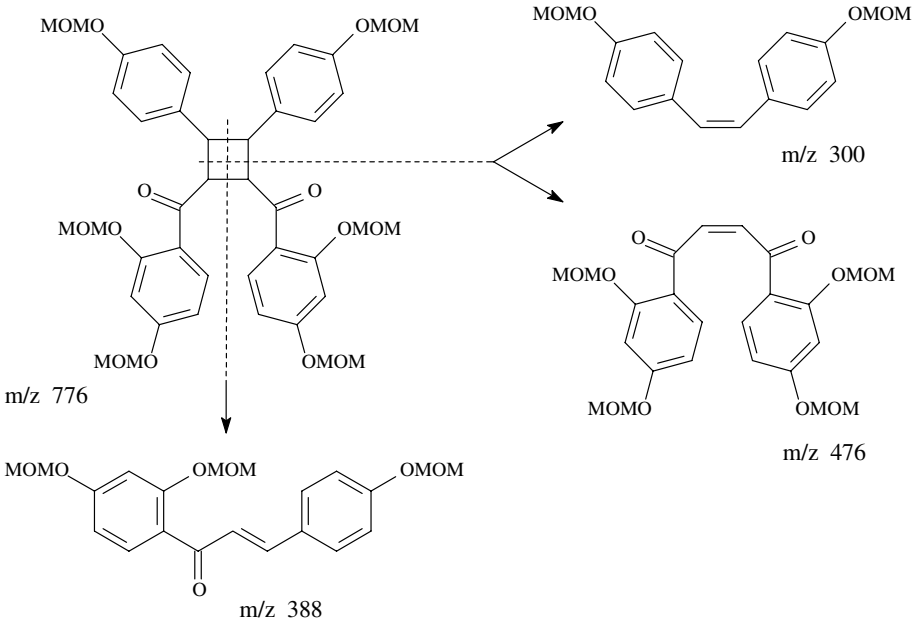
5, R = Ac

6, R = MOM

Fig. 2. (*trans*)-4,2′,4′-Trihydroxychalcone (isoliquiritigenin) (**4**), (*trans*)-4,2′,4′-triacetoxychalcone (**5**), (*trans*)-4,2′,4′-trimethoxymethoxychalcone (**6**).

The two truxinic-type diastereomers (four enantiomers) with C_1 symmetry, probably originating from the addition between an *E*- and an artificial *Z*-isomer, are asymmetrical from a stereochemical viewpoint and may be disregarded as the product on the grounds of the NMR data of (**3**) which is reminiscent of perfect duplication of the molecular components, so requiring a twofold axis of symmetry in the dimer. This prerequisite confines the structure and relative configuration of the synthetic- and identical natural product to one of the two remaining possibilities, namely the δ- (**7**) or μ-truxinic (**8**) type structures, both with C_2 symmetry (see Fig. 3).

^{1}H NMR long-range COSY experiments of (**3**) allow an unambiguous assignment of H-3/4(C) (m, δ 4.10) by coupling ($^4J_{HH}$) with H-2/6(B/B′). The latter is coupled ($^3J_{HH}$) to H-3/5(B/B′) which in turn displays long range coupling ($^4J_{HH}$) to 4-OCH_2OCH_3 (B/B′). The NOESY data for the same compound, however, not only shows strong NOE association expected between H-3/4(C) and H-2/6(B/B′), but also between H-2/6(B/B′) and the second set of methine protons, H-1/2(C) (m, δ 4.30). This association is only possible for a *cis*-relative configuration between H-1/2(C) and the B/B′-rings at C-3/4, implying a 1,4-*trans*-2,3-*trans* relative configuration for the C-ring. Considering the remaining possible δ- (**7**) or μ-truxinic (**8**) type structures only the former conforms to these conditions. Although the methine protons appear as a multiplet in the ^{1}H NMR spectra of both (**2**) and (**3**), the apparent presence of doublets (J = 9.0 Hz) respectively within the multiplet confirms the proposed all-*trans* configuration of the cyclobutane protons (Montaudo and Caccamese, 1973) and are in accord with those in the literature (Katerere et al., 2004), (Cibin et al., 2003 b; Caccamese et al., 1978). Compound (**1**), thus, constitutes a δ-truxinic-type dimeric dihydrochalcone, *rel*-(1β,2α)-di-(2,4-dihydroxybenzoyl)-*rel*-(3β,4α)-di-(4-hyd-roxyphenyl)-cyclobutane.



