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TRITERPENE ALCOHOLS FROM THE FLOWERS OF COMPOSITAE AND THEIR ANTI-INFLAMMATORY EFFECTS

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Key Word Index—Compositae; flower; triterpene alcohol; antioedema; TPA-induced ear oedema.

Abstract—Eleven tabular and nine ligulate flowers from 15 species of Compositae plants were investigated for their triterpene alcohol constituents. This led to the isolation and identification of 11 triterpene alcohols as follows: heliaol, taraxasterol, ψ-taraxasterol, α-amyrin, β-amyrin, lupeol, taraxerol, cycloartenol, 24-methylenecycloartanol, tirucalla-7,24-dienol and dammaradienol. The tabular flowers of *Calendula officinalis*, *Carthamus tinctorius*, *Cosmos bipinnatus*, *Chrysanthemum morifolium*, *Helianthus annuus* and *Matricaria matricarioides* showed a characteristic feature by containing helianol as the most predominant component (29–86%) in the triterpene alcohol fractions. The triterpene alcohols from Compositae flowers were evaluated with respect to their anti-inflammatory activity against 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation (1 μg per ear) in mice. All of these showed marked inhibitory activity, and their 50% inhibitory dose was 0.1–0.8 mg per ear.

INTRODUCTION

In the course of our research on the phytochemical and pharmacological aspects of Compositae plants, we have demonstrated that some sterols [1] and alkane-6,8-diols [2, 3], isolated from the flowers of *Carthamus tinctorius*, and some triterpenes including ψ-taraxasterol (**10**) and taraxasterol (**11**), from the flowers of *C. tinctorius*, *Chrysanthemum morifolium* and *Helianthus annuus* [4], exhibited considerable activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory ear oedema in mice [1, 3, 4] and tumour promotion in mouse skin [1, 3]. Our continued study led to the isolation of helianol (**1**) from the tabular flowers of *H. annuus* and its characterization as 3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-eupha-4,24-dien-3-ol [5]. This paper describes the distribution of **1** and 10 other compounds (**2–11**) in the triterpene alcohol fractions separated from the non-saponifiable lipids (NSL) of the methanol extracts of 11 tabular and 9 ligulate flowers from 15 species of Compositae plants. Furthermore, these triterpene alcohols (**1–11**) from the Compositae flowers were demonstrated to possess marked activity against the TPA-induced inflammation.

RESULTS AND DISCUSSION

Triterpene alcohol fractions of the 11 tabular and nine ligulate flowers from 15 species of Compositae plants were obtained by methanol extraction of the flowers and alkaline hydrolysis of the extracted lipids, followed by silica gel chromatography of the NSL. Table 1 shows the contents of the methanol extract in dried flower materials, and of the NSL and the triterpene alcohols in the methanol extracts, which are expressed as weight per cent. The triterpene alcohol fractions were acetylated and the resulting acetates were subjected to argentation TLC followed by reverse-phase HPLC, which allowed the isolation of individual components as the acetates. Table 2 lists the chromatographic (HPLC and GC) data for the acetyl derivatives and free alcohols of 11 triterpene alcohols identified in the triterpene alcohol derivates and free alcohols of 11 triterpene alcohols identified in the triterpene alcohol fractions of the Compositae flowers in this study. Table 3 shows the abundances, based on the HPLC and GC data, of individual components in the triterpene alcohol fractions of the Compositae flowers. The identifications of the 11 triterpene alcohols (**1–11**) were performed by comparison of the GC and HPLC data and, in addition, of the ^{1}H NMR and mass spectral data with those of reference compounds [5–7].

The contents of the methanol extracts (11–64%) in the dried flowers, and contents of the NSL (2.5–26.1%) and the triterpene alcohol fractions (0.6–2.8%) in the methanol extracts varied significantly, depending of the flower materials and species of the Compositae investigated (Table 1). However, in general the ligulate

Table 1. Compositae flowers investigated, and contents of methanol (MeOH) extracts, nonsaponifiable lipids (NSL) and triterpene alcohol fractions

