

Compositions of Triterpene Alcohols of Seeds and Mature Plants of Family Cucurbitaceae

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Compositions of the triterpene alcohol fractions of the unsaponifiable lipids obtained from 28 seed and mature plant [aerial parts (leaves and stems), pericarp of the fruits, and roots] of 13 genera of the family Cucurbitaceae were determined by gas liquid chromatography (GLC) on an OV-17 glass capillary column. Among the 14 tetracyclic and pentacyclic triterpene alcohols identified, isomultiflorenol was found to be the major component for most of the seed materials. The abundance of this triterpene suggests that it may be a taxonomical marker of the seeds of Cucurbitaceae since only a few higher plants are known to contain isomultiflorenol. The mature plant materials of the Cucurbitaceae, on the other hand, were shown to contain either one or some of the common triterpene alcohols such as α - and β -amyryns, cycloartenol and 24-methylenecycloartanol as the major triterpene constituents.

In the course of our study on the constituents of sterols and triterpene alcohols, it was shown that the seeds of two *Cucumis* species, *C. melo* and *C. sativus*, contained isomultiflorenol (7) as the most predominant component accompanied by its Δ^7 -isomer, multiflorenol (14), in the triterpene alcohol fractions (1). This is a unique characteristic as most of the higher plants contain triterpene alcohols such as β -amyryn (5), α -amyryn (9), cycloartenol (10), lupeol (11) and 24-methylenecycloartanol (13) as major constituents (2). Thus, it was worthwhile to investigate more extensively the triterpene alcohol constituents of other members of Cucurbitaceae. This study presents the capillary GLC analysis of the triterpene alcohol fractions obtained from 28 seed and mature plant materials from 13 genera of Cucurbitaceae. For some plant materials, isolation of individual triterpene alcohols has been undertaken in order to confirm the identifications. The present study complements earlier work on the sterol constituents of the Cucurbitaceae (3-7).

EXPERIMENTAL

General methods. Melting points (m.p.) taken on a heated block were uncorrected. Argentation (AgNO₃-silica gel, 1:4) preparative thin layer chromatography (TLC) was developed four times with CCl₄-CH₂Cl₂ (5:1). GLC was performed on a SCOT OV-17 glass capillary column (30 m \times 0.3 mm i.d., column temp 260 C). High performance liquid chromatography (HPLC) was carried out on a Partisil ODS-2 column

(25 cm \times 10 mm i.d.) with methanol (MeOH) as the mobile phase. The R_c values in the argentation TLC and the relative retention time (RR_t) values in the GLC and HPLC were taken relative to cholesterol acetate. Mass spectra (EI-MS, 70 eV) were recorded by means of a probe injection. ¹H NMR (250 or 400 MHz) were determined in CDCl₃ with tetramethylsilane as internal standard. The other techniques used in this study were described previously (4).

Materials, extraction and separation of triterpene alcohol fraction. Twenty-eight seed and mature plant materials obtained from 21 species of 13 genera of the family Cucurbitaceae used in this study are shown in Table 1. The sources of the plant materials were described in our recent articles (4,8). The 14 (1-14) triterpene alcohols, listed in Table 2, were used as the reference specimens (1,9). Extraction of lipid from air-dried materials was performed by either of the following four methods: (a), extracted with CHCl₃-MeOH (2:1) at room temp under stirring; (b) and (c), with CH₂Cl₂ and CHCl₃ in a Soxhlet extractor, respectively; and (d), with MeOH under reflux, and the extract was then treated with cold acetone to remove insoluble phospholipid; the acetone-soluble portion was subjected to subsequent work. The extracted lipid was saponified, and the triterpene alcohol (with an equatorial 3 β -monohydroxyl group) fraction was separated from the unsaponifiable lipid by preparative TLC as described previously (1,7). The triterpene alcohol fraction was acetylated and the resulting acetate fraction was subjected to GLC analysis and, in some cases, to argentation TLC and HPLC in order to isolate individual components.

