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Synthesis of some symmetrical curcumin derivatives and their antiinflammatory activity

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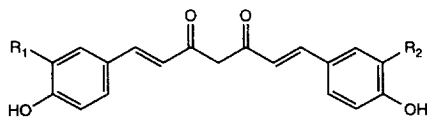
Summary — Curcumin is not only a frequently used food additive, but it is also a well-known constituent of Indonesian traditional medicines. Several beneficial effects are ascribed to curcumin, eg, its antiinflammatory properties. In order to study the antiinflammatory activity, a series of curcumin derivatives were prepared and the inhibition of the carrageenin-induced oedema by these compounds was established. It appeared that the *para* hydroxy groups in curcumin are important for antiinflammatory activity. This activity is enhanced when, in combination with the *para* hydroxy groups, the *meta* positions are occupied with alkyl groups. Since the methyl derivatives are more active than the corresponding ethyl and *tert*-butyl derivatives, it is suggested that sterical hindrance is involved.

curcumin / antiinflammatory activity / carrageenin-induced oedema

Introduction

Several plants, or parts thereof, that grow in Indonesia are used for dyes, cosmetics, spices, daily food or medicines. One of these plants is *Curcuma longa* L, which is known as *kunir* (Javanese) or *kunyit* (Indonesian). Almost all *jamu*-products contain *C longa* L and/or *C xanthorrhiza* Roxb (Javanese *temulawak*). It is known that *C longa* contains curcumin, demethoxycurcumin and bisdemethoxycurcumin, the structures of which are shown in figure 1 [1–3]. *C xanthorriza* contains related compounds, but no bisdemethoxycurcumin [4, 5].

Curcumin was first isolated in 1870. Its chemical structure was determined in 1910 [6], which was subsequently confirmed by synthesis. Thus vanillin and acetylacetone were used in this synthesis which required eight reaction steps. However, the yield is very poor, and therefore this synthesis has little practical value. Pabon [6] mentioned the synthesis of curcumin by heating vanillin, acetylacetone and boric anhydride (2:1:2) over a free flame for 30 min, and claimed a yield of 10% in this one-step procedure. The same author [6] improved this procedure by using tributyl borate and piperidine as catalysts and the freshly prepared complex of acetylacetone and boric anhydride (yield 25%). Furthermore, Pabon synthesized some curcumin derivatives, using vanillin (or benzaldehyde derivatives), tributyl borate, ethyl acetate, the complex of acetylacetone and boric anhydride, and butylamine; this synthesis was carried out at room temperature.



$R_1 = R_2 = OCH_3$	Curcumin
$R_1 = OCH_3$, $R_2 = H$	Demethoxycurcumin
$R_1 = R_2 = H$	Bisdemethoxycurcumin

Fig 1. Chemical structure of the main coloured substances of *C longa* L.

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Curcumin is known to possess various biological activities, such as choleretic, antioxidative, antihepatotoxic, cytotoxic, antiinflammatory and antirheumatic activities [7]. Sodium curcuminate, tetrahydrocurcumin and curcumin have been reported to possess a higher antiinflammatory activity than phenylbutazone [8].

On the basis of the biological activity and the easy synthesis, we used curcumin as a lead compound to design curcumin derivatives with the purpose of examining their antiinflammatory activities.

In this study the synthesis of a series of symmetrical curcumin derivatives and their antiinflammatory activities are described.

Chemistry

The synthesis of curcumin by Pabon was carried out by heating vanillin, acetylacetone and boric anhydride for 30 min over a free flame [6]. The main features in this process (fig 2) are: (a) the protection of the active methylene group by reacting the acetylacetone with boric anhydride in order to produce the acetylacetone–boric anhydride complex (**1**); (b) the less reactive methyl terminals of this complex will react with the aldehyde group of vanillin in order to give curcumin in the form of the complex with boron (**2**); and (c) the complex is then decomposed by using either dilute acids or bases; dilute acid is preferable, since curcumin itself is unstable under alkaline conditions (**3**).

Antiinflammatory activity

Mukhopadhyay et al [8] have studied the antiinflammatory activity of curcumin (C), diacetyl curcumin (DAC), triethyl curcumin (TEC), tetrahydrocurcumin (THC) and phenylbutazone (PB) (fig 3) by using carrageenin-induced rat paw oedema. The curcumin analogues decreased the carrageenin-induced paw oedema at low doses; at higher doses, however, this effect was reversed. So, these curcumin-analogues showed both antiinflammatory (protective) and inflammation-increasing (irritant) effects. The rank of order of potencies of curcumin analogues and PB in the carrageenin-induced inflammation is THC > C > PB > TEC, whereas DAC is devoid of antiinflammatory activity [9].

