Non-Phenolic Linear Diarylheptanoids from *Curcuma xanthorrhiza*: A Novel Type of Topical Anti-Inflammatory Agents: Structure-Activity Relationship

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Abstract: **The topical anti-inflammatory activity of three non-phenolic linear 1,7-diarylheptanoids, previously isolated from a Thai medicinal plant, *Curcuma xanthorrhiza* (Zingiberaceae) and four new semi-synthetic derivatives of the naturally occurring compounds were assessed in the murine model of ethyl phenylpropiolate-induced ear edema. The naturally occurring compound 1*E*,3*E*,1,7-diphenylheptadien-5-one (6) exerted the most potent anti-inflammatory activity, with an ID_{50} value of similar magnitude to that of the reference drug oxyphenbutazone (67 vs. 46 μg/ear, respectively). None of the semi-synthetic diarylheptanoids was more active than 6. The chemical structures and pharmacological data of the natural and semi-synthetic derivatives identified a distinct structure-activity relationship. The degree of unsaturation in positions 1 and 3, and the nature of the oxygenated functional group in position 5 of the C_7-chain were found to play significant roles in determining the observed *in vivo* activity. Based on these findings, the non-phenolic linear 1,7-diarylheptanoids are proposed to represent a novel class of topical anti-inflammatory agents.**

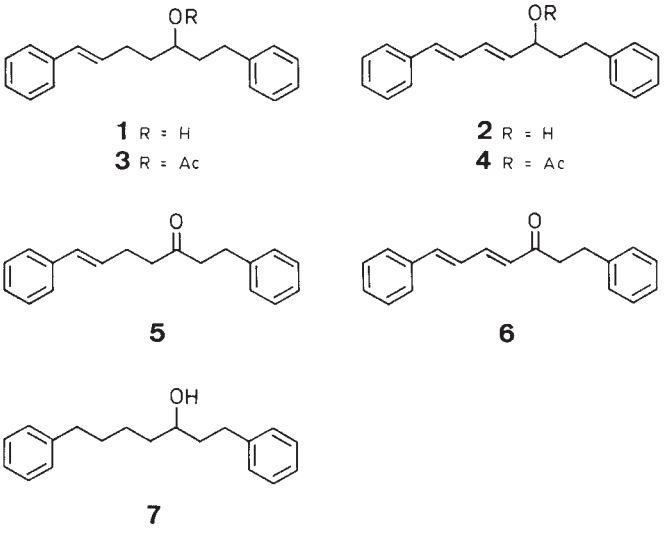
Key words: *Curcuma xanthorrhiza* Roxb., Zingiberaceae, diarylheptanoids, topical anti-inflammatory activity, structure-activity relationship.

Introduction

Major research efforts (1–4) to find new therapeutic agents for a variety of inflammatory skin diseases are motivated mainly by the medical need to find drugs with fewer side effects than the ones currently employed. The numerous, and sometimes serious, adverse effects of e.g. topically applied glucocorticoids are well-known (5). Murine models of artificially induced skin inflammation, similar to the model employed in this study, have been used in the search for drugs for inflammatory skin diseases (1, 2, 4, 6). Some potentially useful drug candidates for treatment of the chronic inflammatory skin disease psoriasis have been identified using this approach (1, 4).

The murine models of skin inflammation are furthermore useful as screening tools for agents with oral anti-inflammatory activity (7). The diarylheptanoid compounds (8) investigated in this study may be of some interest in this aspect, due to the absence of phenolic and other acidic functional groups, which partly have been associated with the ulcerogenic, gastrointestinal side effects common to virtually all clinically used non-steroidal anti-inflammatory drugs (9).