RESULTS AND DISCUSSION

Table 2 showed the 14 triterpene alcohols (1-14) identified in the Cucurbitaceae plants in this study. Identification of 12 triterpenes (1, 2, 4, 5, 7-14) for the seeds of *Cucumis melo* and *C. sativus* already has been performed by GLC, argentation TLC and combined gas chromatography-mass spectrometry (GC-MS), in our previous study (1). This study has further identified 3 and 6 in addition to 4, 5, 7, 9-11 and 13 after isolation from all or any of the three Cucurbitaceae plant materials: *Coccinea grandis* (aerial parts), *Momordica cochinchinensis* (aerial parts), and *Trichosanthes kirilowii* (seeds), as described below. Identification of the other triterpene alcohols from the three cucurbitaceous plant materials, and all of the 14 (cf. Table 2) triterpene alcohols from the other plant materials, is tentative (by GLC) in this study because it can be expected that the triterpene alcohol constituents will be similar among members of the same plant family (2). Although GLC on an OV-17 glass capillary column showed excellent separation among the

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TABLE 1
Cucurbitaceae Plants Investigated

Cucurbitaceae	Plant material	Lipid extraction ^a
<i>Apodanthera undulata</i> Gray	Seeds	(a)
<i>Benincasa cerifera</i> Savi (wax gourd)	Seeds	(b)
<i>Citrullus battich</i> Forskål (watermelon)	Seeds	(b)
	Aerial parts	(d)
<i>Coccinea grandis</i> Voigt (ivy gourd) ^b	Aerial parts	(c)
<i>Cucumis melo</i> L. (melon)	Seeds	(b)
<i>C. sativus</i> L. (cucumber)	Seeds	(b)
	Aerial parts	(d)
<i>Cucurbita digitata</i>	Seeds	(a)
<i>C. foetidissima</i> HBK (buffalo gourd)	Seeds	(a)
<i>C. maxima</i> Duschesne (squash)	Seeds	(b)
<i>C. pepo</i> L. (pumpkin)	Seeds	(b)
<i>Gynostemma pentaphyllum</i> Makino	Aerial parts	(d)
<i>Lagenaria leucantha</i> Rusby var. Gourda Makino (bottle gourd)	Seeds	(b)
<i>L. leucantha</i> Rusby var. <i>depressa</i> Makino	Seeds	(b)
	Pericarp	(b)
<i>Luffa cylindrica</i> Roem. (sponge gourd)	Seeds	(b)
<i>Momordica charantia</i> L. (balsam pear)	Seeds	(b)
	Aerial parts	(c)
<i>M. charantia</i> L. var. <i>Pavel</i> Crantz	Seeds	(b)
<i>M. cochinchinensis</i> Spreng	Seeds	(c)
	Aerial parts	(c)
<i>Sechium edule</i> Sw. (chayote)	Aerial parts	(d)
	Pericarp	(b)
<i>Sicyos angulatus</i> L. (bur cucumber)	Aerial parts	(b)
<i>Trichosanthes dioica</i>	Roots	(c)
<i>T. kirilowii</i> Maxim. ^c	Seeds	(b)
	Roots	(d)

^aSee Experimental section.

^bThis was erroneously identified as "seed material" in ref. (4).

^cThis was erroneously identified as *T. japonica* Regel in refs. (4) and (7).

component peaks of the cucurbitaceous triterpene mixture, the components of each of the following sets of components: cycloartanol (**3**) and taraxerol (**4**); and, in some cases, α -amyirin (**9**) and cycloartenol (**10**), remain unresolved in this study.

The acetylated triterpene alcohol fractions of the aerial parts of *C. grandis* and *M. cochinchinensis* and the seeds of *T. kirilowii* were subjected to argentation TLC followed by HPLC for the isolation of individual components. Identification of the isolated triterpenes was performed based on the chromatographic (GLC, HPLC and argentation TLC), m.p. and MS data, and further by the ¹H NMR data (10) for some triterpenes. See Table 2 for the chromatographic and m.p. data for the acetates of the Cucurbitaceae triterpene alcohols. The acetyl derivatives of the triterpenes isolated and identified from each of the three plant materials are shown below, accompanied with the ¹H NMR data when available.

C. grandis: The acetates of cycloartanol (**3**), β -amyirin (**5**), isomultiflorenol (**7**), α -amyirin (**9**), cycloartenol (**10**), lupeol (**11**) and 24-methylenecycloartanol (**13**). **3**-Acetate: ¹H NMR (400 MHz): δ 0.338 (1H, d, J = 4.2 Hz) and 0.576 (1H, d, J = 3.9 Hz) (19-H₂), 0.846 (3H, s, 30-H₃), 0.861 (3H, d, J = 6.6 Hz, 21-H₃), 0.865 and 0.871 (each 3H, d, and J = 6.6 Hz, 26-H₃, 27-H₃), 0.889 and