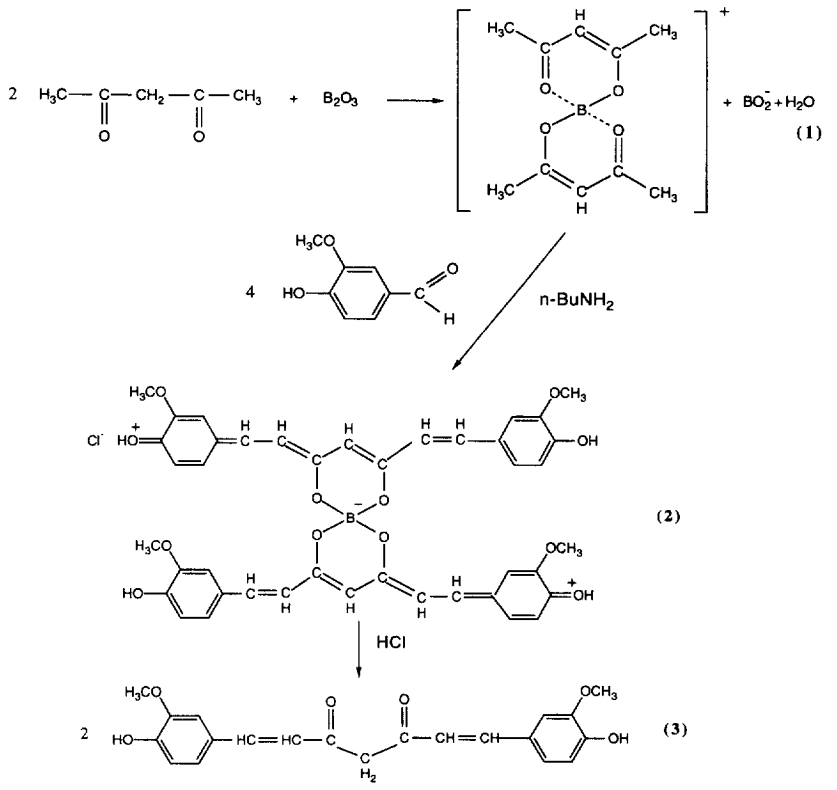


Fig 2. Synthesis of curcumin.

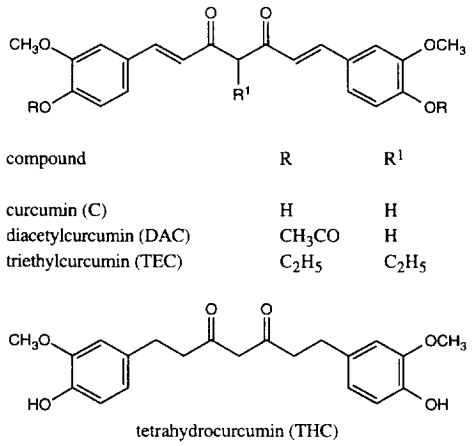


Fig 3. Structure of curcumin analogues with antiinflammatory activity.

The antiinflammatory test we used is based on the inhibition of the carrageenin-induced swelling of the rat paw. The general procedure is to inject a small quantity of a suspension or solution of an oedemogen (eg, carrageenin, kaolin, formalin or mediator) into the plantar tissue of the hind paw of the rat. Assessment of the response is usually made at the time of maximum swelling. Methods for measuring the amount of swelling of the paw include determination of its thickness (by Brownlee), its weight and the volume of water [10] or mercury [11] it displaces.

Results and discussion

According to the pathway depicted in figure 2, in total 16 curcumin analogues were prepared. Some physicochemical properties are summarized in table I, whereas NMR and mass spectral data are included in the *Experimental protocols*. The typical mass fragmentation of curcumin is shown in figure 4.

Out of 16 curcumins, seven show inhibition of the carrageenin-induced oedema. Two hours after treatment with carrageenin the maximum percentage of inhibition was reached in all groups (fig 5); for this reason ED_{50} (table II) was measured after 2 h.

The compound without substituents at the phenyl rings (VUF 9013) showed little inhibition (30% at 80 mg/kg). Substitution at the 4-position of each phenyl ring by hydroxy (VUF 9014), methoxy (VUF 9040) or methyl (VUF 9041) significantly increases the activity over the unsubstituted derivative, whereas the introduction of a chloro atom in the 4-position does not increase the antiinflammatory effect. Probably an electron-donating substituent in the *para* position is favourable for activity. Among the mono-substituted compounds, the hydroxy derivative (VUF 9014) possesses the highest activity (ED_{50} = 73 mg/kg). Moreover, introduction of a methoxy group in the 3- or 2-position instead of the 4-position causes a significant decrease in activity. It may suggest that mono-substitution at *ortho* and *meta* positions is not sufficient for activity.