Tribe	Compositae*	Flowers	Source†	Contents (wt %)		
				MeOH ext. (dried flowers)	NSL (MeOH ext.)	Triterpene fr. (MeOH ext.)
Subfamily Asteroideae						
Anthemideae	*Chrysanthemum morifolium* Ramat. var. *sinense* Makino (chrysanthemum) (I)	Tabular	A	23	9.4	3.0
		Ligulate	A	30	14.2	3.7
	C. morifolium Ramat. var. *sinense* Makino *forma esculentum* Makino (chrysanthemum) (II) [Ryouri-giku]	Edible ligulate	A	36	11.5	2.5
	Matricaria matricarioides Porter. (pineapple weed) [Orosha-giku]	Tabular	B	16	12.0	2.4
		Ligulate	B	26	26.1	9.2
Calenduleae	*Calendula officinalis* L. (pot marigold)	Tabular	C	26	12.1	1.0
		Ligulate	C	33	24.0	4.6
Heliantheae	*Cosmos bipinnatus* Cav. (cosmos)	Tabular	D	35	16.5	2.3
		Ligulate	D	64	2.5	1.2
	Helianthus annuus L. (sunflower)	Tabular	B	18	6.8	1.2
		Ligulate	B	40	4.8	0.6
	H. debilis Nutt. (cucumber-leaf sunflower) [Hime-himawari]	Ligulate	B	40	6.5	0.8
Subfamily Cichoridoideae						
Cardueae	*Arctium lappa* L. (urdock)	Tabular	B	26	7.1	4.2
	Carthamus tinctorius L. (safflower)	Tabular	A	21	5.7	0.6
	Cirsium nipponicum Makino [Tone-azami]	Tabular	E	14	14.5	9.9
	C. tanakae Matsum. [Nohara-azami]	Tabular	E	11	20.2	12.8
	Cynara cardanculus L. (cardoon) [Chosen-azami]	Tabular	B	14	10.6	5.2
	Silybum marianum Gaertn. (St. Mary's thistle) [Maria-azami]	Tabular	B	13	9.7	4.6
Lactuceae	*Taraxacum officinale* Weber. (dandelion) [Seiyo-tanpopo]	Ligulate	D	22	17.2	3.5
	T. platycarpum Dahlst. (dandelion) [Kanto-tanpopo]	Ligulate	D	42	17.3	2.7

* English name in parentheses and Japanese name in square brackets.

† A: cultivated at Mogami, Yamagata; B: cultivated at a Drug Plant Garden of College of Pharmacy, Nihon University; C: purchased at a herb market in London; D: collected locally in Tokyo, Chiba, Gunma or in Fukushima; E: collected at Mt Nyuukasa, Nagano.

Table 2. Chromatographic data of triterpene alcohols identified in the Compositae flowers*

Compound	Triterpene alcohol (systematic name)	Free alcohol (RR_t(I))		Acetyl derivative (RR_t(II))	
		HPLC	GC	HPLC	GC
1	Helianol [3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-eupha-4,24-dien-3-ol]	0.63	1.44	0.63	1.32
2	Taraxerol (tarax-14-en-3β-ol)	1.03	1.82	0.88	1.65
3	Dammaradienol (dammara-20,24-dien-3β-ol)	0.87	1.84	0.61	1.66
4	β-Amyrin (olean-12-en-3β-ol)	1.10	1.88	0.92	1.70
5	Cycloartenol (cycloart-24-en-3β-ol)	1.06	2.08	0.98	1.82
6	Tirucalla-7,24-dienol (tirucalla-7,24-dien-3β-ol)	0.95	2.10	0.83	1.89
7	α-Amyrin (urs-12-en-3β-ol)	1.24	2.14	1.01	1.92
8	Lupeol [lup-20(29)-en-3β-ol]	0.85	2.23	0.74	2.00
9	24-Methylenecycloartanol (24-methylenecycloartan-3β-ol)	1.12	2.23	1.07	2.00
10	ψ-Taraxasterol (taraxast-20-en-3β-ol)	1.22	2.76	1.06	2.48
11	Taraxasterol [taraxast-20(30)-en-3β-ol]	1.07	2.88	0.89	2.58

* Cholesterol was the standard for the determination of RR_t(I) for free alcohols, whereas cholesteryl acetate was the standard for the determination of RR_t(II) for the acetyl derivatives. Triterpene alcohols were arranged in elution order in GC.

flowers afforded more methanol extract than did the tabular flowers within the same species.

Helianol (**1**), a *seco*-triterpene alcohol, constituted the most predominant component (29–86%) in the triterpene alcohol fractions of the tabular flowers of *Calendula officinalis*, *Carthamus tinctorius*, *Cosmos bipinnatus*, *Chrysanthemum morifolium*, *Helianthus annuus* and *Matricaria matricarioides*, as shown in Table 3. The ligulate flowers of the former five species of Compositae, which belong to the subfamily Asteroideae, contained **1** only as a trace or minor amount in their triterpene alcohol fractions. Thus, there was a great diversity in the quantitative compositions of the triterepene alcohol fractions between tabular and ligulate flowers for the Asteroideae species examined. The other Compositae flowers investigated contained either one of the four pentacyclic triterpene alcohols, viz. β-amyrin (**4**), α-amyrin (**7**), ψ-taraxasterol (**10**) or taraxasterol (**11**), as the most predominant component in the triterpene alcohol fractions. The predominance of these pentacyclic compounds and lupeol (**8**) in the triterpene alcohol fractions have so far been reported also in some Compositae plant materials [8–10] including the whole flowers of *C. officinalis* [11, 12].