Scheme 2. Major fragments in the FAB-MS spectrum of the dimeric dihydrochalcone derivative (**3**).

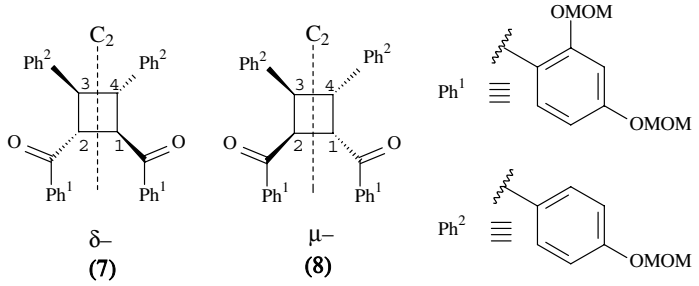


Fig. 3. δ- and μ-Truxinic type structures with C_2-symmetry axes.

3. Experimental

3.1. General

NMR spectra were recorded on a Bruker AVANCE DPX-300 spectrometer with TMS as internal standard. Mass spectrometry was performed on a Kratos MS-80 mass spectrometer in the double focus EI mode. Qualitative thin layer chromatography (TLC) was performed on 3×7 cm Kieselgel $60F_{254}$, 0.25 mm, aluminium plates (Merck). Development of the TLC plates in the appropriate solvent was followed by spraying with formaldehyde (40%)–sulfuric acid (2:98) or with anisaldehyde–sulfuric acid–ethanol (5:5:90) and heated to 120 °C. Compounds were purified by preparative thin layer chromatography (PLC) carried out on 20×20 cm glass plates coated with 1.0 mm Kieselgel PF_{254} (Merck) which were air-dried and used without prior activation. An application of 10–25 mg per plate was used. Small-scale preparative separations were on 20×20 cm precoated silica gel PF_{254}, 0.25 mm glass plates (Merck). Compounds were located by UV light (254 nm) and eluted from the adsorbent with acetone. Column chromatography (CC) was performed on Sephadex LH-20 at a flow speed of 0.5 mL/min, collecting 12 mL fractions. Flash column chromatography (FCC) was executed on Kieselgel $60F_{254}$ (0.040–0.063 mm mesh; Merck) with solvents as specified under N_2 pressure (approximately 50 kPa) 12 mL. Fractions were collected. Solvents were evaporated under reduced pressure at 40 °C on a rotary evaporator or aqueous solutions by freeze-drying on a Virtis 12 SL freezemobile. Acetylations were performed with acetic anhydride/pyridine at 40 °C for 12 h. Methoxy methylations were performed by treatment of the appropriate compound with NaH, followed by addition of chlorodimethyl ether in dry THF at 0 °C, and subsequent stirring at 25 °C for 30 min.

3.2. Plant material

Roots of *A. africanus* were harvested from the gardens of the University of the Free State in the central region of South Africa. Plant material was dried in drying ovens at 40 °C, whereafter it was pulverized. A voucher specimen is kept in the Chemistry Department of the University of the Free State.

3.3. Extraction and purification of compounds from *A. africanus*

Dried pulverized roots (1.96 g) were extracted (3×12.0 L, 24 h each) at ambient temperature (approximately 25 °C) with acetone and the extract evaporated to give a dark brown (45.08 g) solid. The extract was redissolved in water and freeze-dried to give a fine powder. Partitioning (H_2O–*sec*-BuOH–hexane, 5:4:1) of the extract (45.08 g) in a Craig countercurrent assembly (ten tubes, 200 ml of organic and 200 ml of aqueous phase per tube), by 10 transfers of the top phase, gave amongst others combined fractions 8–10 (23.57 g). Column chromatography of the latter with Sephadex LH-20 (4×150 cm column) in ethanol afforded fractions 361–417 which were combined (1.02 g). Acetylation followed by PLC (toluene–Me_2CO–EtOAc, 8:1:1) yielded the pure peracetate derivatives (**5**) (R_f 0.84, 2.2 mg) (Seshadri, 1972) and (**2**) (R_f 0.42, 8.5 mg).