Substitution of the 4-hydroxy derivative in the 3-position with a methoxy group (VUF 9016, curcumin) leads to an increase in activity (ED_{50} = 38 mg/kg). Introduction of methoxy groups in the 3- and 5-position (VUF 9045) reveals a further increase in activity (ED_{50} = 28 mg/kg). Alkylation in the 3- and 5-position appears to affect the antiinflammatory activity as well. Thus the introduction of methyl groups in these positions affords the most active compound of this series (ED_{50} = 13 mg/kg). Upon introduction of larger alkyl groups such as ethyl (VUF 9020), isopropyl (VUF 9021) the activity decreases, while the corresponding *tert*-butyl derivative (VUF 9043) is inactive.

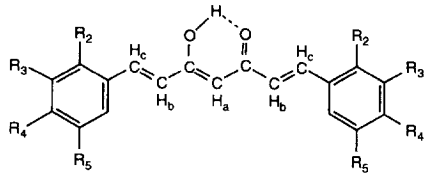
In the series of the 4-methoxy derivatives it is found that introduction of one methoxy group in the 3-position (VUF 9042) increases the activity. However, contrary to what is found in the 4-hydroxy series, here the introduction of an additional methoxy group in the 5-position (VUF 9018) renders the compound completely inactive. The same is found on introduction of benzyloxy groups in the 3- and 4-positions (VUF 9044). Probably sterical reasons may cause this complete loss of activity.

The structure–activity relationships of a series of curcumin analogues show that the presence of olefinic double bonds and 4-hydroxyl groups are important for the antiinflammatory activity of curcumin [8].

Rao et al [13] have studied some other curcumin analogues, viz demethoxycurcumin and bisdemethoxycurcumin (fig 1), which were investigated for their antiinflammatory activity using carrageenin-induced rat paw oedema and compared with sodium curcuminate and phenylbutazone. Demethoxycurcumin was the most potent among the three curcumin analogues. So, besides the olefinic double bonds and the 4-hydroxy groups [8], the presence of one 3-OCH_3 group is also important for the antiinflammatory activity.

In our studies it appeared that the substituents at *meta* positions are important for activity as well. Thus the bigger substituents (*tert*-butyl) lead to inactive compounds, whereas VUF 9019 with 3-methyl groups has the highest activity. The compounds without 4-hydroxy groups have low activity or no longer any activity. These results show that curcumin (VUF 9016) and a number of analogues, viz VUF 9019, VUF 9020 and VUF 9045, are more potent than phenylbutazone in inhibiting the carrageenin-induced rat paw oedema.

Table I. The physical data of the curcumin derivatives.



1,7-bis(*R*-Phenyl)-1,6-heptadiene-3,5-dione (in enol form)

Code VUF	R_2	R_3	R_4	R_5	*Yield (%)*	*Mp (°C)*	*UV (DMSO) λ_{max} (nm)*	*Formula*	*High resolution MS (M^+) found (calcd)*
9013	H	H	H	H	32	140–142[a]	384	$C_{19}H_{16}O_2$	276.1149 (276.1150)
9014	H	H	OH	H	46	209–211	421	$C_{19}H_{16}O_4$	308.1020 (308.1048)
9016	H	OCH_3	OH	H	51	177–178[b]	428	$C_{21}H_{20}O_6$	368.1200 (368.1260)
9017	H	OCH_3	H	H	29	72–74	389	$C_{21}H_{20}O_4$	336.1385 (336.1362)
9018	H	OCH_3	OCH_3	OCH_3	33	108–109	414	$C_{25}H_{28}O_8$	456.1757 (456.1784)
9019	H	CH_3	OH	CH_3	33	185–186	424	$C_{23}H_{24}O_4$	364.1680 (364.1675)
9020	H	C_2H_5	OH	C_2H_5	52	178–179	434	$C_{27}H_{32}O_4$	420.2330 (420.2301)
9021	H	*i*-C_3H_7	OH	*i*-C_3H_7	25	161–162	433	$C_{31}H_{40}O_4$	476.2926 (476.2926)
9022	OCH_3	H	H	H	38	121–122	419	$C_{21}H_{20}O_4$	336.1378 (336.1362)
9039	H	H	Cl	H	8	197–199	332	$C_{19}H_4O_2Cl_2$	344.0364 (344.0371)
9040	H	H	OCH_3	H	25	154–155[c]	398	$C_{21}H_{20}O_4$	336.1368 (336.1362)
9041	H	H	CH_3	H	33	205–206	369	$C_{21}H_{20}O_2$	304.1457 (304.1463)
9042	H	OCH_3	OCH_3	H	40	128–130[d]	427	$C_{23}H_{24}O_6$	396.1606 (396.1573)
9043	H	*t*-C_4H_9	OH	*t*-C_4H_9	28	190–192	423	$C_{33}H_{48}O_4$	532.3566 (532.3553)
9044	H	[e]	[e]	H	44	155–156	419	$C_{47}H_{39}O_6$	–
9045	H	OCH_3	OH	OCH_3	22	188–190	368	$C_{23}H_{24}O_8$	428.1466 (428.1471)