In the search for novel therapeutic agents, plant derived substances have become increasingly important (10, 11). *Curcuma xanthorrhiza* Roxb. (Zingiberaceae) is a medicinal plant widely spread in South East Asia. In Thai traditional medicine it is commonly used externally for the treatment of acne and skin inflammations. As a result of a bioassay guided fractionation of a *n*-hexane extract of the rhizomes of this plant, we recently isolated and identified the three closely related non-phenolic linear diarylheptanoids **1**, **2**, and **6** (12). The same investigation also indicated that the three compounds exerted low-potent, but significant, anti-inflammatory activity in the assay of carrageenin-induced hind paw edema in rats. In a continuation of this study, we have prepared and spectroscopically characterized four new semi-synthetic derivatives (**3**–**5**, **7**), and assessed the topical anti-inflammatory activities of all seven compounds in the assay of ethyl phenylpropiolate-induced ear edema in rats. The present paper describes the structure-activity relationship of these compounds.



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Materials and Methods

Chemistry

The instrumentation and medium pressure liquid chromatography (MPLC) employed were as described previously (12). The purity of compounds isolated was verified by TLC (ethyl acetate : hexane, 2 : 8), using precoated fluorescent silica gel plates (Merck), in addition to high resolution MS and/or elemental analysis.

Compounds **1** (1*E*-1,7-diphenylhepten-5-ol), **2** (1*E*,3*E*-1,7-diphenylheptadien-5-ol), and **6** (1*E*,3*E*-1,7-diphenylheptadien-5-one) were isolated from an *n*-hexane extract of the rhizomes of *C. xanthorrhiza*, by flash chromatography and MPLC; and the spectral data of these compounds were identical in all aspects to the ones previously reported (12).

The semi-synthetic derivatives **3**–**5** and **7** were prepared from chromatographic fractions of the plant extract, highly enriched in the corresponding starting materials (> 80 %). Thus no yields of the reactions are reported. All reactions were carried out at room temperature and on a 2 g scale.

Compounds **3** and **4** were prepared by treatment of **1** and **2**, respectively, with excess acetic anhydride in the presence of catalytic amounts of 4-dimethylaminopyridine. Pure **3** and **4** were obtained by MPLC. The following conditions were used: column size: 2.6 × 23 cm; silica gel Si 60, 40–63 μm (Merck); flow rate 50 ml/min; combined isocratic and gradient elution, consisting of 3 segments [quoted as % ethyl acetate in hexane at the beginning and at the end of the segment, and (in brackets), the duration of each segment]: 1) 0 %–0 % (20 min), 2) 0 %–40 % (40 min), 3) 40 %–40 % (30 min).

1E-5-Acetoxy-1,7-diphenylheptene (**3**). Pale yellow liquid; $[\alpha]_D$: − 3.8° ($CHCl_3$; *c*, 1.2); UV: λ_{max} (log ε) = 251 nm (4.31); IR: ν_{max} = 3030, 2930, 1725, 1600, 1495, 1445, 1370, 1220, 1010, 955, 735, 685 cm^{-1}; ^{1}H-NMR: δ = 1.75 (2H, m, H-4), 1.90 (2H, m, H-6), 2.04 (3H, s, OAc), 2.24 (2H, m, H-3), 2.64 (2H, m, H-7), 5.00 (1H, m, H-5), 6.18 (1H, dt, *J* = 15.9, 6.8 Hz, H-2), 6.37 (1H, d, *J* = 15.9 Hz, H-1), 7.2 (10 H, m, arom. H); EI-MS *m/z* (rel. int. %) = 308 ([M$^+$], 2), 248 (67), 157 (32), 144 (100), 129 (64), 117 (42), 91 (73), 43 (38); HR-MS: Calculated for $C_{21}H_{24}O_2$: 308.1775. Found: 308.1785.

