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CYTOTOXIC COMPONENTS OF *ZINGIBER ZERUMBET*, *CURCUMA ZEDOARIA* AND *C. DOMESTICA**

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Key Word Index – *Zingiber zerumbet*; *Curcuma zedoaria*; *C. domestica*; Zingiberaceae; cytotoxicity; sesquiterpenes; curcuminoids; flavonol 3-rhamnoside; synthesis; ^{13}C NMR.

Abstract—One new and five known compounds, which all showed cytotoxic activity, were isolated from the rhizomes of *Zingiber zerumbet*. The new compound was 3″,4″-*O*-diacetylafzelin. The known compounds were zerumbone, zerumbone epoxide, diferuloylmethane, feruloyl-*p*-coumaroylmethane and di-*p*-coumaroylmethane. Several substituted cinnamoylmethanes were synthesized and tested for cytotoxic properties. Among these were tricinnamoylmethane and triferuloylmethane. The structures were elucidated mainly by spectroscopic methods and ^{13}C NMR data are given.

INTRODUCTION

As part of our continuing search for antitumour principles in Chinese and other traditional drugs [1], we have investigated three plants from the Zingiberaceae: *Zingiber zerumbet* Smith, *Curcuma zedoaria* Roscoe and *C. domestica* Valet.† The rhizomes of *Curcuma zedoaria* are used clinically in China in the treatment of several types of tumour [2].

We tested the cytotoxicity of extracts of the roots of the plants on 'hepatoma tissue culture' (HTC), a neoplastic rat liver cell strain cultured *in vitro* [1c]. We isolated six cytotoxic compounds, which were tested on normal mouse fibroblasts (3T3), to indicate general or selective cytotoxicity. One of the compounds, 3″,4″-*O*-diacetylafzelin (**7**), is a new natural product. Of the known compounds, two, zerumbone (**2**) and zerumbone epoxide (**3**) are already known in *Z. zerumbet* [5, 6], while the three curcuminoids, diferuloylmethane (**4**), feruloyl-*p*-coumaroylmethane (**5**) and di-*p*-coumaroylmethane (**6**) have not previously been described in *Z. zerumbet*, but are well-known in *Curcuma* species [7, 8]. We have also prepared several analogues of **4**, 'synthetic curcuminoids' and examined their cytotoxicity.

RESULTS AND DISCUSSION

Two cytotoxic compounds, zerumbone (**2**) and zerumbone epoxide (**3**), were isolated from the pentane extract of *Z. zerumbet* [5, 6]. The ether extract yielded three highly cytotoxic compounds: diferuloylmethane (**4**), feruloyl-*p*-coumaroylmethane (**5**) and di-*p*-coumaroylmethane (**6**) and one slightly cytotoxic compound (**7**) (Table 1 and Fig. 1).

Table 1. Cytotoxicity measured by the HTC and 3T3 models

Structure	Activity (HTC)‡	Activity (3T3)§
1	0 (33 μg/ml)	
2	2 (33 μg/ml)	+ (33 μg/ml)
3	3 (33 μg/ml)	÷ (30 μg/ml)
		+ (120 μg/ml)
4	4 (33 μg/ml)	+ (33 μg/ml)
	2 (13 μg/ml)	
	1 (8 μg/ml)	
	0 (3 μg/ml)	
5	4 (33 μg/ml)	
6	4 (33 μg/ml)	
7	1 (33 μg/ml)	
4a	1 (8 μg/ml)	
9	3 (33 μg/ml)	
	1 (8 μg/ml)	
	0 (3 μg/ml)	
11	3 4 (8 μg/ml)	
15a	3–4 (13 μg/ml)	
16	3 (8 μg/ml)	
18	4 (8 μg/ml)	

‡Activity 0: No of cells alive after 3 days' incubation: 200–800%.
Activity 1: No. of cells alive after 3 days' incubation: 100–200%.
Activity 2: No. of cells alive after 3 days' incubation: 50–100%.
Activity 3: No. of cells alive after 3 days' incubation: 0–50%.
Activity 4: No. of cells alive after 2 days' incubation: 0%.
§ ÷ (non toxic): the fibroblasts were identical with the control after 3 days' incubation. + (toxic): the fibroblasts were morphologically different from the control. The cells were generally round and detached. Cellular debris was also present.

* Part VI in the series "Chemistry and Biochemistry of Chinese Drugs". For Parts I–V see ref. [1].

† Used by many authors as synonymous with *C. longa* L., which, according to Holttum [3, 4], is incorrect.

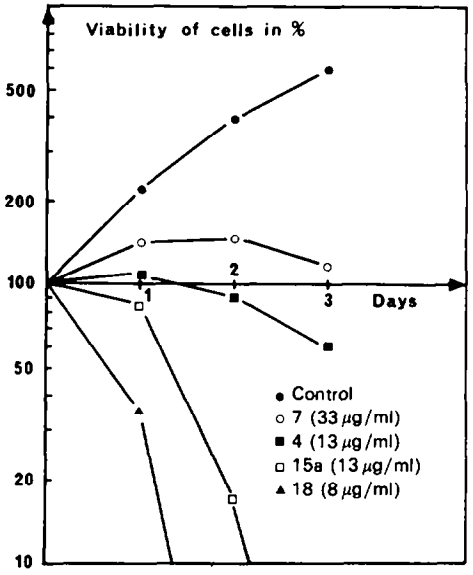


Fig. 1. Effect of compounds **4**, **7**, **15a** and **18** on the growth of hepatoma tissue cultures (HTC).

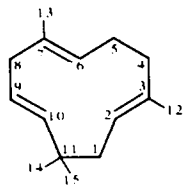
Kaempferol-3-α-L-(3'',4''-*O*-diacetyl)rhamnopyranoside (diacetylafzelin) (**7**), was isolated as a pale yellow amorphous solid. Field desorption MS gave evidence for a MW of 516. The colour reactions in acid, base and with Fe(III) chloride showed the compound to be phenolic. The UV spectra and bathochromic shifts at different pH values and with Al(III) chloride were typical of kaempferol-3-*O*-glycosides [9]. This was confirmed by ^{1}H NMR, which strongly suggested a kaempferol derivative: a low-field proton (δ = 12.65) was assigned to the 5-OH group (Table 2). This and two other broad signals at δ 9.4 and 4.8 disappeared on addition of D_2O. The integration indicates four free OH groups. The four doublets at 7.87 (J = 9 Hz), 7.07 (J = 9 Hz), 6.48 (J = 2 Hz) and 6.29 (J = 2 Hz) ppm were assigned to the protons at C-6' + C-2', C-5' + C-3', C-8 and C-6 according to the additivity rules for substituted benzenes. Two singlets, each integrating for three protons at δ 1.97 and 2.02, showed that the compound contained two non-phenolic acetates. The five protons in the region 3.5–5.6 and the three protons forming a doublet (J = 6 Hz) at 0.83 could be explained by a deoxypyranose residue, e.g. rhamnose. Decoupling experiments established the assignments of the sugar protons at carbons 1'', 2'', 3'', 4'', 5'' and 6''. The proton at C-2'', which is at 0.8 ppm higher field than the protons at C-3'' and C-4'', indicated the position of the acetyl groups at C-3 and C-4. After peracetylation, the 2''-proton moved 1.1 ppm downfield. After deacetylation, the protons at C-3'' and C-4'' moved 1.5 and 1.7 ppm upfield. ^{13}C NMR confirmed that the sugar was situated at the 3-position of kaempferol, the chemical shift at C-2 being 12 ppm higher for **7** than for kaempferol [10]. Acid hydrolysis yielded an aglycone (**8**) which, by mp, IR, ^{1}H NMR and MS, was shown to be kaempferol [11, 12]. The sugar moiety was determined by GLC after methanolysis and trifluoracetylation by the method of Zanetta [13] and was identified as rhamnose. Hydrolysis under mild basic conditions yielded afzelin (**7a**), whose mp, IR, UV and $[\alpha]_D$ were in accordance with published data [9, 14, 15].