0.896 (each 3H and s, 31-H₃, 32-H₃), 0.959 (3H, s, 18-H₃), 2.051 (3H, s, 3 β -OAc), 4.567 (1H, dd, J = 11.2, 5.6 Hz, 3 α -H). **5**-Acetate: ¹H NMR (250 MHz): δ 0.830 (3H, s, 28-H₃), 0.873 (12H, s, 23-H₃, 24-H₃, 29-H₃, 30-H₃), 0.967 (6H, s, 25-H₃, 26-H₃), 1.130 (3H, s, 27-H₃), 2.044 (3H, s, 3 β -OAc), 4.503 (1H, dd, J = 7.7, 7.3 Hz, 3 α -H), 5.182 (1H, dd, J = 3.3, 3.3 Hz, 12-H). **7**-Acetate: ¹H NMR (400 MHz): δ 0.874 and 0.884 (each 3H and s, 23-H₃, 24-H₃), 0.955 and 0.972 (each 3H and s) and 0.977 (6H, s) (25-H₃, 27-H₃, 29-H₃, 30-H₃), 1.055 (3H, s, 26-H₃), 1.071 (3H, s, 28-H₃), 2.050 (3H, s, 3 β -OAc), 4.496 (1H, dd, J = 11.5, 5.0 Hz, 3 α -H). **9**-Acetate: ¹H NMR (250 MHz): δ 0.796 (3H, d, J = 5.8 Hz, 29-H₃), 0.799 (3H, s, 28-H₃), 0.867 and 0.876 (each 3H and s, 23-H₃, 24-H₃), 0.903 (3H, d, J = 5.5 Hz, 30-H₃), 0.981 (3H, s, 25-H₃), 1.010 (3H, s, 26-H₃), 1.066 (3H, s, 27-H₃), 2.048 (3H, s, 3 β -OAc), 4.512 (1H, dd, J = 7.9, 7.3 Hz, 3 α -H), 5.126 (1H, dd, J = 4.0, 4.0 Hz, 12-H). *M. cochinchinensis*: The acetates of **5**, butyrospermol (**6**), **9**, **10** and **11**. **6**-Acetate: ¹H NMR (400 MHz): δ 0.765 (3H, s, 19-H₃), 0.803 (3H, s, 18-H₃), 0.849 (3H, d, J = 6.0 Hz, 21-H₃), 0.851 (3H, s, 30-H₃), 0.933 (3H, s, 31-H₃), 0.974 (3H, s, 32-H₃), 1.606 (3H, s, 27-H₃), 1.688 (3H, s, 26-H₃), 2.054 (3H, s, 3 β -OAc), 5.418 (1H, dd, J = 11.0, 3.8 Hz, 3 α -H), 5.098 (1H, tt, J = 6.5, 1.1 Hz, 24-H), 5.247 (1H, dd, J = 6.6, 2.7 Hz, 7-H). *T. kirilowii*: The ace-

TRITERPENE ALCOHOLS OF CUCURBITACEAE

TABLE 2

Chromatographic and Mp Data of Some Triterpene Alcohols from Cucurbitaceae

Triterpene alcohol (systematic name) ^a	Data for the acetyl derivatives			
	RRt ^b		Rc ^b	Mp ^c
	GLC	HPLC	Argentation TLC	(C)
1 Euphol (eupha-8,24-dienol)	1.30	—	—	—
2 Tirucallol (tirucalla-8,24-dienol)	1.47	—	—	—
3 Cycloartanol (9 β ,19-cyclolanostanol)	1.54	1.15	1.24	132-133
4 Taraxerol (D-friedoolean-14-enol)	1.56	0.71	0.76	304-306
5 β -Amyrin (olean-12-enol)	1.63	0.72	1.35	244-245
6 Butyrospermol (eupha-7,24-dienol)	1.68	0.64	0.19	146-148
7 Isomultiflorenol (D:C-friedoolean-8-enol)	1.71	0.80	1.20	221-222
8 24-Methylene-24-dihydrolanosterol (24-methylenelanost-8-enol)	1.76	—	—	—
9 α -Amyrin (urs-12-enol)	1.84	0.77	1.42	227-230
10 Cycloartenol (24-dehydrocycloartanol)	1.86	0.89	0.83	117-118
11 Lupeol (1up-20(29)-enol)	1.92	0.57	0.27	211-212
12 24-Methylene-24-dihydroparkeol (24-methylenelanost-9(11)-enol)	2.00	—	—	—
13 24-Methylenecycloartanol	2.07	0.96	0.16	119-120
14 Multiflorenol (D:C-friedoolean-7-enol)	2.12	—	—	—

^aAll triterpene alcohols are 5 α -compounds. The hydroxyl group at C-3 of all triterpene alcohols is β -oriented (equatorial).