1E,3E-5-Acetoxy-1,7-diphenylheptadiene (**4**). Pale yellow liquid; $[\alpha]_D$: − 1.5° ($CHCl_3$; *c*, 1.9); UV: λ_{max} (log ε) = 309 sh (4.17), 288 (4.54), 280 sh (4.53), 233 sh (4.19), 226 sh nm (4.34); IR: ν_{max} = 3330, 2920, 1725, 1600, 1495, 1447, 1362, 1225, 1005, 978, 738, 685 nm; ^{1}H-NMR: δ = 1.96–2.05 (2H, m, H-6), 2.07 (3H, s, OAc), 2.67 (2H, m, H-7), 5.36 (1H, apparent q, *J* = 7.0 Hz, H-5), 5.75 (1H, dd, *J* = 15.4, 7.3 Hz, H-4), 6.42 (1H, dd, *J* = 15.4, 10.4 Hz, H-3), 6.58 (1H, d, *J* = 15.7 Hz, H-1), 6.74 (1H, dd, *J* = 15.7, 10.4 Hz, H-2), 7.3 (10 H, m, arom. H); EI-MS *m/z* (rel. int. %) = 306 ([M$^+$], 0.6), 246 (58), 155 (100), 142 (24), 129 (15), 115 (17), 91 (25), 77 (10), 60 (8), 43 (10); HR-MS: Calculated for $C_{21}H_{22}O_2$: 306.1619. Found: 306.1612.

Compound **1** was oxidised with excess pyridinium dichromate (molar ratio 1 : 3) in the presence of molecular sieves (3 Å) in dichloromethane (13). Filtration through a short column of silica gel (Si 60, 40–63 μm) eluted by diethyl ether followed by further purification by MPLC [conditions as for **3** and **4**, but with the following gradient program: 1) 0 %–0 % (15 min), 2) 0 %–5 % (30 min), 3) 5 %–5 % (30 min), 4) 5 %–10 % (30 min)] afforded 1*E*-1,7-diphenylhepten-5-one (**5**). Colourless liquid; UV: λ_{max} (log ε) = 251 nm (4.32); IR: ν_{max} = 3028, 3020, 1713, 1496, 1454, 1369, 1221, 966 cm^{-1}: ^{1}H-NMR: δ = 2.47 (2H, m, H-3), 2.55 (2H, m, H-4), 2.74 (2H, t, *J* = 7.2 Hz, H-6), 2.90 (2H, t, *J* = 7.2 Hz, H-7), 6.15 (1H, dt, *J* = 16.0, 6.8 Hz, H-2), 6.38 (1H, d, *J* = 16.0 Hz, H-1), 7.3 (10H, m, arom. H); EI-MS *m/z* (rel. int. %): 264 ([M]$^+$, 11), 146 (10), 130 (18), 117 (20), 105 (62), 91 (100), 83 (8), 77 (19), 65 (10), 51 (8), 43 (7), 32 (89), HR-MS: Calculated for $C_{19}H_{20}O$: 264.1512. Found: 264.1549.

Catalytic hydrogenation (H_2, room temperature, atmospheric pressure, 10 % Pd on charcoal) of **1** in ethyl acetate, followed by filtration through Celite and recrystallization (hexane) yielded 1,7-diphenylheptan-3-ol (**7**). White needles, m.p. 61.5–62 °C; $[\alpha]_D$: − 6.5° ($CHCl_3$; *c*, 1.2); UV: λ_{max} (log ε) = 254 (2.65), 260 (2.71), 269 nm (2.58); IR: ν_{max} = 3600, 3500, 3000, 2940, 2850, 1603, 1498, 1455, 1025, 695 cm^{-1}: ^{1}H-NMR: δ = 1.37 (1H, m, H-5a), 1.48 (1H, m, H-4a), 1.49 (1H, m, H-5b), 1.51 (1H, m, H-4b), 1.63 (2H, m, H-6), 1.73 (1H, m, H-2a), 1.77 (1H, m, H-2b), 2.61 (2H, t, $J_{7,6a}$ = $J_{7,6b}$ = 7.5 Hz, H-7), 2.66 (1H, ddd, $J_{1a,1b}$ = 13.5 Hz, $J_{1a,2a}$ = 9.5 Hz, $J_{1a,2b}$ = 6.7 Hz, H-1a), 2.78 (1H, ddd, $J_{1a,1b}$ = 13.5 Hz, $J_{1b,2a}$ = 9.5 Hz, $J_{1b,2b}$ = 5.5 Hz, H-1b), 3.66 (1H, m, H-3), 7.3 (10H, m, arom. H); EI-MS: *m/z* (rel. int. %) = 250 ([M − H_2O]$^+$, 12), 159 (4), 145 (4), 131 (6), 117 (25), 104 (56), 91 (100), 77 (11), 65 (16), 51 (6), 40 (82); Elemental analysis: Calculated for $C_{19}H_{24}O$: C, 85.02; H, 9.01. Found: C, 84.82; H, 9.00.