The ^{13}C NMR data for afzelin (**7a**) diacetylafzelin (**7**) and hexaacetylafzelin (**7b**) are given in Table 5. For all compounds the assignments were based on broad-band proton-decoupled spectra, as well as selective proton-decoupled spectra for carbons: 6' + 2', 5' + 3', 6, 8,

Table 2. ^{1}H NMR data of compounds **7**, **7a**, **7b** and **8** (250 MHz, acetone-d_6, ppm from TMS as internal standard, J(Hz) in parentheses)

H	7	7a	7b	8
6	6.29, 1 H, *d* (2)	6.28, 1 H, *d* (2)	6.99, 1 H, *d* (2)	6.27, 1 H, *s*
8	6.48, 1 H, *d* (2)	6.48, 1 H, *d* (2)	7.45, 1 H, *d* (2)	6.53, 1 H, *s*
2',6'	7.87, 2 H, *d* (9)	7.87, 2 H, *d* (9)	8.07, 2 H, *d* (9)	8.16, 2 H, *d* (9)
3',5'	7.07, 2 H, *d* (9)	7.07, 2 H, *d* (9)	7.39, 2 H, *d* (9)	7.02, 2 H, *d* (9)
1''	5.57, 1 H, *d* (1.2)	5.55, 1 H, *d* (1.0)	5.57, 1 H, *d* (1.3)	..
2''	4.43, 1 H, *br. s*	4.22, 1 H, *dd* (1,3)	5.64, 1 H, *dd* (1.5,2)	-
3''	5.18, 1 H, *dd* (3,10)	3.70, 1 H, *dd* (3,9)	5.21, 1 H, *dd* (2,9)	-
4''	5.08, 1 H, *dd* (9,10)	3.35, 1 H, *dd* (9,9)	4.89, 1 H, *dd* (9,9)	-
5''	3.50, 1 H, *dq* (6,9)	3.31, 1 H, *dq* (6,9)	3.30, 1 H, *dd* (6.9)	
6''	0.83, 3 H, *d* (6)	0.90, 3 H, *d* (6)	0.82, 3 H, *d* (6)	—
3-OH				9.68, 1 H, *br. s**
5-OH	12.65, 1 H, *br. s*	12.73, 1 H, *br. s*		12.18, 1 H, *br. s*
7-OH	9.4, 1 H, *br. s*	9.4, 1 H, *br. s*	—	9.03, 1 H, *br. s**
4'-OH	9.4, 1 H, *br. s*	—	—	8.03, 1 H, *br. s**
2''-OH	4.8, 1 H, *br. s*	—	—	
3''-OH	—	—		-
4''-OH		—	—	—
5-OAc	—	—	2.32, 3 H, *s*†	-
7-OAc	..	..	2.34, 3 H, *s*†	—
4'-OAc	—	—	2.35, 3 H, *s*†	
2''-OAc	—	—	1.95, 3 H, *s**	—
3''-OAc	1.97, 3 H, *s**	—	1.97, 3 H, *s**	—
4''-OAc	2.02, 3 H, *s**	—	2.10, 3 H, *s**	—

*,† Chemical shifts with same sign may be interchanged.



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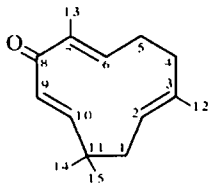

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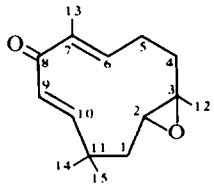

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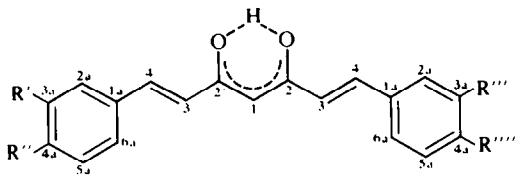
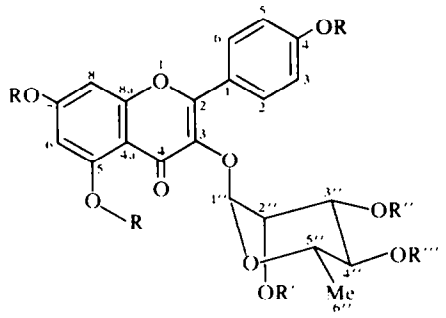
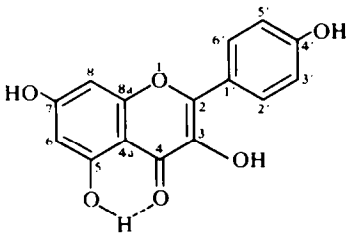


	4	5	6	4a	9
R′	OMe	OMe	H	OMe	H
R″	OH	OH	OH	OAc	H
R‴	OMe	H	H	OMe	H
R⁗	OH	OH	OH	OAc	H



	7	7a	7b
R	H	H	Ac
R′	H	H	Ac
R″	Ac	H	Ac
R‴	Ac	H	Ac



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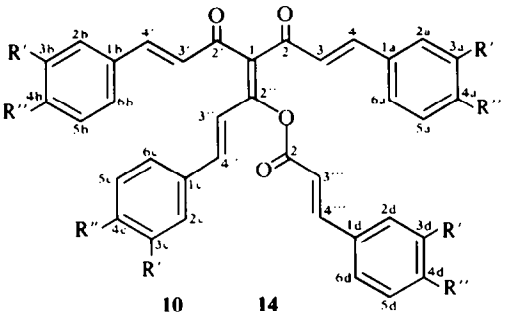
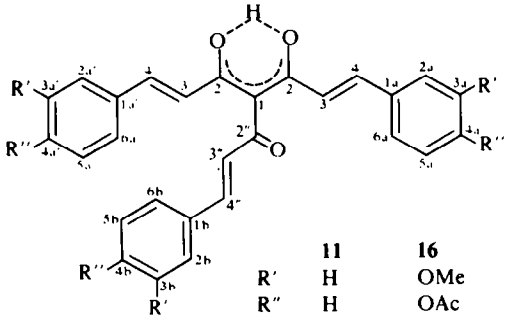
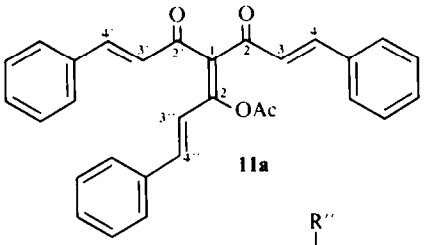