[a]Lit 140.5 °C [6]; [b]lit 176–178 °C [6]; [c]lit 164–165 °C [6]; [d]lit 128–130 °C [12]; [e]$C_6H_5CH_2O$.

Conclusion

Several symmetrically substituted curcumin derivatives were found to possess antiinflammatory activity (carrageenin oedema test). It appeared that some of them have higher activities than curcumin itself. Particularly the combination of 4-hydroxy groups and 3,5-di- (lower) alkyl groups is favourable.

Experimental protocols

Chemistry

Melting points were obtained using a Mettler FP 52 microscope and heating table. NMR spectra were recorded on a Bruker AC 200 and mass spectra were obtained with a Finnigan MAT 90 (San Jose, CA, USA) with EI ionization using 70 eV electrons. All benzaldehydes and reagents were commercially available.

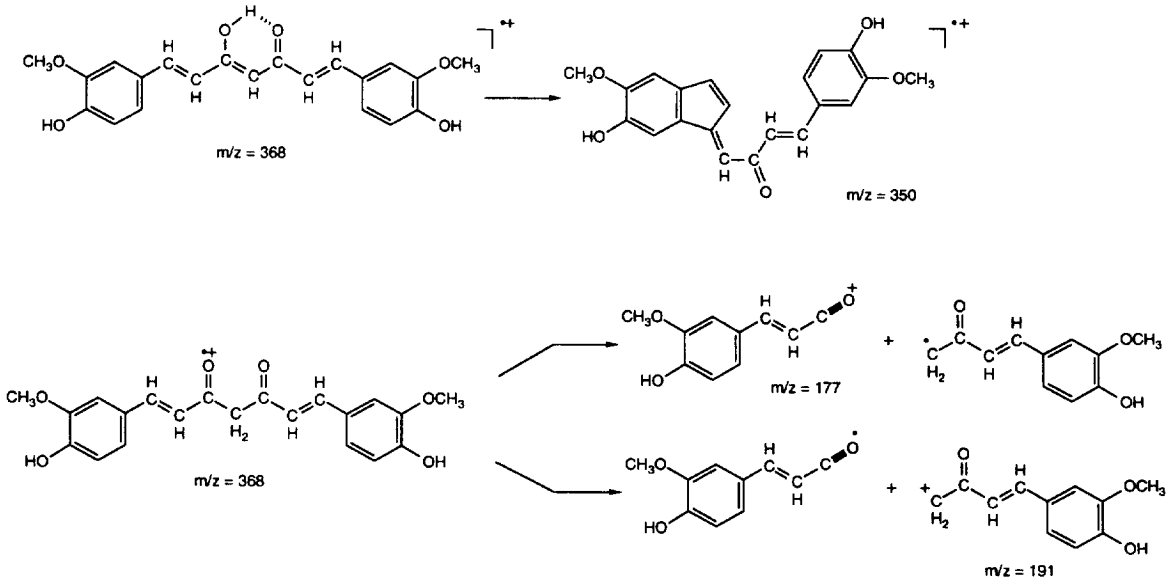


Fig 4. Typical mass fragmentation of curcumin.

General synthesis of curcumin

The appropriate benzaldehyde (0.4 mol) and 210 mL (184 g) of tributyl borate (0.8 mol) were dissolved in 200 mL of dry ethyl acetate. The complex then formed from 20 g of acetylacetone (0.2 mol) and 10 g of boric anhydride (0.14 mol) was added and the reaction mixture was stirred for 5 min. While stirring, 1 mL of butylamine was added dropwise every 10 min (total 4 mL). Stirring was continued for 4 h, after which the mixture was allowed to stand overnight.