Pharmacology

Topical anti-inflammatory activity in rats was assessed by a slightly modified version of the method of Brattsand et al. (14). Male Sprague-Dawley rats (National Laboratory Animal Center, Salaya, Thailand), with a body weight of 50–70 g, were used. They were maintained on a standard pellet diet and water ad libitum. All experiments were performed in an air conditioned laboratory (26 ± 1 °C). The inflammogen ethyl phenylpropiolate (EPP) was dissolved in acetone of analytical grade and ear edema was induced by application of 1 mg EPP/ear in a volume of 10 μl to the inner and outer surfaces of both ears (20 μl/ear). The test substances (20 μl/ear) were administered topically concomitantly with the inflammogen in the same acetone solution, which was prepared immediately before use. Before, and 30 min and 1 h after edema induction, the thickness of each ear was measured with a pair of callipers (Mitutoyo, Japan). The individual gain of ear thickness was calculated and considered as edema. The increase in ear thickness was compared to the vehicle (acetone) treated group and the percent inhibition calculated. The ID_{30} and ID_{50} values were determined 30 min and 1 h after edema induction. Oxyphenbutazone dissolved in acetone was used as a positive reference drug.

Statistical analysis

The results of the ear edema assay are expressed as mean values ± SEM. Student's t-test for unpaired samples (2-tailed) was used to determine statistical significance, which was accepted when $p < 0.01$. Linear regression analysis was used to calculate ID_{30} and ID_{50} values.

Results

The naturally occurring diarylheptanoids **1**, **2**, and **6** were isolated from *C. xanthorrhiza* as previously described (12). The semi-synthetic derivatives **3**–**5** and **7** were prepared by well-known synthetic methods. All compounds exhibited UV, IR, NMR, and mass spectral data consistent with the proposed structures.

Table 1 Effects of topically applied diarylheptanoids and oxyphenbutazone on ethyl phenylpropiolate-induced ear edema in rats.

Treatment Compound No.	Dose (μg/ear)	Edema thickness (μm) — 30 min	1 h	Inhibition (%) — 30 min	1 h
	Vehicle	170 ± 10	201 ± 10	–	–
1	0.1	145 ± 6	161 ± 7*	15	20
	1	137 ± 7	155 ± 8*	19	23
	10	125 ± 6*	143 ± 5*	26	29
	100	112 ± 5*	137 ± 4*	34	32
	1000	104 ± 7*	136 ± 9*	39	32
	Vehicle	181 ± 8	209 ± 10	–	–
2	0.1	136 ± 8*	166 ± 13	25	20
	1	136 ± 12*	144 ± 13*	25	31
	10	100 ± 5*	127 ± 4*	45	39
	100	99 ± 5*	122 ± 4*	45	42
	1000	72 ± 5*	93 ± 6*	60	55
	Vehicle	164 ± 11	210 ± 13	–	–
3	0.1	184 ± 8	205 ± 11	−12	2
	1	175 ± 8	216 ± 10	− 7	−3
	10	152 ± 6	190 ± 6	7	9
	100	141 ± 8	162 ± 7*	14	23
	1000	126 ± 3*	162 ± 5*	23	23
	Vehicle	178 ± 11	211 ± 12	–	–
4	0.1	154 ± 6	169 ± 5*	13	20
	1	135 ± 8*	158 ± 10*	24	25
	10	131 ± 4*	157 ± 6*	26	25
	100	122 ± 8*	146 ± 6*	31	31
	1000	78 ± 5*	112 ± 7*	56	47
	Vehicle	177 ± 6	224 ± 8	–	–
5	0.1	150 ± 6*	165 ± 7*	15	26
	1	135 ± 6*	154 ± 5*	24	31
	10	111 ± 9*	136 ± 8*	37	39
	100	101 ± 9*	121 ± 10*	43	46
	1000	87 ± 9*	111 ± 9*	51	50
	Vehicle	207 ± 9	234 ± 5	–	–
6	0.1	142 ± 10*	152 ± 11*	31	35
	1	128 ± 5*	138 ± 6*	38	41
	10	128 ± 5*	138 ± 5*	38	41
	100	101 ± 8*	118 ± 8*	51	49
	1000	79 ± 6*	94 ± 7*	62	60
	Vehicle	169 ± 10	221 ± 12	–	–
7	0.1	176 ± 7	214 ± 10	−4	3
	1	152 ± 4	167 ± 4*	10	24
	10	149 ± 8	178 ± 8*	12	19
	100	156 ± 10	169 ± 9*	8	23
	1000	141 ± 8	167 ± 10*	16	24
	Vehicle	174 ± 5	211 ± 6	–	–
Oxyphen-	0.1	121 ± 8*	149 ± 12*	30	29
butazone	1	110 ± 8*	136 ± 10*	37	35
	10	98 ± 7*	134 ± 6*	44	36
	100	81 ± 8*	103 ± 7*	53	51
	1000	37 ± 3*	67 ± 4*	79	68