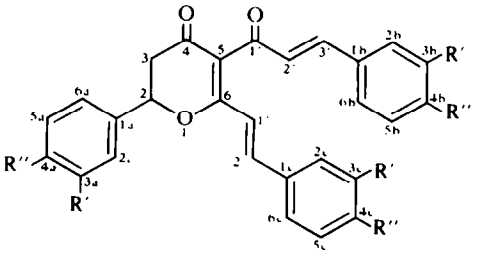
	10	14
R′	H	OMe
R″	H	OAc



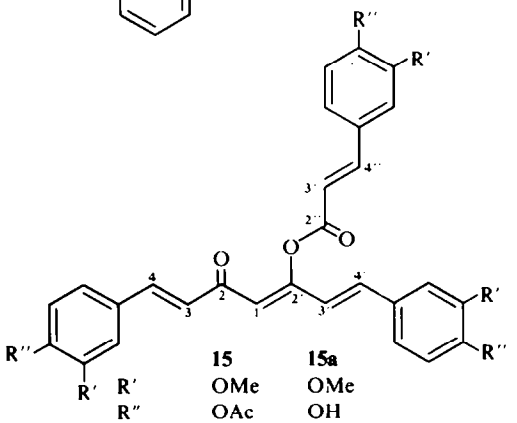
	11	16	18
R′	H	OMe	OMe
R″	H	OAc	OH



11a



	12	17	19
R′	H	OMe	OMe
R″	H	OAc	OH

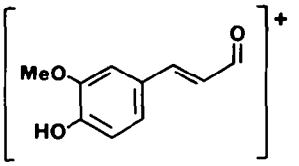


	15	15a
R′	OMe	OMe
R″	OAc	OH

1″, 2″, 3″, 4″ and 5″. The carbons 5, 7, 8a, 2 and 4′ were assigned according to their long-range coupling in gated decoupled spectra, taking the nuclear Overhauser effect into consideration. In the case of **7**, a gated decoupled spectrum recorded in acetone-d_6 and traces of D_2O left the aromatic long-range couplings $^2J_{CH}$ more easily visible [16]. The chemical shifts are in general agreement with literature values for flavonols and rhamnosides [17–20].

Compounds **2**–**6** were all tested on non-tumoural 3T3 mouse fibroblasts (Table 1). Compounds **2**, **4**, **5** and **6** were all toxic at our standard dose (33 μg/ml), while zerumbone epoxide (**3**) showed no toxicity at this dose. *C. zedoaria* showed a slight HTC-toxicity in its ether extract, which was due to small amounts of the curcuminoids **4**, **5** and **6** [21]. No other extract showed cytotoxic activity in the HTC-system. *C. domestica* was only examined for HTC-toxicity in the crude extracts. The pentane extract showed moderate toxicity and the ether extract high toxicity because of the curcuminoids present [7].

The reddish-yellow coloured fractions from *Z. zerumbet* and *C. domestica*, which showed higher cytotoxicity than the isolated crystalline curcuminoids, were assumed to contain minor unknown curcuminoids [7] with highly cytotoxic properties. It was confirmed by MS that this is indeed the case and that there exist small quantities of such compounds which are of higher mass than the known compounds. In highly cytotoxic fractions, we detected compounds of $M^{+\cdot}$ *m/e* 544 and 586, which we were not able to isolate, but which appear to be curcuminoids, since their fragmentation pattern, *m/e* 177, 150 and 137, was similar to that of curcumin (**4**).



m/e 177

Triferuloylmethane, MW 544, could arise biogenetically by extension of the biosynthesis of curcumin proposed by Geissman and Crout [22], although this could not be proved [23]. Furthermore, the isolation of a natural β-triketone has recently been reported [24].

This led us to synthesize a series of curcumin analogues to try to identify the minor natural products and to attempt to find a structure–cytotoxicity relationship. We prepared dicinnamoylmethane (**9**), tricinnamoylmethane (**11**), diferuloylmethane (**4**), triferuloylmethane (**18**) and diferuloylmethane enol ferulate (**15a**). These and the by-products **12** and **17**, formed by intramolecular Michael addition, were characterized by ^{1}H NMR and ^{13}C NMR. All compounds were highly cytotoxic at 33 μg/ml. Triferuloylmethane was effective even at 8 μg/ml and is the most active pure substance in this series.

It seemed probable that the unsaturated β-diketone (or triketone) group was partly responsible for the cytotoxicity and that the free phenolic group had an additive effect. The MS of triferuloylmethane and diferuloylmethane enol ferulate both showed a M^+ at *m/e* 544, but in spite of the similarities they were not identical with the MS of the natural product of same M^-, which remains unidentified.

The electron impact (EI)-MS of diferuloylmethane enol ferulate (**15a**) was the closest to that of the natural product $M^{+\cdot}$ *m/e* 544. The main difference was the much lower intensity of the $M^{+\cdot}$. A peak *m/e* 368 was shown by a defocussing technique to have no observable parent ion and therefore appears to be itself a $M^{+\cdot}$. Furthermore, it is a parent ion of the base peak at *m/e* 150. This could be explained by thermal decomposition of **15a** to diferuloylmethane (*m/e* 368) before entering the electron source. **15a**, being little volatile, was further shown to give different intensities of the main peaks depending on the speed with which the sample was heated to the final temperature above 200°. Such thermal effects in the mass spectrometer could explain the difficulty of identification of the natural product $M^{+\cdot}$ 544 by comparison of EI-MS. However, even though the unidentified substance is an isomer of the synthetic triferuloylmethane enol ferulate **15a** and certainly closely related, we cannot take their identity as established. Lack of material has made it impossible to pursue this study.

Dicinnamoylmethane and diferuloylmethane were prepared by the procedure of Pabon [25]. Dicinnamoylmethane was treated with sodium hydride in anhydrous DME. The anion formed was treated with two equivalents of cinnamoyl chloride to give the enolic ester of tricinnamoylmethane, which since it is a stronger acid than dicinnamoylmethane, transferred the enolic proton to the weaker acid. The enolic ester **10** was hydrolysed under acidic or basic conditions to tricinnamoylmethane (**11**). Under acidic conditions (trifluoracetic acid), a by-product (**12**) was formed. **12** could be transformed to **11** under basic conditions.

As tricinnamoylmethane and curcumin seemed reasonably stable under basic conditions, the synthesis of triferuloylmethane (**18**) was attempted by protection as the acetate. Phenolic acetates hydrolyse very easily under mild basic conditions.

Diacetyldiferuloylmethane was treated with sodium hydride in anhydrous DME and two equivalents of freshly prepared acetylferuloyl chloride were added. Contrary to the unsubstituted case, some *O*-acylated product (**15**) was formed together with acetylferuloyltriacetyltriferuloylmethane (**14**). **14** was hydrolysed to triacetyltriferuloylmethane (**16**) with formic acid. When trifluoracetic acid was used, some of the by-product **17** was formed.

16 was hydrolysed to triferuloylmethane (**18**) with barium hydroxide in methanol solution and was purified by preparative TLC after acidification.

