Pergamon

PII: S0031-9422(97)00433-0

Phytochemistry, Vol. 46, No. 7, pp 1261–1266, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

7-OXODIHYDROKAROUNIDIOL-3-BENZOATE AND OTHER TRITERPENES FROM THE SEEDS OF CUCURBITACEAE

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(*Received* 27 *February*, 1997)

Key Word Index—Cucurbitaceae; seeds; triterpene; 7-oxodihydrokarounidiol-3-benzoate; isokarounidiol-3-*p*-methoxybenzoate.

Abstract—The highly-polar fractions of the nonsaponifiable lipids obtained from the methanol extracts of 10 Cucurbitaceae seed materials were investigated for their components. Fourteen dihydroxy triterpenes and their derivatives, and one oxo-sterol were characterized. They are 7-oxodihydrokarounidiol-3-benzoate (7-oxomultiflor-8-ene-3α,29-diol-3-benzoate), isokarounidiol-3-*p*-methoxybenzoate (multiflora-6,8-diene-3α,29-diol-3-*p*-methoxybenzoate), karounidiol-3-benzoate, karounidiol, isokarounidiol, 5-dehydrokarounidiol, 7-oxodihydrokaroudnidiol, bryonolol, 3-epibryonolol, loranthol, betulin, 29-hydroxylupeol, erythrodiol, (23*Z*)-cycloart-23-ene-3β,25-diol and 7-oxositosterol among which the first two were the new naturally occurring compounds. Karounidiol and 7-oxodihydrokarounidiol were detected in all of the investigated seed materials.

INTRODUCTION

Our recent study on the constituents of highly-polar fraction of the non-saponifiable lipids (NSL) obtained from the *Trichosanthes kirilowii* Maxim. seed extract has led to the isolation and structural elucidation of 12 novel triterpenes: nine multifloranes (D:C-friedo-oleananes) [1–5], one cucurbitane [6] and two cycloartanes [7]; and five novel hydroxylated sterols [8]. These triterpenes [5, 6, 9] and hydroxylated sterols [10] showed marked inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in mice. Moreover, karounidiol (**4**), a major component of the saponified neutral extract of *T. kirilowii* seeds, markedly suppressed TPA-induced tumour promotion following initiation by 7,12-dimethylbenz[*a*]anthracene in mice [9]. Our further study on the constituents of highly-polar fractions from the non-saponifiable lipid of the seed extracts from 10 species of Cucurbitaceae plants led to the isolation of two novel triterpenes, **1** and **2**, in addition to 12 known triterpenes **3**–**14** and an oxo sterol **15**. This paper describes the characterization of the novel triterpenes, **1** and **2**, as 7-oxodihydrokarounidiol-3-benzoate and isokarounidiol-3-*p*-methoxybenzoate, respectively, and the distribution of the compounds **1**–**15** in the extracts from 10 species of Cucurbitaceae plant seeds.

RESULTS AND DISCUSSION

Dried and ground seeds from 10 species of Cucurbitaceae plants were extracted with methanol. The non-saponifiable lipid obtained from the methanol extracts by alkaline hydrolysis were subjected to preparative TLC on silica gel which yielded highly-polar fractions containing dihydroxy triterpenes and their derivatives as the major components. The fractions were then acetylated and the resulting acetates were subjected to preparative reverse-phase HPLC, which allowed the isolation of 15 components as the acetates. Identification of the compounds, **3**–**9**, **11**–**13** and **15**, was performed by chromatographic (HPLC and GC) and spectral (MS and ^{1}H NMR) comparison as the acetyl derivatives with authentic compounds, whereas the other two compounds, **10** [11, 12] and **14** [13], were identified as the acetyl derivatives by NMR and mass spectral comparison with the literature data. Table 1 shows the names of the compounds and their chromatographic data as the acetyl derivatives. The characterization of two triterpenes, **1** and **2**, the natural occurrence of which has so far been unreported, is described below.