The following day, 300 mL of 0.4 N hydrochloric acid (60 °C) was added and the mixture was stirred for 60 min. The organic layers were separated and the aqueous fraction was extracted three times with 100 mL of ethyl acetate. The combined organic layers were washed with water and evaporated to ca 150 mL. Then 100 mL of methanol were added, followed by cooling in the refrigerator for 3 h. The curcumin or its derivative was filtered off, washed with cold methanol and dried. All prepared curcumin derivatives gave one spot on TLC with CH_3OH/H_2O (7:3) as eluent. The physical data of the prepared curcumin derivatives are summarized in table I. The following NMR data and mass spectral data were obtained:

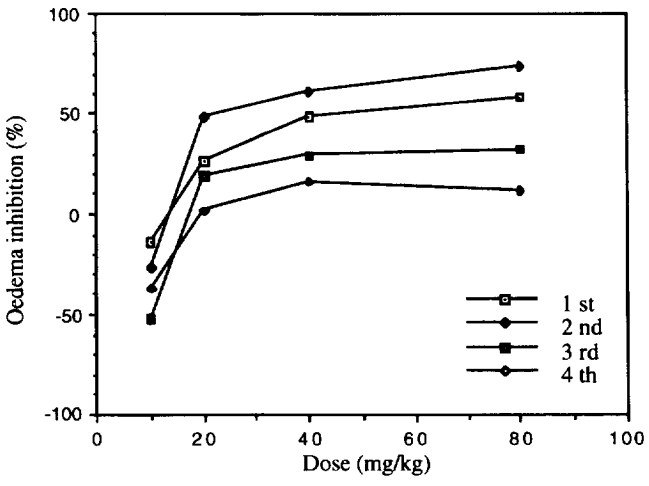


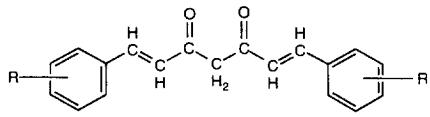
Fig 5. Inhibition of carrageenin-induced rat paw oedema by VUF 9020 measured 1, 2, 3, and 4 h after application of carrageenin. Standard errors are < 4.1%.

1,7-Diphenyl-1,6-heptadiene-3,5-dione (VUF 9013). NMR: (δ, ppm, $CDCl_3$), 5.85 (s, 1H, $=CH_a$), 6.59 (d, J = 13 Hz, 2H, 2 x $-CH_b=CH_c-$), 7.35 (m, 10H, 2 x $-C_6H_5$), 7.63 (d, J = 13 Hz, 2H, 2 x $-CH_c=CH_b-$). Mass spectrum (EI, *m/z*), 276 (M^+, $C_{19}H_{16}O_2$, 100%), 199 ($C_{13}H_{11}O_2$, 15%), 145 ($C_{10}H_9O$, 20%), 131 (C_9H_7O, 48%), 103 (C_8H_7, 30%), 77 (C_6H_5, 18%) mass units.

1,7-bis(4-Hydroxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9014). NMR: (δ, ppm, $CDCl_3$), 2.65 (broad, 2H, 2 x $HO-C_6H_4-$), 5.62 (s, 1H, $=CH_a-$), 6.26 (d, J = 13 Hz, 2H, 2 x $-CH_b-=CH_c-$), 7.25 (AA'BB', 8H, 2 x $-C_6H_4-$), 7.38 (d, J = 13 Hz, 2H, 2 x $-CH_c=CH_b-$). Mass spectrum (EI, *m/z*), 308 (M^+, $C_{19}H_{16}O_4$, 30%), 290 [($M^+ - H_2O$), $C_{19}H_{14}O_3$, 13%], 161 ($C_{10}H_9O_2$, 65%), 119 (C_8H_7O, 30%), 147 ($C_9H_7O_2$, 100%) mass units.

1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9016). NMR: (δ, ppm, $CDCl_3$), 3.95 (s, 6H, 2 x $CH_3OC_6H_3-$), 5.80 (s, 1H, $=CH_a-$), 5.86 (s, 2H, 2 x $HO-C_6H_3-$),

Table II. Oedema inhibiting activity of curcumin and some derivatives.