We recorded ^{13}C NMR spectra of most of the compounds isolated and synthesized. The assignments were based on broad-band proton-decoupled spectra, decoupled spectra and in some cases on selective proton-decoupling (**1**, **2**, **3**, **7**, **7a** and **7b**). The assignments of the curcumin analogues were in part based on the empirical rules for substituted benzenes [26], using in addition the following values for *trans* Ar—CH=CH—COR:

C-1	*ortho*	*meta*	*para*
6.0	−0.4	+0.4	+2.0

These were based on the chemical shifts of cinnamic acid, dicinnamoylmethane and tricinnamoylmethane. These values helped us to assign the chemical shifts of most of the more complex compounds.

Our results show the presence of very cytotoxic compounds in all three investigated drugs, of which one, *C. domestica* (turmeric), is the main constituent of curry powder. None of the compounds have yet been tested *in vivo*, though one, zerumbone epoxide, according to results obtained in our model systems, shows important cytotoxicity against tumour cells, but little activity against normal fibroblasts.

EXPERIMENTAL

The drugs were bought from Coopérative Pharmaceutique Française, Melun. A histological examination of the drugs confirmed the identity of *C. zedoaria* and *C. domestica*. *Z. zerumbet* could not easily be distinguished from *C. zedoaria* by this method. The identity of drugs was further confirmed by comparison of GC–MS data of pentane fractions with those of authentic samples: the MS obtained could, in most cases, be attributed to compounds reported to be present in the essential oil of the drug in question.

Mps were corr. ^{1}H NMR spectra were recorded at 250 MHz with TMS as int. standard. ^{13}C NMR at 62.8 MHz, int. standard TMS. Gated decoupled spectra were run with a pulsing time of 5 μsec and a recovery time of 1 sec. EI-MS: 70 eV, direct inlet. FD-MS: 80°, accelerating voltage 4 KV. GC–MS was carried out on a 2 m × 2 mm i.d. glass column filled with 1% OV 101. Prep. TLC Merck Fertigplatten, thickness 2 or 0.5 mm.

The powdered drugs were extracted with the following solvents: 1 pentane, 2 Et_2O, 3 CH_2Cl_2, 4 MeOH, 5 H_2O. The extracts were tested for HTC-cytotoxicity at 33 μg/ml of culture [1c]. Active extracts were chromatographed on a Si gel column and the fractions tested under the standard conditions. Continuing this procedure, pure, active compounds were isolated.

For all known compounds, physical and spectroscopic data are in accordance with lit. values. In some cases ^{1}H NMR data are given where 250 MHz data give more information than older 60 MHz data.

1. *Z. zerumbet*. From the pentane extract (2.7% dry wt), one inactive (**1**) and two (**2** and **3**) HTC-active compounds were isolated cyclohexane–EtOAc, 9:1). *Humulene* (**1**) [27]. ^{1}H NMR ($CDCl_3$): δ 1.06 (6 H, *s*, 14-H, 15-H), 1.42 (3 H, *s*, 12-H), 1.63 (3 H, *s*, 13-H), 1.9 (2 H, *d*, J = 16 Hz, 1-H), 2.08 (4 H, *br. s*, 4-H, 5-H), 2.5 (2 H, *dd*, J = 7 and 2 Hz, 8-H), 4.87 (1 H, *t*, J = 7 Hz, 2-H), 4.95 (1 H, *br. t*, J = 7 Hz, 6-H), 5.15 (1 H, *d*, J = 16 Hz, 10-H), 5.59 (1 H, *ddd*, J = 16, 8 and 7 Hz, 9-H). *Zerumbone* (**2**). 70% of pentane extract. Recrystallized from heptane, mp 67.5–68.0° [5]. ^{1}H NMR ($CDCl_3$): δ 1.08 (3 H, *s*, 14-H or 15-H), 1.21 (3 H, *s*, 14-H or 15-H), 1.55 (3 H, *s*, 12-H), 1.8 (3 H, *s*, 13-H), 1.9 (1 H, *d*, J = 16 Hz, 1-H), 2.2–2.5 (5 H, *m*, 1-H, 4-H and 5-H), 5.25 (1 H, *br. d*, J = 16 Hz, 2-H), 5.87 (1 H, *d*, J = 16 Hz, 10-H), 5.93 (1 H, *d*, J = 16 Hz, 9-H), 6.02 (1 H, *br. d*, J = 10 Hz, 6-H). ^{13}C NMR: see Table 3.

Table 3. ^{13}C NMR data of compounds **1**–**3** (62.8 MHz, $CDCl_3$, ppm from TMS as internal standard)

Carbon	1	2	3
1	42.1 *t*	42.2 *t*	42.4 *t*
2	125.0 *d*	125.0 *d*	62.6 *d*
3	133.0 *s*	136.1 *s*	61.2 *s*
4	39.8 *t*	39.4 *t*	38.2 *t*
5	23.4 *t*	24.3 *t*	24.6 *t*
6	126.0 *d*	148.5 *d*	147.5 *d*
7	139.0 *s*	137.8 *s*	139.4 *s*
8	40.4 *t*	203.8 *s*	202.6 *s*
9	127.7 *d*	127.1 *d*	128.2 *d*
10	140.9 *d*	160.4 *d*	159.2 *d*
11	37.3 *s*	37.8 *s*	35.9 *s*
12	15.1 *q*	15.2 *q*	15.6 *q*
13	17.9 *q*	11.7 *q*	12.0 *q*
14	27.1 *q*	24.1 *q**	23.5 *q**
15	27.1 *q*	29.4 *q**	29.8 *q**

* Signals may be reversed.

Table 4. ^{13}C NMR data of compounds **4**, **4a** and **16** (62.8 MHz, $CDCl_3$, ppm from TMS as internal standard)

Carbon	4	4a	16
1	101.1, *d*	101.7, *d*	116.6
2,2′	183.3, *s*	183.1, *s*	183.6
3,3′	122.9, *d*	124.4, *d*	121.7
4,4′	140.6, *d*	139.9, *d*	142.6
2″	—	—	193.7
3″	-	-	129.6
4″	-		145.9
1a, 1b	127.8, *s*	134.0, *s*	133.8
2a, 2b	109.7, *d*	111.7, *d*	111.9
3a, 3b	146.8, *s*	151.5, *s*	151.5
4a, 4b	147.9, *s*	141.5, *s*	141.9
5a, 5b	114.9, *d*	123.3, *d*	123.4
6a, 6b	121.9, *d*	121.1, *d*	121.7
1c		-	133.1
2c			111.9
3c			151.6
4c	- -	—	142.1
5c		-	123.4
6c	—	—	121.7
$-O\underline{C}H_3$	56.0, *q*	56.0, *q*	56.0
$-\underline{C}OMe$	—	168.7, *q*	168.7
$-CO\underline{C}H_3$	-	20.6, *q*	20.6

Zerumbone epoxide (**3**). Mp: 96°, $[\alpha]_D^{20}$ 0° [6]. ^{1}H NMR ($CDCl_3$): δ 1.09 (3 H, *s*, 14-H or 15-H), 1.23 (3 H, *s*, 14-H or 15-H), 1.3 (3 H, *s*, 12-H), 1.46 (2 H, *dd*, J = 3 and 11 Hz, 1-H), 1.86 (3 H, *s*, 13-H), 1.94 (1 H, *d*, J = 13 Hz, 5-H), 2.2–2.45 (3 H, *m*, 2 × 4-H and 5-H), 2.75 (1 H, *dd*, J = 11 and 1.5 Hz, 2-H), 6.1 (1 H, *br. d*, J = 10 Hz, 6-H), 6.12 (2 H, *d*, AB system, A ≃ B, $\Delta\nu$ = 0.7 Hz, 9-H and 10-H). ^{13}C NMR: see Table 3. GC MS: The following compounds were identified by comparison with reference samples: α-pinene, camphene, caryophyllene, humulene, caryophyllene epoxide, two humulene epoxides, humulene diepoxide, zerumbone and zerumbone epoxide. Of unidentified products we detected two monoterpenes, M^+ 136 and 4 sesquiterpenes, M^+ 206, 218, 220, 220.