The mass spectrum of the acetate of **1** showed $[M]^+$ at *m/z* 602 ($C_{39}H_{54}O_5$) accompanied with the fragmentations at *m/z* 542 $[M-HOAc]^+$, 480 [M-HOC-

Table 1. Chromatographic data for the acetyl derivatives of oxygenated triterpene alcohols and a oxo sterol from Cucurbitaceae seeds

Code	Compound	Acetate RR_t HPLC I†	HPLC II‡	GC
1	7-Oxodihydrokarounidiol-3-benzoate [7-oxomultiflor-8-ene-3α,29-diol-3-benzoate]	0.12	0.10	no peak
2	Isokarounidiol-3-*p*-methoxybenzoate [multiflora-6,8-diene-3α,29-diol-3-*p*-methoxybenzoate]	0.40	0.36	no peak
3	Karounidiol-3-benzoate [multiflora-7,9(11)-diene-3α,29-diol-3-benzoate]	0.54	0.50	no peak
4	Karounidiol [multiflora-7,9(11)-diene-3α,29-diol]	0.34	0.30	4.32
5	Isokarounidiol [multiflora-6,8-diene-3α,29-diol]	0.25	0.25	4.22
6	5-Dehydrokarounidiol [multiflora-5,7,9(11)-triene-3α,29-diol]	0.23	0.22	4.71
7	7-Oxodihydrokarounidiol [7-oxomultiflor-8-ene-3α,29-diol]	0.06	0.06	8.28
8	Bryonolol [multiflor-8-ene-3β,29-diol]	0.40	0.40	4.94
9	3-Epibryonolol [muiltiflor-8-ene-3α,29-diol]	0.33	0.31	4.25
10	Loranthol [lup-20(30)-ene-3β,7β-diol]	0.25	0.21	3.02
11	Betulin [lup-20(30)-ene-3β,28-diol]	0.27	0.23	4.80
12	29-Hydroxylupeol [lup-20(30)-ene-3β,29-diol]	0.39	0.32	5.18
13	Erythrodiol [olean-12-ene-3β,28-diol]	0.31	0.28	4.09
14	(23*Z*)-Cycloart-23-ene-3β,25-diol	0.20	0.18	2.72
15	7-Oxositosterol [7-oxostigmast-5-en-3β-ol]	0.32	0.29	3.45

*Standard cholesterol acetate (RR_t = 1.00).
†Ultrasphere ODS column.
‡Superiorex ODS column.

OPh]$^+$ and 420 [M-HOAc-HOCOPh]$^+$. This, in combination with an acetyl methyl [δ_H 2.08 (*s*)] and a phenyl [δ_H 7.46 (2H, *ddt*, *J* = 7.4, 8.0, 1.4 Hz), 7.58 (1H, *ddt*, *J* = 7.4, 7.4, 1.4 Hz), and 8.02 (2H, *dt*, *J* = 8.0, 1.4 Hz)] signals in the ^{1}H NMR spectrum, suggested that it possessed acetoxyl and benzoxyl groups in the molecule. This compound was further shown to possess an equatorially oriented oxymethine [δ_H 4.95 (1H, *dd*, *J* = 2.5, 2.5 Hz); δ_C 77.7 (*d*)], an oxymethylene [δ_H 3.78 (2H, *s*); δ_C 74.7(*t*)], a ketone (δ_C 198.3) conjugated with a tetrasubstituted double bond [δ_C 142.9 (*s*) and 163.3 (*s*)] (λ 250 nm) [2], and seven tertiary methyl groups [δ_H 0.79, 0.96, 1.04, 1.08, 1.09, 1.20, and 1.40 (each *s*)]. The close similarity of the ^{1}H and ^{13}C NMR spectra of **1**-acetate with those of 7-oxodihydrokarounidiol (**7**) diacetate [2], with the exception of the signals arising from the functional group at C-3α, suggested that this possesses the structure 7-oxodihydrokarounidiol-3-benzoate-29-acetate. The diagnostic mass spectral fragmentations $[C_{26}H_{31}O_3]^-$ (*m/z* 391, ABC rings+C-26, C-27), $[C_{22}H_{28}O_3]^+$ (*m/z* 340, AB ring+C-11, C-12), $[C_{15}H_{22}O]^+$ (*m/z* 218; *m/z* 340-HOCOPh), and $[C_{17}H_{27}O_2]^+$ (*m/z* 263, DE ring+C-26, C-27 [2, 14] supported the proposed structure. On alkaline hydrolysis, **1**-acetate yielded a 3-benzoate **1** (*m/z* 560 [M]$^+$ $C_{37}H_{52}O_4$) and a diol **7**.