Code VUF	*R*	*Oedema inhibition (%) on dose (mg/kg; ip)*				*ED_{50}[a] (mg/kg)*
		10	*20*	*40*	*80*	
9013	H	–74 ± 3.3	–46 ± 2.9	–18 ± 2.1	30 ± 2.4	–
9014	4-OH	0.5 ± 0.4	15 ± 1.6	22 ± 2.0	55 ± 4.9	73 ± 5
9016	3-OCH_3-4-OH	12 ± 1.8	29 ± 2.9	52 ± 4.7	44 ± 4.2	38 ± 4[c]
9017	3-OCH_3	–27 ± 3.0	–17 ± 2.1	35 ± 3.1	21 ± 2.3	–
9018	3,4,5-TriOCH_3	–25 ± 2.8	15 ± 2.0	27 ± 2.8	44 ± 4.2	–
9019	3,5-DiCH_3-4-OH	41 ± 3.9	62 ± 4.7	63 ± 4.1	64 ± 5.2	13 ± 2
9020	3,5-DiC_2H_5-4-OH	–27 ± 2.8	48 ± 3.5	61 ± 5.0	74 ± 5.7	22 ± 6
9021	3,5-Di-*i*-C_3H_7-4-OH	–15 ± 1.8	20 ± 1.9	47 ± 3.8	55 ± 4.8	58 ± 21
9022	2-OCH_3	–109 ± 8.5	–77 ± 6.4	–37 ± 2.6	5 ± 1.8	–
9039	4-Cl	–34 ± 3.0	–6 ± 1.5	16 ± 2.1	28 ± 2.6	–
9040	4-OCH_3	–32 ± 2.5	–5 ± 1.8	28 ± 2.5	49 ± 3.7	82 ± 7
9041	4-CH_3	7 ± 1.5	30 ± 2.9	41 ± 3.6	50 ± 4.1	80 ± 18
9042	3,4-DiOCH_3	18 ± 1.6	40 ± 3.2	48 ± 3.8	55 ± 4.0	50 ± 22
9043	3,5-Di-*t*-C_4H_9-4-OH	–9 ± 1.8	18 ± 1.6	28 ± 2.5	23 ± 2.0	–
9044	3,4-Di$C_6H_5CH_2O$	10 ± 1.5	28 ± 2.2	44 ± 3.1	47 ± 3.8	–
9045	3,5-DiOCH_3-4-OH	34 ± 2.6	42 ± 3.4	58 ± 4.3	67 ± 4.6	28 ± 5
	Phenylbutazone	14 ± 2.3	26 ± 2.0	50 ± 4.2	67 ± 3.9	43 ± 10[d]
	Indomethacin	22 ± 1.8 (1[b])	55 ± 3.9 (5[b])	85 ± 3.7 (10[b])		3 ± 0.4

[a]ED_{50} = effective dose 50%; [b]dose of indomethacin (mg/kg); [c]lit ED_{50} = 36 mg/kg [8]; [d]lit ED_{50} = 48 mg/kg [8]. Negative values indicate that the compound did not afford any protection; on the contrary, it contributed to the inflammatory reaction.

6.43 (d, J = 13 Hz, 2H, =CH_b=CH_c-), 6.91 (m, 6H, 2 x -C_6H_3-), 7.55 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 368 (M^+, $C_{21}H_{20}O_6$, 100%), 350 [(M^+ – H_2O), $C_{21}H_{18}O_5$, 69%], 191 ($C_{11}H_{11}O_3$, 40%), 177 ($C_{10}H_9O_3$, 60%) mass units.

1,7-bis(3-Methoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9017). NMR: (δ, ppm, $CDCl_3$), 3.85 (s, 6H, 2 x $CH_3OC_6H_4$-), 5.86 (s, 1H, =CH_a-), 6.56 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_a-), 6.90 (m, 8H, 2 x -C_6H_4-), 7.60 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 336 (M^+, $C_{21}H_{20}O_4$, 100%), 318 [(M^+ – H_2O), $C_{21}H_{18}O_3$], 175 ($C_{11}H_{11}O_2$, 40%), 161 ($C_8H_2O_4$, 60%), 133 (C_9H_9O, 30%) mass units.

1,7-bis(3,4,5-Trimethoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9018). NMR: (δ, ppm, $CDCl_3$), 3.89 (s, 6H, 2 x $CH_3OC_6H_2$-), 3.93 (s, 12H, 2 x $(CH_3O)_2C_6H_2$-), 5.86 (s, 1H, =CH_a-), 6.50 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 6.78 (s, 4H,

2 x -C_6H_2-), 7.60 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 456 (M^+, $C_{25}H_{28}O_8$, 100%), 234 ($C_9H_{14}O_7$, 77%), 181 ($C_{10}H_{13}O_3$, 63%), 161 ($C_6H_9O_5$, 21%) mass units.