Ether extract. The crude Et_2O extract (1.3%) was highly active (HTC). After column chromatography on Si gel with $CHCl_3$–EtOH (10:1), we isolated **4**, **5** and **6** (highly active) and **7** (less cytotoxic in the HTC system, see Table 1). *Diferuloylmethane* (**4**). 0.06% of crude drug. Mp 182–184°. MS *m/e* (rel. int.): 368 (M^+, 33), 350 (33), 190 (35), 177 (100), 150 (27), 145 (38), 137 (43). *Feruloyl-p-coumaroylmethane* (**5**). 0.03%. Amorphous. MS *m/e* (rel. int.): 338 (M^+, 21). *Di-p-coumaroylmethane* (**6**). 0.03%. Mp 218°. MS *m/e* (rel. int.): 308 (M^+, 35). *Kaempferol-3-α-L-(3,4,O-diacetyl) rhamnopyranoside* (**7**). Slightly yellow, amorphous solid, mp 155°. $[\alpha]_D^{20}$ −208° (c = 0.13, EtOH). Found: C, 58.1; H, 4.7. $C_{25}H_{24}O_{13}$ requires: C, 58.14; H, 4.68%. FD-MS *m/e* (rel. int.): 518 (M^+ + 2, 20), 517 (M^+ + 1, 55), 516 (M^+, 35), 286 (aglycone$^+$, 16), 285 (10), 284 (12), 231 (100), 220 (25), 74 (13), 59 (32), 58 (100). IR ν_{max}^{KBr} cm^{-1}: 3400 (*br.*, OH), 1720–1740 (acetate), 1655 (C=O), 1610 (C=C), 1500, 1450, 1360, 1175. UV λ_{max}^{EtOH} nm (log ε): 266 (4.38), 318 (*sh.*, 4.17), 345 (4.21); + NaOAc: 274 (4.43), 300 (*sh.*, 4.16), 352 (4.16); + NaOH: 276 (4.45), 327 (4.18), 396 (4.42); + $AlCl_3$: 276 (4.36), 303 (4.12), 344 (4.20), 397 (4.10). ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 5. *Kaempferol-3-α-L-rhamnopyranoside* (**7a**) [14] (afzelin) was obtained from **7** by treatment overnight with dil K_2CO_3 in MeOH, acidification with HOAc, washing with a little H_2O, drying and evapn.

Table 5. ^{13}C NMR data of compounds **7**–**8** (62.8 MHz, acetone-d_6, ppm from TMS as internal standard, J(Hz) in parentheses)*

Carbon	7	7a	7b	8(lit.) [10]
2	158.6 *br. s*	158.8 *st* (0.4)	156.5 *br. s*	146.8
3	135.2 *s*	135.9 *s*	138.0 *s*	135.6
4	178.7 *s*	179.3 *s*	173.0 *s*	175.9
4a	105.7 *br. s*	105.9 *br. s*	116.0 *s*	103.1
5	162.9 *br. s*	163.3 *br. s*	151.4 *br. s*	156.2‡
6	99.6 *dd* (163, 3)†	99.6 *dd*(165, 5)	114.8 *dd*(168.6)	98.2
7	164.8 *s*	165.0 *br. s*	155.5 *br. s*	163.9
8	94.5 *dd*(166, 4)	94.6 *dd*(165, 2)	110.0 *dd*(170, 5)	93.5
8a	157.7 *sd*(0, 5)†	158.1 *sd*(0, 4)	157.8 *br. s*	160.7‡
1′	122.1 *st*(0, 8)	122.7 *st*(0, 7)	128.5 *st*(0, 8)	121.7
2′,6′	131.4 *dd*(162, 7)	131.7 *dd*(160, 7)	131.2 *dd*(165, 7)	129.5
3′,5′	116.2 *dd*(161, 4)	116.4 *dd*(160, 3)	123.2 *dd*(166, 4)	115.4
4′	160.8 *st*(0, 8)	160.9 *sm*	154.1 *sm*	159.2
1″	101.7 *d*(179)	102.8 *d*(175)	99.2 *d*(180)	
2″	69.1 *d*(150)	71.6 *d*(148)	70.0 *d*(157)	
3″	72.1 *d*(150, *br.*)	72.3 *d*(140, *br.*)	69.8 *d*(150, *br.*)	
4″	71.1 *d*(150, *br.*)	73.2 *dd*(145, 5)	70.9 *d*(152, *br.*)	
5″	68.9 *d*(150, *br.*)	71.4 *d*(145, *br.*)	69.4 *d*(148, *br.*)	
6″	17.4 *q*(128)	17.8 *q*(127)	17.5 *q*(130)	
$-\underline{C}H_3$	20.6 *q*(129) 20.8 *q*(129)	–	20.6 *q*(128) 21.8 *q*(129)	
$-\underline{C}OMe$	170.3 *sm* 170.7 *sm*	–	170.2 *br. s*, 169.3 *br. s* 170.0 *br. s*, 168.8 *br. s*	

* First letter *s*, *d*, *t* or *q* indicates $^1J_{CH}$ from the gated decoupled spectrum. Second letter the visible long-range couplings $^2J_{CH}$ and $^3J_{CH}$. *br.* means that the peaks are broadened by small long-range couplings.

† According to gated decoupled spectrum in acetone-d_6 + D_2O.

‡ Should probably be reversed.

Recrystallization from EtOH, H_2O, mp 171–173°. $[\alpha]_D^{20}$ −177°. The IR spectrum was identical with that of authentic afzelin. ^1H NMR: see Table 2. ^{13}C NMR: see Table 5. *Hexaacetylafzelin* (**7b**) was obtained by acetylation of **7** with Ac_2O–pyridine. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740–1760 (acetate), 1640 *sh.*, 1624. ^1H NMR: see Table 2. ^{13}C NMR: see Table 5. *Identification of rhamnose by GC.* The sugar moiety of **7** and **7a** was confirmed as rhamnose after methanolysis, trifluoracetylation and GC by the method of ref. [13]. *Kaempferol* (**8**) (from **7** by hydrolysis with TFA EtOAc) Mp 282–284°. EI-MS *m/e* (rel. int.): 287 (M^+ + 1, 18), 286 (M^+, 100), 285 (29), 258 (13), 257 (10), 229 (11), 205 (12), 143 (16), 129 (16), 121 (42). The IR spectrum was identical with that of an authentic sample. ^1H NMR: see Table 2. A minor unidentified product was found in a very HTC-active fraction. MS *m/e* (rel. int.): 586 (M^+, 20), 461 (5), 369 (17), 285 (7), 209 (8), 208 (8), 177 (100), 150 (20), 145 (21), 137 (38). The CH_2Cl_2 and MeOH extracts from *Z. zerumbet* were only slightly active and were not investigated further.