* $p < 0.01$ (in comparison with vehicle).

The inflammogen EPP, in a dose of 1 mg/ear, evoked a clear edematous response when topically applied on the rat ear. The average edema thickness in the vehicle treated (i.e. acetone treated) animal groups amounted to 178 (164–207) μm and 215 (201–234) μm, 30 min and 1 h after application, respectively (Table **1**). The effects of increasing doses of topically applied diarylheptanoids and oxyphenbutazone on the EPP-induced ear edema are shown in Table **1**. Compounds **1**, **2**, **4**–**6**, and oxyphenbutazone produced significant and dose-dependent inhibitions of the edema in the dose-range 0.1–1000 μg/ear, 30 min and/or 1 hour post edema induction. Compound **3** exerted weak inhibitory effects (<25%) only at the two highest doses tested. Compound **7** only slightly influenced (<25% inhibition) edema formation and a clear dose-response relationship could not be established.

In Table **2** the calculated ID_{30} and ID_{50} values of the seven diarylheptanoids and oxyphenbutazone, 30 min and 1 h after EPP challenge, have been listed. The log dose-response plots showed good linear relationships for all compounds, except **3** and **7**, with an average linear correlation coefficient of 0.96 (0.92–1.00).

Table 2 ID_{30} and ID_{50} values of diarylheptanoids and oxyphenbutazone on ethyl phenylpropiolate-induced rat ear edema 30 min and 1 h after inflammogen challenge.

Compound No.	30 min ID_{30} (μg/ear)	30 min ID_{50} (μg/ear)	1 h ID_{30} (μg/ear)	1 h ID_{50} (μg/ear)
1	35	–[a]	28	–[a]
2	0.8	129	1.2	312
3	–[b]	–[a]	–[b]	–[a]
4	10	–[a]	12	–[a]
5	3.6	573	0.5	694
6	0.1	63	0.02[c]	67
7	–[b]	–[a]	–[b]	–[a]
Oxyphenbutazone	0.2	12	0.3	46

[a] 50% inhibition was not reached at a dose of 1000 μg/ear.
[b] 30% inhibition was not reached at a dose of 1000 μg/ear.
[c] Extrapolated value.

Using the ID_{50} values 30 min after edema induction (Table **2**), the diarylheptanoids can be ranked in the following order of decreasing potency: **6** > **2** > **5**. The same ranking order is valid using the ID_{50} values 1 h after edema induction. Making use of the ID_{30} values, both at 30 min and 1 h post EPP challenge, allows the decreasing potency rank order to be continued with compounds **4** and **1**.

Using the ID_{50} values 30 min after edema induction (Table **2**), the reference compound oxyphenbutazone was about five times more potent than compound **6**, but 1 h after edema induction the two compounds were approximately equipotent. A comparison of the ID_{30} values (both 30 min and 1 h post EPP challenge), however, gives compound **6** as more potent than oxyphenbutazone, due to a steeper slope of the log dose-response curve of oxyphenbutazone compared to that of compound **6**.