1,7-bis(4-Hydroxy-3,5-dimethylphenyl)-1,6-heptadiene-3,5-dione (VUF 9019). NMR: (δ, ppm, $CDCl_3$), 2.24 (s, 12H, 2 x (CH_3)$_2$-C_6H_2-), 4.91 (broad, 2H, 2 x HO-C_6H_2), 5.73 (s, 1H, =CH_a-), 6.40 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 7.19 (s, 4H, 2 x -C_6H_2-), 7.48 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 364 (M^+, $C_{23}H_{24}O_{41}$, 48%), 346 [(M^+ – H_2O), $C_{23}H_{22}O_3$, 45%], 268 ($C_{18}H_{20}O_2$, 26%), 189 ($C_{12}H_{13}O_2$, 38%), 175 ($C_{11}H_{11}O$, 40%), 135 ($C_9H_{11}O$, 40%) mass units.

1,7-bis(3,5-Diethyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9020). NMR: (δ, ppm, $CDCl_3$), 1.25 (t, 12H, 2 x (CH_3-CH_2)$_2$-C_6H_2-), 2.64 (q, 8H, 2 x (CH_3-CH_2)$_2$-C_6H_2), 4.98 (broad, 2H, 2 x HO-C_6H_2-), 5.78 (s, 1H, =CH_a-), 6.43 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 7.21 (s, 4H, 2 x -C_6H_2-), 7.53 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 420 (M^+, $C_{27}H_{32}O_4$, 43%), 402 [(M^+ – H_2O), $C_{27}H_{30}O_3$, 77%], 175 ($C_{11}H_{11}O_2$, 42%), 147 ($C_{10}H_{11}O_4$, 20%) mass units.

1,7-bis(4-Hydroxy-3,5-diisopropylphenyl)-1,6-heptadiene-3,5-dione (VUF 9021). NMR: (δ, ppm, $CDCl_3$), 1.28 (d, J = 13 Hz, 24H, 2 x {(CH_3)$_2$CH}$_2$-C_6H_2), 3.15 (m, 4H, 2 x (CH_3)$_2$$CH$-), 5.09 (broad, 2H, 2 x HO-C_6H_2-), 5.86 (s, 1H, =CH_a-), 6.47 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 7.28 (s, 4H, 2 x -C_6H_2-), 7.58 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 476 (M^+, $C_{31}H_{40}O_4$, 42%), 458 [(M^+ – H_2O), $C_{31}H_{38}O_3$, 70%], 231 ($C_{15}H_{19}O_2$, 47%), 189 ($C_{12}H_{13}O_2$, 52%) mass units.

1,7-bis(2-Methoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9022). NMR: (δ, ppm, $CDCl_3$), 3.90 (s, 6H, 2 x $CH_3OC_6H_4$-), 5.87 (s, 1H, =CH_a-), 6.68 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 6.90 (m, 8H, 2 x -C_6H_4-), 7.94 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 336 (M^+, $C_{21}H_{20}O_4$, 83%), 318 [(M^+ – H_2O), $C_{21}H_{18}O_3$, 6%], 175 ($C_{11}H_{11}O_2$, 28%), 161 ($C_{10}H_9O_2$, 100%), 121 (C_8H_9O, 47%), 91 (C_7H_7, 32%) mass units.

1,7-bis(4-Chlorophenyl)-1,6-heptadiene-3,5-dione (VUF 9039). NMR: (δ, ppm, $CDCl_3$), 5.82 (s, 1H, =CH_a-), 6.55 (d, J = 13 Hz, 2H, 2 x -CH_b-=CH_c-), 7.34 (AA'BB', 8H, 2 x -C_6H_4-), 7.57 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 346 [(M + 2)$^+$, $C_{19}H_{14}O_2{}^{35}Cl^{37}Cl$, 54%], 344 ($M^+$, $C_{19}H_{14}O_2Cl_2$, 85%), 222 ($C_{12}H_{11}O_2Cl$, 47%), 165 (C_9H_6OCl, 100%) mass units.

1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9040). NMR: (δ, ppm, $CDCl_3$), 3.84 (s, 6H, 2 x $CH_3OC_6H_4$), 5.78 (s, 1H, =CH_a-), 6.45 (d, J = 13 Hz, 2H, 2 x -CH_b-=CH_c-), 6.89 (AA'BB', 8H, 2 x -C_6H_4-), 7.58 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectra (EI, *m/z*), 336 (M^+, $C_{21}H_{20}O_4$, 69%), 318 [(M^+ – H_2O), $C_{21}H_{18}O_3$, 47%], 240 ($C_{16}H_6O_2$, 28%), 175 ($C_{11}H_{11}O_2$, 61%), 161 ($C_{10}H_9O_2$, 100%).