2. *C. zedoaria.* The pentane extract (1.6%) showed no activity (HTC) at the standard dose (33 μg/ml). The GC–MS data are in accord with the composition of the essential oil reported in ref. [28]. We detected camphor, two monoterpenes M^+ 136, four sesquiterpenes M^+ 204, furanodiene and curzerene (M^+ 216), one unknown sesquiterpene M^+ 218 (17%), 135 (85%), 107 (100%), 67 (64%), a sesquiterpene M^+ 220, six compounds M^+ 230, one M^+ 232, three M^+ 234 and one M^+ 246. The Et_2O extract was slightly active due to the presence of small quantities of **4**, **5** and **6** [21]. The CH_2Cl_2 extract (0.3%) and the MeOH extract (1.0%) showed no activity at the standard dose in the HTC system.

3. *C. domestica.* The crude extracts were examined for HTC-cytotoxicity. The activities are given according to the definition in Table 1. Pentane extract (HTC-activity) (1), Et_2O (3) CH_2Cl_2 (1) MeOH (1) H_2O (0). The curcuminoids **4**, **5** and **6** were isolated from the Et_2O extract by prep. TLC (eluant $CHCl_3$–EtOH, 20:1). A minor compound M^+ 544 was also detected by MS in this fraction, EI-MS *m/e* (rel. int.): 544 (M^+, 35), 516 (M^+ − 28, 28), 460 (5), 379 (6), 368 (45), 350 (26), 285 (23), 177 (100), 150 (43), 145 (41), 137 (57), 124 (67), 109 (71), 81 (37). We were not able to isolate enough of this compound for further investigation

4. *Synthesis. Dicinnamoylmethane* (**9**) was prepared from benzaldehyde and acetylacetone by the method of refs. [25, 29]. Yield: 65% of greenish-yellow needles, mp 140–141° ^1H NMR ($CDCl_3$). δ 5.86 (1 H, *s*, 1-H), 6.64 (2 H, *d*, J = 16 Hz, 3-H and 3′-H), 7.67 (2 H, *d*, J = 16 Hz, 4-H and 4′-H) and 7.3–7.6 (10 H, aromatic protons), 15.91 (1 H, *br. s*, enolic proton). ^{13}C NMR: see Table 6. UV λ_{max}^{EtOH} nm (log ε): 392 (4.56), 300 (4.08), 233 (4.06), $\lambda_{max}^{EtOH(1\%HOAc)}$: 392 (4.58), $\lambda_{max}^{EtOH(1\%NaOH)}$: 408 (4.40), 292 (4.35). MS *m/e* (rel. int.): 276 (M^+, 59). *Tricinnamoylmethane enol cinnamate* (**10**). 50% NaH in oil (100 mg) was washed with dry hexane under Ar. **9** (500 mg) was dissolved in dry dimethoxyethane (DME) (10 ml) and added to the NaH. A vigorous reaction followed. The red soln was left for 10 min, then cinnamoyl chloride (600 mg) dissolved in dry DME was added slowly under stirring. Finally a ppt. was formed. After further stirring for 1 hr, HOAc was added until the reaction mixture was acidic. After addition of H_2O and EtOAc, the aq. phase was discarded and the organic phase washed twice with H_2O, dried ($MgSO_4$), evapd and crystallized by adding a little Me_2CO. Yield 820 mg of TLC pure product. Eluant $CHCl_3$–hexane (7:3). Mp 194–195°. Found: C, 82.9; H, 5.3. $C_{37}H_{28}O_4$ requires: C, 82.81; H, 5.26%. ^1H NMR ($CDCl_3$): δ 6.63 (1 H, *d*, J = 16 Hz, 3‴-H), 6.96 (1 H, *d*, J = 16 Hz, 3′-H), 6.99 (1 H, *d*, J = 16 Hz, 3-H), 7.08 (1 H, *d*, J = 16 Hz, 3″-H), 7.6 (1 H, *d*, J

Table 6. ^{13}C NMR data of compounds **9** and **11** (62.8 MHz, $CDCl_3$, ppm from TMS as internal standard, J(Hz) in parentheses)*

Carbon	9	11
1	101.8, *d*(164)	116.8, *s*
2,2′	183.3, *d*(6)	183.5, *br. s*
3,3′	124.1, *d*(157)	121.8, *d*(161)
4,4′	140 5, *d*(155)	143.1, *d*(157)
2″	-	194.2, *s*
3″	—	129.7, *d*(157)
4″		146.5, *d*(155)
1a, 1b	135.0, *d*(1)	135.0, *s*(*br*)
2a,6a,2b,6b	128.1, *dd*(159, 5)	128.6, *d*(159), *br.*
3a,5a,3b,5b	128.9, *dd*(161, 5)	129.0, *d*(163), *br.*
4a, 4b	130.1, *dt*(161, 7)	130.6, *d*(157)
1c	-	134.4, *s*
2c, 6c	-	128.7, *d*(159)
3c, 5c	-	129.1, *d*(163)
4c		131.6, *d*(157)

* Coupling constants from gated proton-decoupled spectra.