3.3.1. *rel-(1β,2α)-di-(2,4-diacetoxybenzoyl)-rel-(3β,4α)-di-(4-acetoxyphenyl)-cyclobutane (dimeric 4,2′,4′-triacetoxydihydrochalcone) (**2**)*

Compound (**2**) (R_f 0.42, 8.5 mg) proved to be the peracetate of (**1**). ^{1}H NMR, Table 1. Circular dichroism (CD): $[\theta]_{284.5}$ 1.422×10, $[\theta]_{276.5}$ -1.088×10, $[\theta]_{233.5}$ 1.169×10, $[\theta]_{227.5}$ -1.723×10, $[\theta]_{220.0}$ 1.556×10, $[\theta]_{209.5}$ 2.434×10, $[\theta]_{204.5}$ -3.638×10. Following deacetylation of (**2**) by hydrolysis, the free phenol (**1**) gave FAB-MS: [M^+] *m/z* 512 (8.1), 207 (21.1), 147 (100), 109 (36.5).

3.4. Synthesis of dimeric dihydrochalcone

3.4.1. *2,4-Dimethoxymethoxyacetophenone (**9**)*

Acetophenone (1.5 g, 9.835 mmol) in a suspension of NaH (0.75 g, 31.25 mmol, 3.2 equiv.) in dry THF (30 ml) was stirred (30 min) until the colour changed from pink to orange. Chloromethylmethylether (1.27 g, 15.82 mmol, 1.6 equiv.) was introduced and the mixture stirred for 7 h. Crushed ice and 1.0 N HCl (10 ml) were

Table 1
^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data (**2**) and ^{1}H NMR (300 MHz) (**3**)

Compound **2**						Compound **3**
Ring	Position	^{1}H multiplicity *J* (Hz)	^{13}C	^2J (H → C)	^3J (H → C)	^{1}H multiplicity *J* (Hz)
A/A′	1	–	127.2			
	2	–	150.0	–		
	3	6.92 *d* (2.5)	117.0	150.0, 151.1	119.2, 127.2	6.75 *d* (2.5)
	4	–	151.1	–	–	
	5	6.85 *dd* (8.5, 2.5)	119.2	132.8, 151.1	117.0, 127.2	6.66 *d* (8.5, 2.5)
	6	7.58 *d* (8.5)	132.8	119.2, 127.2	119.2, 150.0, 198.2	7.70 *d* (8.5)
B/B′	1	–	139.2	–	–	
	2	7.28 *d* (8.5)	128.5	122.4, 139.2	47.3, 155.1	7.20 *d* (8.5)
	3	7.02 *d*, (2.5)	122.4	128.5, 155.1	139.2	6.94 *d* (2.5)
	4	–	155.1	–		
	5	7.02 *d* (2.5)	122.4	128.5, 155.1	139.2	6.94 *d* (2.5)
	6	7.28 *d* (8.5)	128.5	122.4, 139.2	47.3, 155.1	7.20 *d* (8.5)
C	1/2	4.34 *m*	50.8	47.3, 198.2	127.2, 139.2	4.30 *m*
	3/4	3.91 *m*	47.3	50.8, 139.2	128.5, 198.2	4.08 *m*
	(2*x*) >*C*=O		198.2			
	(2*x*)-OAc	2.28 *s*				
	(2*x*)-OAc	2.31 *s*				
	(2*x*)-OAc	2.37 *s*				
	(6*x*)-OCO*C*$_3$		22.1			
	(6*x*)-O*C*OCH$_3$		169.0–171.1			
	(2*x*)-OC*H*$_2$					5.16 *s*
	(2*x*)-OC*H*$_2$					5.13 *s*
	-OC*H*$_2$					4.85 *d* (7.0)
	-OC*H*$_2$					4.84 *d* (7.0)
	(4*x*)-OMe					3.45 *s*
	(2*x*)-OMe					3.17 *s*