1,7-bis(4-Methylphenyl)-1,6-heptadiene-3,5-dione (VUF 9041). NMR: (δ, ppm, $CDCl_3$), 2.38 (s, 6H, 2 x $CH_3C_6H_4$-), 5.82 (s, 1H, =CH_a-), 6.54 (d, J = 13 Hz, 2H, 2 x -CH_b-=CH_c-), 7.18 (AA'BB', 8H, 2 x -C_6H_4-), 7.60 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectra (EI, *m/z*), 304 (M^+, $C_{21}H_{20}O_2$, 100%), 159 ($C_{11}H_{11}O$, 61%), 115 (C_9H_7, 42%) mass units.

1,7-bis(3,4-Dimethoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9042). NMR: (δ, ppm, $CDCl_3$), 3.92 (s, 6H, 2 x $CH_3OC_6H_3$-), 3.93 (s, 6H, 2 x $CH_3OC_6H_3$-), 5.82 (s, 1H, =CH_a-), 6.45 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 6.86 (m, 6H, 2 x -C_6H_3-), 7.57 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 396 (M^+, $C_{23}H_{24}O_6$, 97%), 378 [(M^+ – H_2O), $C_{23}H_{22}O_5$], 204 ($C_{12}H_{12}O_3$, 56%) mass units.

1,7-bis(3,5-Di-tert-butyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9021). NMR: (δ, ppm, $CDCl_3$), 1.46 (s, 36H, 2 x [((CH_3)$_3$C)$_2$$C_6H_2$-], 5.86 (s, 1H, =$CH_a$-), 5.51 (s, 2H, 2 x HO-C_6H_2), 6.44 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 7.40 (s, 4H, 2 x -C_6H_2-), 7.57 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 532 (M^+, $C_{35}H_{48}O_4$, 28%), 436 ($C_{30}H_{44}O_2$, 100%), 301 ($C_{19}H_{25}O_3$, 14%), 259 ($C_{17}H_{23}O_2$, 33%), 219 ($C_{15}H_{23}O$, 19%) mass units.

1,7-bis(3,4-Dibenzyloxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9044). NMR: (δ, ppm, $CDCl_3$), 5.19 [d, 8H, 2 x ($C_6H_5CH_2O$-)$_2$], 5.75 (s, 1H, =CH_a-), 6.37 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 6.90 [m, 26H, 2 x ($C_6H_5CH_2O$-)$_2$-C_6H_3], 7.49 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 700 (M^+, $C_{47}H_{39}O_6$, 60%), 701 [(M + H)$^+$, 100%], 718 [(M + NH_4)$^+$, 60%] mass units.

1,7-bis(4-Hydroxy-3,5-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (VUF 9045). NMR: (δ, ppm, $CDCl_3$), 3.93 (s, 12H, 2 x [(CH_3O)$_2$$C_6H_2$-], 5.82 (s, 1H, =$CH_a$-; s, 2H 2 x HOC_6H_2-), 6.43 (d, J = 13 Hz, 2H, 2 x -CH_b=CH_c-), 6.78 (s, 4H, 2 x -C_6H_2-), 7.52 (d, J = 13 Hz, 2H, 2 x -CH_c=CH_b-). Mass spectrum (EI, *m/z*), 428 (M^+, $C_{23}H_{24}O_8$, 10%), 222 ($C_{12}H_{14}O_4$, 100%), 207 ($C_{11}H_{11}O_4$, 75%), 180 ($C_{10}H_{12}O_3$, 69%) mass units.

Inhibition of oedema

Materials

Phenylbutazone, carrageenin and amylum were purchased from Farmitalia, Carlo Erba (Milan, Italy), indomethacin was obtained from Sigma Chemical Co (Poole, Dorset, UK). Male Wistar rats (160–200 g) were obtained from Harlan, CPB, Zeist, The Netherlands.

Methods

For each compound in this experiment 30 rats were used. The 30 rats were divided into five groups: one group as control and four groups were given graded doses of the test compounds. To the four groups of rats the test compounds were given intraperitoneally as 1% suspension in 1% amylum [14], with graded doses of 10, 20, 40 and 80 mg/kg body weight, and to the control group 1% amylum solution was given.

One hour later each rat was subcutaneously injected with 0.05 mL of 1% carrageenin suspension in a physiological salt solution into the plantar tissue of one hind paw [11].

The oedema volume was measured by inserting the rat hind paw into a mercury bath. The mercury pushed a rhodamine B solution into the volume pipette and so the volume of mercury that was displaced by the hind paw could be measured. The volume of the paw was observed every hour, starting from zero hour – this being immediately after the given carrageenin suspension until the fourth hour [15]. The difference of oedema volume after treatment was compared with the difference in the control group at the corresponding time-point. The inhibition of oedema volume 2 h after the carrageenin injection was used to calculate the ED_{50} being the calculated dose which should provide 50% inhibition, with the probit analysis program.

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