Discussion

A series of plant derived natural and semi-synthetic non-phenolic linear diarylheptanoids was examined for topical anti-inflammatory properties. The chemical distinction "non-phenolic" is made to differentiate this class of compounds from the phenolic diarylheptanoids, of which curcumin, the major orange plant pigment of *C. longa* (turmeric), is the most well-known (8, 15). Several phenolic diarylheptanoids, including curcumin, have been reported to exhibit potent *in vitro* inhibitory effects on enzymes involved in the biosynthesis of the inflammatory mediators prostaglandins and leukotrienes (16–20). The phenolic nature of these compounds, rendering them entirely different properties from the presently investigated diarylheptanoids, was, however, identified in some of these studies (18, 19) as an indispensable structural feature for potent *in vitro* enzyme inhibiting effect. It is thus reasonable to assume that the presently observed *in vivo* effects of non-phenolic diarylheptanoids represent a different type of interaction with the inflammatory process than the ones previously reported for curcuminoids.

Inhibition of inflammatory edema, induced by topical application of EPP, was used as a model for assessment of anti-inflammatory activity. An inhibition of edema in this model due to a direct chemical reaction between EPP and the test substances appears very unlikely. Introductory experiments (data not shown) in which some of the test compounds were applied prior or post EPP-challenge clearly demonstrated inhibitory effects of similar magnitudes as the ones reported here. The dosage regimen of concomitant application of inflammogen and test compound was adopted due to a lower statistical variation in the results compared to the regimens involving repeated separate applications of the agents. Several of the compounds investigated here did not reach a 50 % inhibition in the dose-range tested. The ID_{30} values of the compounds were therefore estimated in order to make possible a comparison and potency ranking of the less potent compounds as well.

The common structural feature of the compounds evaluated is phenyl-C_7-phenyl. Structural variations are on the C_7-chain i.e. the functional group on carbon 5 and the degree of saturation in positions 1 and 3. Some structure-activity relationship (SAR) trends of these compounds can be commented. The three alcohols **2**, **1**, and **7** form a homologous series in which the degree of saturation of the C_7-chain increases. Compound **2** is an intermediately potent compound (cf. Table **2**), whereas **1** is weakly active (ID_{50} not reached), and the fully saturated analogue **7** is virtually inactive thus showing that the potency increases with the number of double bonds. Furthermore, compounds **1**, **3**, and **5** all have one double bond in the C_7-chain (denoted "monoenes"), whereas **2**, **4**, and **6**, respectively, have two conjugated double bonds (denoted "dienes"), but pairwise respectively, the functional groups on carbon 5 of the C_7-chain are identical. Invariably, the "diene" of each pair has a higher potency than the corresponding "monoene" (cf. Table **2**).

The nature of the functional group at carbon 5 of the C_7-chain also appears to be of importance for the activity (cf. Tables **1** and **2**). A comparison of the potencies among the "monoenes" shows that the ketone **5** is more potent than the alcohol **1**, and that the acetate **3** is almost inactive. The same order of potency is observed for the corresponding "dienes". The higher activities of the ketones **5** and **6**, compared to the corresponding alcohols **1** and **2**, may be due to their more lipophilic characters, and thus probably better skin penetrating properties. However, masking the hydroxy groups of **1** and **2** by acetylation, forming the more lipophilic derivatives **3** and **4**, surprisingly reduced the activity (Table **2**).

The main result of the present study is the finding that the naturally occurring "diene ketone" **6**, the most potent compound in the series studied, showed a topical anti-inflammatory activity in the same dose-range as oxyphenbutazone. None of the synthetic modifications resulted in diarylheptanoids with more potent action than **6**. However, the combined chemical and pharmacological data on natural and semi-synthetic derivatives made some distinct SAR trends discernible, and we propose that these compounds represent a novel class of topical antiinflammatory agents. Compound **6** has been selected for further work directed toward characterization of the nature of the interaction(s) with the inflammatory process, and the results of these studies will be reported subsequently.

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