Data obtained in $CDCl_3$.

added and the reaction mixture extracted with $CHCl_3$ (3×30 ml). The extract was neutralized with saturated *aq*. $NaHCO_3$ (30 ml), washed with water (30 ml) and dried over $MgSO_4$. Following FCC (hexane-Me_2CO, 8:2) of the extract, the combined fractions 60–80 gave the product (**9**) as a yellow oil (1.2 g, 50%). ^1H NMR ($CDCl_3$) (δ_H): 7.62 (1 H, d, $J = 8.5$ Hz, H-6), 6.55 (1 H, dd, $J = 2.5$, 8.5 Hz, H-5), 6.51 (1 H, d, J 2.5 Hz, H-2), 5.20 (4 H, s, OCH_2), 3.45 (6 H, s, OCH_3), 2.20 (3 H, s, CH_3).

3.4.2. 4-Methoxymethoxybenzaldehyde (*10*)

Benzaldehyde (1.0 g, 8.18 mmol) was stirred with a suspension of NaH (0.60 g, 25.0 mmol, 3.0 equiv.) in dry THF (30 ml) until the colour changed from purple to brown (30 min). Chloromethylmethylether (0.84 g, 10.46 mmol, 1.3 equiv.) was added and stirring maintained for 7 h, followed by the addition of crushed ice and 1.0 N HCl (10 ml). The reaction mixture was extracted with $CHCl_3$ (3×30 ml), the extract neutralized with saturated *aq*. $NaHCO_3$ (30 ml), washed with water (30 ml) and dried over $MgSO_4$. The product (**10**) was collected from the combined fractions 15–40 as a yellow oil (0.8 g, 58.8%) after FCC (hexane-Me_2CO, 8:2). ^1H NMR ($CDCl_3$) (δ_H): 9.82 (1 H, s, C*H*O), 7.74 (1 H, d, $J = 8.5$ Hz, H-2,6), 7.10 (1 H, d, $J = 8.5$ Hz, H-3,5), 5.20 (2 H, s, OCH_2), 3.45 (3 H, s, OCH_3).

3.4.3. 4,2′,4′-Trimethoxymethoxychalcone (*6*)

50% (m/v) aq. KOH (5 ml) was added to a solution of 2,4-dimethoxymethoxyacetophenone (**9**) (1.2 g, 4.99 mmol) in ethanol (20 ml) and the mixture stirred at room temperature for 30 min. Excess 4-methoxymethoxybenzaldehyde (**10**) (1 g, 6.018 mmol, 1.2 equiv.) was added and the agitation continued for 18-24 hrs. The reaction mixture was subsequently treated with water (50 ml) and extracted with diethyl ether (4×30 ml). Drying of the combined ethereal extracts with $MgSO_4$ followed by evaporation and FCC (hexane-Me_2CO, 7:3) gave a pure yellow oil (fractions 46–65), the chalcone (**6**) (1.30 g, 67%). ^1H NMR ($CDCl_3$) (δ_H): 7.84 (1 H, d, $J = 8.5$ Hz, H-6′), 7.85 (1 H, d, $J = 15.8$ Hz, β-H), 7.60 (2 H, d, $J = 8.5$ Hz, H-2,6), 7.45 (1 H, d, $J = 15.8$ Hz, α-H), 7.09 (2 H, d, $J = 8.5$ Hz, H-3,5), 6.64 (1 H, d, $J = 2.5$ Hz, H-3′), 6.59 (1 H, dd, $J = 8.5$ Hz, 2.5 Hz, H-5′), 5.23 (6 H, s, OCH_2), 3.50 (9 H, s, OCH_3).