= 16 Hz, 4‴-H), 7.68 (1 H, *d*, *J* = 16 Hz, 4′-H), 7.91 (1 H, *d*, *J* = 16 Hz, 4-H) and 7.3-7.6 (21 H, *m*, 4″-H and aromatic protons). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 1735 (ester), 1630, 1615, 1595, 1575, 1495. UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 354 (4.50), 295 (4.64). MS *m/e* (rel. int.): 536 (M^+, 4), 406 (6), 405 (5), 148 (10), 131 (100), 103 (25), 77 (12). *Tricinnamoylmethane* (**11**). **10** (100 mg) in EtOAc was treated with K_2CO_3 soln for a few min. HOAc and toluene were added, the reaction mixture evapd to dryness and the product was purified by prep. TLC with $CHCl_3$–hexane (7:3) as eluant. Crystallization from Me_2CO gave yellow prisms (50 mg), mp 145–6°. Found: C, 82.6; H, 5.4. $C_{28}H_{22}O_3$ requires: C, 82.74; H, 5.46%. ^1H NMR ($CDCl_3$): δ 6.63 (1 H, *d*, *J* = 16 Hz, 3‴-H), 6.85 (2 H, *d*, *J* = 16 Hz, 3-H and 3′-H), 7.11 (1 H, *d*, *J* = 16 Hz, 4‴-H), 7.84 (2 H, *d*, *J* = 16 Hz, 4-H and 4′-H), 7.87 (1 H, *d*, *J* = 16 Hz, 4‴-H) and 7.3 7.6 (15 H, *m*, aromatic protons), 17.63 (1 H, *br. s*, enolic proton). ^{13}C NMR: See Table 6. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1615, 1590, 1575, 1495. UV λ_{max}^{EtOH} nm (log ε): 397 (4.63), 305 (4.46): + NaOH: 384 (4.43), 298 (4.61). MS *m/e* (rel. int.): 406 (M^+, 20), 388 ($M^+ - H_2O$, 9), 315 (10), 301 (12), 206 (21), 131 (100), 103 (65). *Tricinnamoylmethane enol acetate* (**11a**) was obtained by treating **11** overnight with Ac_2O–pyridine. Mp 55°. ^1H NMR ($CDCl_3$): δ 6.93 (2 H, *d*, *J* = 16 Hz, 3-H and 3′-H), 7.86 (2 H, *d*, *J* = 16 Hz, 4-H and 4′-H), 7.3-7.6 (15 H, *m*, aromatic protons); 3″-H and 4″-H gave very *br.*, dispersed peaks. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (ester), 1615, 1595, 1575. 5-*Cinnamoyl*-2-*phenyl*-6-(2″-*phenylvinylene*)-2,3-*dihydro*-4 H-*pyrane*-4-*one* (**12**). When the hydrolysis of **10** was performed under acidic conditions in CH_2Cl_2 with a few drops of TFA, two products, **11** and **12**, were formed which could be separated by prep. TLC with $CHCl_3$ - hexane (7:3). **12** crystallized from Me_2CO, mp 166–167°. Found: C, 82.8; H, 5.4. $C_{28}H_{22}O_3$ requires: C, 82.74; H, 5.46%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1655 (4-C=O), 1615 (5,6-C=C), 1595, 1510, 1490. UV λ_{max}^{EtOH} nm (log ε): 348 (4.52), 301 (4.48): + NaOH: 384 (4.47), 299 (4.63): + NaOH, + HOAc: 396 (4.65), 306 (4.48): see **11**. MS *m/e* (rel. int.): 406 (M^+, 22), 388 (6), 315 (5), 301 (13), 206 (28), 131 (100), 103 (80), 77 (45). ^1H NMR ($CDCl_3$): δ 2.86 (1 H, *dd*, *J* = 16 and 3 Hz, A of ABX system, 3-H), 3.08 (1 H, *dd*, *J* = 16 and 14 Hz, B of ABX system, 3-H), 5.61 (1 H, *dd*, *J* = 14 and 3 Hz, X of ABX system, 2-H), 7.19 (1 H, *d*, *J* = 16 Hz, 3′-H or 3″-H), 7.21 (1 H, *d*, *J* = 16 Hz, 3″-H or 3′-H), 7.6 (1 H, *d*, *J* = 16 Hz, 4′-H or 4″-H), 7.63 (1 H, *d*, *J* = 16 Hz, 4″-H or 4′-H) and 7.3-7.6 (15 H, *m*, aromatic protons). ^{13}C NMR ($CDCl_3$): 43.4 (*t*, 3-C), 80.3 (*d*, 2-C), 118.7 (*s*, 5-C), 119.6 (*d*, 1″-C), 126.3 (*d*, 2′-C), 127.9 (*d*), 128.4 (*d*), 128.6 (*d*), 128.8 (*d*), 128.9 (*d*), 129.0 (*d*), 129.1 (*d*), 130.3 (*d*) aromatic carbons 2a, 6a, 3a, 5a, 4a, 2b, 6b, 3b, 5b, 4b, 2c, 6c, 3c, 5c, 4c), 135.2 (*s*, 1b-C, 1c-C), 137.8 (*s*, 1a-C), 141.1 (*d*, 2″-C), 142.9 (*d*, 3′-C), 170.3 (*s*, 6-C), 190.7 (*s*, 1′-C), 191.6 (*s*, 4-C). *Acetylferulic acid* (**13**) was prepared by the Perkin reaction [30]. Mp: 194–196°. ^1H NMR ($CDCl_3$): δ 2.34 (3 H, *br. s*), $-COCH_3$), 3.88 (3 H, *br. s*, $-OCH_3$), 6.41 (1 H, *d*, *J* = 16 Hz, 3-H), 7.08 (1 H, *d*, *J* = 8 Hz, 5a-H), 7.13 (1 H, *d*, *J* = 2 Hz, 2a-H), 7.15 (1 H, *dd*, *J* = 8 and 2 Hz, 6a-H), 7.75 (1 H, *d*, *J* = 16 Hz, 4-H). *Diferuloylmethane* (**4**) was prepared according to ref. [25]. Mp: 185 186°. Spectroscopic data as for the natural product. *Diacetyldiferuloylmethane* (**4a**) was prepared by a modification of the method of ref. [23]. **4** was dissolved in 10% NaOH containing crushed ice Ac_2O was added slowly until yellow crystals were formed and Ac_2O addition was continued until the crystals aggregated. Recrystallization by dissolving in a small vol. of Me_2CO, adding hot EtOH (50 ml/g **4**) and allowing the soln to cool to give yellow needles, mp 171 172°. ^1H NMR ($CDCl_3$): δ 2.33 (6 H, *s*, $-COCH_3$), 3.88 (6 H, *s*, $-OCH_3$), 5.86 (1 H, *s*, 1-H), 6.57 (2 H, *d*, *J* = 16 Hz, 3-H and 3′-H), 7.06 (2 H, *d*, *J* = 8 Hz, 5a-H and 5b-H), 7.13 (2 H, *d*, *J* = 2 Hz, 2a-H and 2b-H), 7.16 (2 H, *dd*, *J* = 8 and 2 Hz, 6a-H and 6b-H), 7.62 (2 H, *d*, *J* = 16 Hz, 4-H and 4′-H) and 15.87 (1 H, *br. s*, enolic proton). ^{13}C NMR, see Table 4. *Triacetyltriferuloylmethane enol acetylferulate* (**14**). **13** (472 mg) was treated with excess ($COCl_2$) in dry DME under stirring for 2 hr. Excess ($COCl_2$) was removed *in vacuo*. The residue was dissolved in a small vol. of dry DME, which was distilled off to give crystalline acid chloride, mp 134° [30] (lit.: 133°). **4a** (225 mg) in dry DME (10 ml) under argon was treated with a slight excess of NaH. After stirring for 5 min, the freshly prepared acid chloride, dissolved in a little dry DME, was added slowly under stirring, which was continued for 2 hr. The reaction mixture was worked-up as for **10**. It contained mainly **14**, which was purified on a Si gel column using toluene–EtOAc (4:1) as eluant. **14** was isolated as an amorphous, yellow solid (450 mg). ^1H NMR ($CDCl_3$): δ 2.31 (9 H, *s*, 3x-$COCH_3$), 2.34 (3 H, 1x-$COCH_3$), 3.81 (3 H, *s*, $-OCH_3$), 3.84 (3 H, *s*, $-OCH_3$), 3.85 (3 H, *s*, $-OCH_3$), 3.87 (3 H, *s*, $-OCH_3$), 6.56 (1 H, *d*, *J* = 16 Hz, 3‴-H), 6.83 (1 H, *d*, *J* = 16 Hz, 3′-H), 6.90 (1 H, *d*, *J* = 16 Hz, 3-H), 6.96 (1 H, *d*, *J* = 16 Hz, 3″-H), 7.0-7.2 (12 H, *m*, aromatic protons), 7 29 (1 H, *d*, *J* = 16 Hz, 4″-H), 7.63 (1 H, *d*, *J* = 16 Hz, 4′-H), 7.8 (1 H, *d*, *J* = 16 Hz, 4‴-H) and 7.82 (1 H, *d*, *J* = 16 Hz, 4-H). MS: No M^+ in EI- or FD-MS. *Diacetyldiferuloylmethane enol acetylferulate* (**15**) was occasionally obtained as by-product in the preparation of **14**. ^1H NMR ($CDCl_3$): δ 2.33 (3 H, *s*, $-COCH_3$), 2.34 (3 H, *s*, $-COCH_3$), 2.36 (3 H, *s*, $-COCH_3$), 3.88 (3 H, *s*, $-OCH_3$), 3.89 (3 H, *s*, $-OCH_3$), 3.92 (3 H, *s*, $-OCH_3$), 6.39 (1 H, *s*, 1-H), 6.62 (1 H, *d*, *J* = 16 Hz, 3-H), 6.83 (1 H, *d*, *J* = 16 Hz, 3′-H), 7.61 (1 H, *d*, *J* = 16 Mz, 4′-H), 7.88 (1 H, *d*, *J* = 16 Hz, 4-H), 8.18 (1 H, *d*, *J* = 16 Hz, 4″-H) and 7.0 7.25 (10 H, *m*, 3″-H and aromatic protons). *Diferuloylmethane enolferulate* (**15a**) was obtained from **15** by hydrolysis with $BaCO_3$ in MeOH. ^1H NMR ($CDCl_3$): δ 3.94 (6 H, *s*, 2 - OCH_3), 3.95 (3 H, *s*, $-OCH_3$), 5.83 (1 H, *br. s*, $-OH$), 5.89 (1 H, *br. s*), $-OH$, 5.96 (1 H, *br. s*, $-OH$), 6.3 (1 H, *s*, 1-H), 6.59 (1 H, *d*, *J* = 16 Hz, 3-H), 6.75 (1 H, *d*, *J* = 16 Hz, 3′-H), 6.8 7.2 (9 H, *m*, aromatic protons), 7.04 (1 H, *d*, *J* = 16 Hz, 3″-H), 7.59 (1 H, *d*, *J* = 16 Hz, 4′-H), 7.85 (1 H, *d*, *J* = 16 Hz, 4-H), and 8.14 (1 H, *d*, *J* = 16 Hz, 4″-H). EI-MS *m/e* (rel. int.): 544 (M^+, 1.5), 516 ($M^+ - 28$, 1), 410 (10), 368 (21), 350 (14), 272 (17), 205 (38), 177 (55), 150 (100), 137 (48), 135 (76). *Triacetyltriferuloylmethane* (**16**). **14** (110 mg) in EtOAc was treated with a few drops HCO_2H for 1 hr. Toluene was added before evapn at red. pres. and the residue was purified by prep. TLC (toluene–EtOAc, 4:1) to give **16** (60 mg), which was crystallized from EtOH to furnish yellow prisms mp: 158 160° (melted first at