The 11 triterpene alcohols isolated from the Compositae flowers in this study were examined for their inhibitory effects on TPA-induced inflammation in mice. The inhibitory effects were compared with those of the commercially availabe anti-inflammatory drugs, indomethacin and hydrocortisone (Table 4). All of the triterpene alcohols markedly inhibited the TPA-induced inflammation with 0.1–0.8 mg per ear of the 50% inhibitory dose, and among which **1** exhibited the strongest inhibitory effect (0.1 mg per ear). The inhibitory effects evaluated for five triterpenes, **4**, **5** and **7**–**9**, in this study were consistent with our previous results [13]. While the inhibitory effects of these compounds were weaker than that of hydrocortisone, the inhibition of **1** and eight other triterpenes (**2**, **4**, **5** and **7**–**11**) was at a level comparable to that of indomethacin. Inhibitors of TPA-induced inflammation have been demonstrated to be almost in parallel with their inhibitory activities against tumour promotion [1, 3, 13, 14]; the triterpenes, especially **1**, described here are, therefore, expected to be potent anti-tumour agents. Biological activities of two taraxastanes (**10** and **11**) from Compositae have recently been reported. Thus, **10** has been demonstrated to be the most active against croton oil inflammation in mice among several pentacyclic monohydroxytriterpenes isolated from *C. officinalis* flowers [12]. On the other hand, **11**, as the acetyl derivative, isolated from *Inula britannica* flowers, was shown to have preventive effects on experimental hepatitis caused by either immunologically induced injuries of hepatotoxic chemicals [15]. The inhibitory effects against TPA-induced oedema as well as against carrageenan- and ethyl phenylpropiolate-induced oedema for β-anyrin (**4**), α-anyrin (**7**) and lupeol (**8**) have also been reported recently [16].

EXPERIMENTAL

HPLC: C_{18} silica column (Superiorex ODS S-5 μm column, 25 cm × 10 mm i.d.; Shiseido Co., Tokyo), MeOH as mobile phase (flow rate 4 ml min^{-1}); GC: DB-17 fused silica capillary column (30 m × 0.3 mm i.d.), column temp. 275°. RR_t on HPLC and GC expressed relative to cholesterol (cholest-5-en-3β-ol) as for free triterpene alcohols whereas relative to cholesteryl acetate as for the acetyl derivatives. EIMS (70 eV): probe. ^{1}H NMR (400 MHz): in $CDCl_3$ with TMS as int. standard. Acetylation: Ac_2O–pyridine at room temp. overnight. Hydrolysis: 5% KOH in MeOH at toom temp. overnight. Sources of the Compositae plant materials used in this study shown in Table 1. Eleven triterpene alcohols (**1**–**11**) were used as ref. compounds [5–7]. Indomethacin and hydrocortisone were purchased from Sigma (St Louis, MO). Female ICR mice were obtained from Japan SLC (Shizuoka).

General isolation procedure. Fresh flowers were air

Table 3. Compositions (%) of the triterpene alcohol fractions separated from the nonsaponifiable lipids of the methanol extracts of Compositae flowers

Tribe	Compositae	Flowers	Compositions (%)*											Others (unidentified)
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	
Subfamily Asteroideae														
Anthemideae	*Chrysanthemum morifolium* (I)	Tabular	86.0			2.8	0.3		3.9	0.3	0.2	1.6	0.6	4.3
		Ligulate	2.1	0.2	2.7	30.5	0.2	1.3	12.3	1.7	0.3	33.0	14.4	1.1
	C. morifolium (II)	Edible ligulate	0.1	0.4	4.1	24.5	0.3	2.8	15.4	0.2	2.6	35.6	13.3	0.7
	Matricaria matricarioides	Tabular	34.2	0.7	1.5	7.9	0.4		5.1	1.0	1.0	1.5	9.5	37.2
		Ligulate	0.2	0.1	1.5	19.0		0.2	2.1	6.1	1.2	8.2	61.4	
Calenduleae	*Calendula officinalis*	Tabular	58.5		0.8	7.7	0.3	0.5	3.7	4.2	0.2	22.8	0.9	0.4
		Ligulate	trace		4.2	15.7			11.0	11.4		52.7	5.0	
Heliantheae	*Cosmos bipinnatus*	Tabular	77.2	1.6		2.3	1.1		3.9	0.2	0.6			13.1
		Ligulate	5.2		1.9	21.3		3.4	63.7	0.6	0.9	1.4	1.1	0.5
	Helianthus annuus	Tabular	78.3		0.5	11.1	0.6	0.4	3.9	0.5	1.0	0.7		3.0
		Ligulate	2.5		5.9	40.5	6.4		2.8	2.4	3.4	25.9	4.6	5.6
	H. debilis	Ligulate	9.5		2.4	34.9	0.3		36.1	10.6	4.2	0.8		1.2
Subfamily Cichoridoideae														
Cardueae	*Arctium lappa*	Tabular	0.8			9.9			8.9	5.7	11.8	21.9	39.8	1.2
	Carthamus tinctorius	Tabular	28.7	0.6	0.6	17.7	1.4		13.1	4.6	3.3	1.2	0.7	28.1
	Cirsium nipponicum	Tabular	0.4			18.7			49.7	8.6	8.5	8.4	2.3	3.4
	C. tanakae	Tabular				16.9			37.6	4.7	6.5	12.7	21.6	
	Cynara cardanculus	Tabular	3.2			16.6			11.5	2.6	5.3	18.9	41.5	0.4
	Silybum marianum	Tabular				27.1			23.3	4.3	5.9	14.1	25.3	
Lactuceae	*Taraxacum officinale*	Ligulate	12.2		4.2	11.3	4.9	2.4	14.1	9.6	3.8	5.7	16.5	15.3
	T. platycarpum	Ligulate	9.8	0.5	2.5	8.0	14.4	1.0	13.0	0.8	2.1	8.0	17.4	22.5