^bRRt and Rc values were expressed relative to cholesterol acetate.

^cThe data corresponding to the acetates of 3, 5, 7, 9, 10, 11 and 13 isolated from the aerial parts of *Coccinea grandis*; 4-acetate from the seeds of *Trichosanthes kirilowii*; and 6-acetate from the seeds of *Momordica cochinchinensis*.

TABLE 3

Compositions (%) of Triterpene Alcohols of the Seed Materials of Cucurbitaceae

RRt (acetate) Estimated compd.	Compositions (%)																			
	1	1.34	1.41	1.47	1.55	1.60	1.63	1.68	1.71	1.76	1.84	1.86	1.92	1.95	2.00	2.07	2.12	2.24	2.36	Others
Cucurbitaceae	1		2	3,4		5	6	7	8	9	10	11		12	13	14				
<i>Apodanthera undulata</i>	2.0		8.0	1.2	0.1	0.5	5.6		27.7	1.9	1.1	4.1	34.0		1.3	2.7	1.2	2.1	5.4	1.1
<i>Benincasa cerifera</i>	1.1	1.1	8.7	1.8	1.6	1.3	9.3	4.4	28.4	2.8	10.9	1.8		2.5	17.0	6.4				0.9
<i>Citrullus battich</i>	2.2		0.3				9.0		39.1	3.1	2.2	35.5				1.7	1.3	0.4	5.2	
<i>Cucumis melo</i>	1.8	0.6	0.4	1.5	1.2	1.2	9.9		31.6	9.4	4.6	4.8	5.1	2.8	2.5	17.6	5.0			
<i>C. sativus</i>	3.9			2.2	1.0	0.4	2.6	0.7	37.6	3.0	15.6	0.9				27.2	2.1			2.8
<i>Cucurbita digitata</i>			8.2	6.8	1.4		18.9	7.8	17.1	6.9	1.5	15.6				1.6	4.1			10.2
<i>C. foetidissima</i>	3.9		9.7	7.3	0.8	0.3	8.6		42.9	5.4	1.6	4.3	3.2		0.6	3.7	5.5	0.6		1.6
<i>C. maxima</i>	0.2		0.2	1.4	1.0	0.5	8.3		42.0	3.6	21.2	1.7			1.3	15.0	3.6			
<i>C. pepo</i>	1.0			3.1	0.6	0.5	7.6	0.4	17.4	1.9	51.9	3.3	0.9		1.6	5.4	4.4			
<i>Lagenaria leucantha</i> var. <i>gourda</i>	4.3		3.8	5.1	2.9		0.8		22.7	9.0	5.1	12.1	7.6		1.3	17.3	6.0	0.5	0.4	1.1
<i>L. leucantha</i> var. <i>depressa</i>	6.8		20.5	6.5	0.9	0.8	7.4	2.4	22.5	1.6	9.8	4.9			1.1	9.5	3.2	0.6		1.5
<i>Luffa cylindrica</i>	2.2	1.5	0.4	3.2	1.4	1.0	13.0	14.7	18.2	1.3	1.5	1.8	26.1		1.3	3.2	2.0	1.9	0.8	4.5
<i>Momordica charantia</i>	0.3			0.8	2.1	0.8	6.6	15.9	10.7	0.4	37.1	9.1				11.4	1.3			1.5
<i>M. charantia</i> var. <i>Pavel</i>	0.3		1.5	0.7	2.9	1.7	12.8	4.1	7.6	1.1	4.7	18.8	5.3			36.2	1.7	0.6		
<i>M. cochinchinensis</i>	4.0	3.1			2.1	1.1	39.0	1.2	0.7		2.2	31.2			1.5	13.6	tr.			0.3
<i>Trichosanthes kirilowii</i>	2.6		3.5	1.6	2.9	1.8	7.9		29.8	4.3	5.6	16.7	4.0		0.4	11.2	3.9	0.2	1.4	2.1

TABLE 4

Compositions (%) of Triterpene Alcohols of the Mature Plant Materials of Cucurbitaceae