125°, but subsequently became crystalline again). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (acetyl), 1630, 1595, 1585, 1500. UV λ_{max}^{EtOH} nm: 402, 425 (*sh.*), 310. +NaOH: 408, 290 (*br.*); $\lambda_{max}^{CHCl_3}$ nm (log ε): 407 (4.70), +HOAc: 407 (4.72). ^{1}H NMR ($CDCl_3$): δ 2.31 (9 H, *s*, 3 $-COCH_3$), 3.78 (6 H, *s*, 2 $-OCH_3$), 3.82 (3 H, *s*, $-OCH_3$), 6.81 (2 H, *d*, *J* = 16 Hz, 3-H, 3′-H), 7.02 (2 H, *d*, *J* = 8 Hz, 5a-H, 5b-H), 7.04 (1 H, *d*, *J* = 16 Hz, 3″-H), 7.05 (1 H, *d*, *J* = 8 Hz, 5c-H), 7.18 (1 H, *dd*, *J* = 8 and 2 Hz, 5c-H), 7.6 (1 H, *d*, *J* = 16 Hz, 4″-H), 7.8 (2 H, *d*, *J* = 16 Hz, 4-H and 4′-H) and 17.71 (1 H, *br. s*), enolic proton). ^{13}C NMR: see Table 4. 5-(4-*Acetoxy*-3-*methoxy-cinnamoyl*)-2-(4-*acetoxy*-3-*methoxyphenyl*)-6-[2″-(4-*acetoxy*-3-*methoxyphenyl*)*vinylene*]-2,3-*dihydro*-4H-*pyrane*-4-*one* (**17**) was formed as a by-product from **16**, when the hydrolysis was performed with TFA. ^{1}H NMR ($CDCl_3$): δ 2.32 (3 H, *s*, $-COCH_3$), 2.33 (3 H, *s*, $-COCH_3$), 2.36 (3 H, *s*, $-COCH_3$), 2.82 (1 H, *dd*, *J* = 16 and 3 Hz, A of ABX system, 3-H), 3.09 (1 H, *dd*, *J* = 16 and 14 Hz, B of ABX system, 3-H), 3.86 (3 H, *s*, $-OCH_3$), 3.88 (3 H, *s*, $-OCH_3$), 3.90 (3 H, *s*, $-OCH_3$), 5.55 (1 H, *dd*, *J* = 14 and 3 Hz, X of ABX system, 2-H), 7.0–7.25 (11 H, *m*, 2′-H, 1″-H and aromatic protons), 7.59 (1 H, *d*, *J* = 16 Hz, 3′-H or 2″-H), 7.62 (1 H, *d*, *J* = 16 Hz, 2″-H or 3′-H). *Triferuloylmethane* (**18**). **16** (50 mg) in EtOAc was treated with a soln of $Ba(OH)_2$ in MeOH to give a brownish ppt. After stirring for 20 min at room temp., the mixture was acidified with HOAc, washed with H_2O, dried and purified by prep. TLC with $CHCl_3$–EtOH (10:1) to give **18** (20 mg) as an orange-red amorphous solid. MS *m/e* (rel. int.): 544.173 (M^+, 5) ($C_{31}H_{28}O_9$ requires: 544.173), 408 (6), 197 (3), 286 (7), 272 (24), 177 (15), 150 (100), 137 (16), 135 (85), 107 (38), 77 (35), 44 (60). UV λ_{max}^{EtOH} nm (log ε): 434 (4.79), 450 (*sh.*), 365 (*sh.*); +NaOH: 424 (4.86), 250. ^{1}H NMR ($CDCl_3$): δ 3.83 (6 H, *s*, 2 $-OCH_3$), 3.87 (3 H, *s*, $-OCH_3$), 5.88 (2 H, *br. s*, phenolic $-OH$), 5.94 (1 H, *br. s*, phenolic $-OH$), 6.73 (2 H, *d*, *J* = 16 Hz, 3-H and 3′-H), 6.89 (2 H, *d*, *J* = 8 Hz, 5a-H and 5b-H), 6.96 (1 H, *d*, *J* = 8 Hz, 5c-H), 6.96 (2 H, *d*, *J* = 2 Hz, 2a-H, 2b-H), 6.97 (1 H, *d*, *J* = 16 Hz, 3″-H), 7.05 (1 H, *d*, *J* = 2 Hz, 2c-H), 7.09 (2 H, *dd*, *J* = 2 and 8 Hz, 6a-H and 6b-H), 7.15 (1 H, *dd*, *J* = 2 and 8 Hz, 6c-H), 7.57 (1 H, *d*, *J* = 16 Hz, 4″-H), 7.76 (2 H, *d*, *J* = 16 Hz, 4-H and 4′-H) and 17.83 (1 H, *br. s*, enolic proton).

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