* Determined based on the HPLC and GC data.

Table 4. Inhibitory effect of 11 triterpene alcohols (**1–11**) isolated from the Compositae flowers and two reference compounds on TPA-induced inflammation in mice*

Compound	Triterpene alcohol	ID_{50} (mg per ear)†	IR‡ (%)
1	Helianol	0.1	90§
2	Taraxerol	0.4	49§
3	Dammaradienol	0.8	57
4	β-Amyrin	0.4	71‖
5	Cycloartenol	0.3	64
6	Tirucalla-7,24-dienol	0.8	63
7	α-Amyrin	0.2	86‖
8	Lupeol	0.6	87‖
9	24-Methylenecycloartanol	0.2	85‖
10	ψ-Taraxasterol	0.4	77
11	Taraxasterol	0.3	90
Reference compounds			
	Indomethacin	0.3	96
	Hydrocortisone	0.03	99

* Compounds dissolved in $CHCl_3$–MeOH (1:1) were applied 30 min before TPA treatment. Ear thickness was determined at 8 hr after TPA treatment.

† 50% Inhibitory dose.

‡ If not otherwise specified, inhibition ratio (IR) was at 1 mg per ear.

§ IR at 0.5 mg per ear.

‖ IR at 2 mg per ear.

dried and then extracted 3× for 3 days each with MeOH. The NSL obtained from the MeOH extract by alkaline hydrolysis (5% KOH in MeOH, reflux 3 hr) were subjected to CC on silica gel using the gradient solvent system (*n*-hexane–EtOAc, 1:0–1:4) to yield a triterpene alochol fr. which, on acetylation, gave the triterpene acetate fr. Isolation of individual triterpene acetates was achieved by argentation TLC [7] followed by prep. HPLC. Alkaline hydrolysis of the isolated triterpene acetates yielded free alcohols which were used for confirmation of identification for bioassay.

Assay of TPA-induced inflammation in mice. Female ICR mice were housed in an air-conditioned room (22–23°) lit from 08:00 to 20:00. Food and H_2O were available *ad libitum*. TPA (1 μg per ear) dissolved in Me_2CO (20 μl) was applied to the right ear only of ICR mice by means of micropipette. A vol. of 10 μl was delivered to both the inner and outer surfaces of the ear. The sample, or its vehicle, MeOH–$CHCl_3$ (1:1; 20 μl), as a control, was applied topically *ca* 30 min before each TPA treatment. Application of the sample completely inhibited TPA-induced inflammation and this inhibitory activity was reduced in a dose-dependent manner. For ear thickness determinations, a pocket thickness gauge (Mitsutoyo, Tokyo) with a range of 0–9 mm, graduated at 0.01-mm intervals and modified so that the contact surface area was increased, thus reducing the tension, was applied to the tip of the ear.

The ear thickness was measured before treatment (a). The oedema was measured 8 hr after TPA-treatment (b: TPA alone; b′: TPA plus sample). The inhibition ratio (IR) was calc. as follows, where oedema A: oedema was induced by TPA alone (b − a); oedema B: oedema was induced by TPA plus sample (b′ − a),

$$IR = \frac{A - B}{A} \times 100$$

Each value was the mean of individual determinations for 5 mice, and 50% inhibitory dose (ID_{50}) values were determined by the method of probit–graphic interpolation for at least 4 dose levels.

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