3.4.4. rel-(1β,2α)-di-(2,4-dimethoxymethoxybenzoyl)-rel-(3β,4α)-di-(4-methoxymethoxyphenyl)-cyclobutane (dimeric 4,2′,4′-trimethoxymethoxydihydrochalcone) (*3*)

trans-4,2′,4′-Trimethoxymethoxychalcone (**6**) (200 mg, 0.781 mmol) was dissolved in methanol and irradiated with UV-light (354 nm) for 4 days. PLC (hexane-Me_2CO, 7:3) yielded two bands (R_f 0.41 and 0.25). The R_f 0.41 band was obtained as a colorless oil (120 mg, 19.8 %). ^1H NMR, Table 1. FAB-MS: [M^+] *m*/*z* 776 (6.8), 476 (2.8), 388 (14.6), 300 (14.6), 225 (100).

The second product (R_f 0.25) was obtained as a colorless oil (23 mg, 3.8%). ^1H NMR ($CDCl_3$) (δ_H): 7.67 (2 H, d, $J = 8.5$ Hz, H-6, A/A′), 6.95 (4 H, d, $J = 8.5$ Hz, H-2,6, B/B′), 6.82 (4 H, dd, $J = 8.5$ Hz, H-3,5, B/B′), 6.69 (2 H, d, $J = 2.5$ Hz, H-2, A/A′), 6.67 (2 H, dd, $J = 2.5$, 8.5 Hz, H-5, A/A′), 5.17 (4 H, s, OCH_2), 5.10 (4 H, s, OCH_2), 4.88 (2 H, d, $J = 7.0$ Hz, OCH_2), 4.73 (2 H, d, $J = 7.0$ Hz, OCH_2); C-ring, 4.64 (2 H, m, H-1/2), 4.25 (2 H, m, H-3/4), 3.47 (6 H, s, OCH_3), 3.44 (6 H, s, OCH_3), 3.25 (6 H, s, OCH_3); FAB-MS: [M^+] *m*/*z* 776 (4.1), 256 (3.3), 154 (49.4), 136, (100).

3.4.5. rel-(1β,2α)-di-(2,4-dihydroxybenzoyl)-rel-(3β,4α)-di-(4-hydroxyphenyl)-cyclobutane (dimeric 4,2′,4′-trihydroxydihydrochalcone) (*1*)

To the solution of (**2**) in minimum methanol, 3 N HCl (2 ml) was added and the mixture refluxed for 1.5 h. Water (10 ml) was added and the reaction mixture was extracted with $CHCl_3$ (3×20 ml). The organic extracts were neutralized with saturated aq. $NaHCO_3$ (20 ml), washed with water (20 ml) and dried over $MgSO_4$. Following FCC (hexane–Me_2CO–MeOH, 6:3:1), compound (**1**) was obtained (fractions 186–226) as a yellow oil (120 mg, 19.8%). ^1H NMR ($CDCl_3$) (δ_H): 7.45 (2 H, d, $J = 8.5$ Hz, H-6, A/A′), 7.24 (4 H, d, $J = 8.5$ Hz, H-2,6, B/B′), 6.82 (4 H, dd, $J = 8.5$ Hz, H-3,5, B/B′), 6.31 (2 H, d, $J = 2.5$ Hz, H-3, A/A′), 6.20 (2 H, dd, $J = 2.5$, 8.5 Hz, H-5, A/A′); C-ring, 4.54 (2 H, m, H-1/2), 3.82 (2 H, m, H-3/4).

3.4.6. rel-(1β,2α)-di-(2,4-diacetoxybenzoyl)-rel-(3β,4α)-di-(4-acetoxyphenyl)-cyclobutane (dimeric 4,2′,4′-triacetoxydihydrochalcone) (*2*)

Dry dimeric 4,2′,4′-trihydroxydihydrochalcone (**1**) (120 mg) was dissolved in a minimum volume of pyridine and twice the amount of acetic anhydride added. After 8–12 h at ambient temperatures, crushed ice was added to precipitate the acetylated material (78 mg, 66.0%) which was filtered and excess pyridine washed out with cold water (^1H NMR, Table 1).

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