RRt (acetate) Estimated compd. Cucurbitaceae	Compositions (%)																			
	1.30 1	1.34	1.41	1.47	1.55 2	1.60 3,4	1.63 5	1.68 6	1.71 7	1.76 8	1.84 9	1.86 10	1.92 11	1.95	2.00 12	2.07 13	2.12 14	2.24	2.36	Others
<i>Citrullus battich</i> ^a	tr			tr			2.7		44.3	2.7	8.5	6.4				3.7	2.1	29.2		0.4
<i>Coccinea grandis</i> ^a	0.3	0.5		0.4	1.0		8.5	4.0	2.3	0.9	20.8	16.5	19.0	7.2		3.2	1.1	0.2	1.2	12.9
<i>Cucumis sativus</i> ^a	0.2		0.5	1.7	0.5	1.6	19.3		3.2	19.3	10.4	8.0	3.8		2.8	23.9	4.1	0.7		
<i>Gynostemma pentaphyllum</i> ^a	0.5				9.5		22.7	2.3	2.0	2.2	3.4	11.4	6.2		0.7	21.2	1.0	0.3	3.2	13.4
<i>Lagenaria leucantha</i> var. <i>depressa</i> ^b	0.5	1.0	4.0	2.1	1.5	0.3	10.3	2.4	1.4	6.9	10.0	6.7	16.2		4.1	26.2	3.5	1.0		1.9
<i>Momordica cochinchinensis</i> ^a	0.9	1.0			4.0		72.0	9.7	0.6		1.4	1.5	0.6			tr	tr	tr		8.3
<i>M. charantia</i> ^a			3.4	1.0	3.0		13.8		4.9	8.6	17.5	3.3	1.0		37.8	5.7				
<i>Sechium edule</i> ^a	1.6	0.3			0.4	1.3	28.5	1.0	1.3	0.2	31.5	4.6	2.6	1.1	15.0	3.5	3.3			3.8
<i>S. edule</i> ^b				1.2	1.4	4.0	33.5		2.4	1.8	11.0	8.9	23.4		9.6	1.0		0.7		1.1
<i>Sicyos angulatus</i> ^a		0.6		0.3	0.3	0.2	4.1	0.1	7.1	3.1	40.7	7.9	3.0		27.3	tr	0.1			5.2
<i>Trichosanthes dioica</i> ^c	4.5	1.9		6.1	1.4	0.7	4.8	1.9	24.6	3.5	11.3			6.0	26.8	0.8				2.2
<i>T. kirilowii</i> ^c	0.6			0.1	0.5	0.1	1.4	0.3	0.9	0.6	65.4			0.5	25.7	3.3	0.4			0.2

^aAerial parts.^bPericarp of the fruits.^cRoots.

tates of taraxerol (5), 7, 9 and 10.

The approximate compositions of the triterpene mixtures separated from the unsaponifiable lipids of various plant parts (Table 1) are shown in Tables 3 and 4, respectively. Several components with the RRts smaller than that of cholesterol acetate, which were observed for some plant materials and were supposed to be the acetates of aliphatic alcohols, were omitted from the tables. The most significant feature is the occurrence of isomultiflorenol (7) and its Δ^1 -isomer, multiflorenol (14), in all of the Cucurbitaceae investigated. The occurrence of these triterpenes is known to be rare, and only a few higher plants, besides Cucurbitaceae (1), have been reported to contain these D:C-friedooleanane type triterpenes: 7 in *Pelargonium hortorum* (Geraniaceae) (11) and *Zanthoxylum decaryi* (Rutaceae) (12), and 14 in *Gelonium multiflorum* (Euphorbiaceae) (13). The triterpene 7 was found to be the major triterpene component for all of the Cucurbitaceae seed materials examined with the exception of the three *Momordica* species (*M. charantia*, *M. charantia* var. *Pavel* and *M. cochinchinensis*). This suggests that the predominance of 7 may be a taxonomical marker of the seeds of Cucurbitaceae. As for the mature plant materials, although 7 occurred abundantly in the aerial parts of *Citrullus battich* and the roots of *Trichosanthes dioica*, this triterpene constituted only a minor portion for the other mature plant materials examined. The major triterpenes observed for these mature plants were either β -amyrin (35), α -amyrin (9), cycloartenol (10), lupeol (11) or 24-methylenecycloartanol (13), which are the common triterpene alcohols of higher plants (2).

Although this study has concerned the compositions of the triterpene alcohols with an equatorial 3β -monohydroxyl, it is important to recall that both the seed and mature plant materials of the Cucurbitaceae contain another type of triterpene alcohol which pos-

sesses an axially oriented 3β -monohydroxyl group such as anhydrolitsomentol (6,7). These cucurbitane-type triterpenes, which separated on TLC from the above mentioned usual triterpene alcohols, were determined in another study (7).

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