The mass spectrum of the acetate of **2** showed [M]$^+$ at *m/z* 616 ($C_{40}H_{56}O_5$). The fragmentations at *m/z* 464 [M-HOCOPhOMe]$^+$ and *m/z* 152 [HOCOPhOMe]$^+$ and the NMR signals at δ_C 165.7 (*s*; C-1′), 123.3 (*s*; C-2′), 131.5 [*d*; C-3′ and C-7′; δ_H 8.00 (2H, *d*, *J* = 8.8 Hz)], 133.7 [*d*; C-4′ and C-6′; δ_H 6.93 (2H, *d*, *J* = 8.8 Hz)], 163.3 (*s*; C-4′) and 55.5 [*q*; 5′-OMe; δ_H 3.86 (3H, *s*)] suggested the presence of a *p*-methoxybenzoxyl moiety in the molecule. This compound was further shown to possess an equatorially oriented oxymethine [δ_H 4.90 (1H, *dd*) *J* = 3.0, 3.0 Hz; δ_C 77.4 (*d*)], an acetoxymethylene [δ_H 3.80 (2H, *s*) and 2.08 (3H, *s*)], a disubstituted double bond [δ_H 5.76 (1H, *br dd*) *J* = 3.0, 9.6 Hz, and 6.10 (1H, *dd*) *J* = 3.0, 9.6 Hz] conjugated with a tetrasubstituted double bond [δ_C 136.5 (*s*) and 137.0 (*s*)] (λ 255 nm) [4], and seven tertiary methyl groups [δ_H 0.81, 1.00, 1.04, 1.05, 1.10, 1.18, and 1.20 (each *s*)]. The ^{1}H and ^{13}C NMR spectral data were very close to those of isokarounidiol (**5**) diacetate [4],

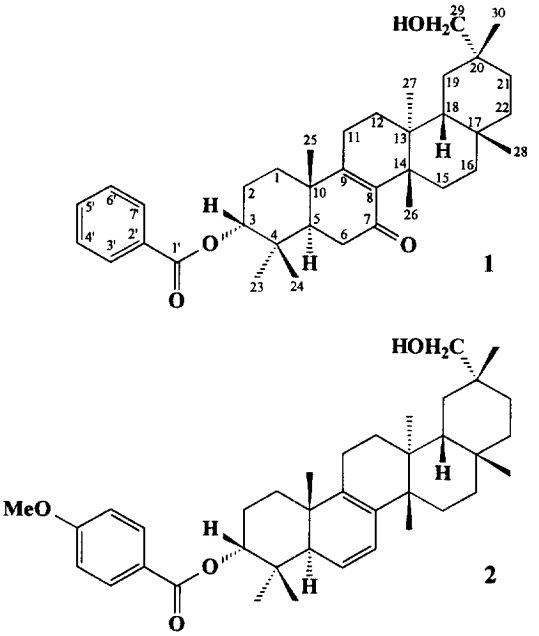

Fig. 1.

with the exception of the signals arising from the functionality at C-3α, and hence, the structure isokarounidiol-3-*p*-methoxybenzoate-29-acetate was proposed for **2**-acetate. The diagnostic mass spectral fragmentations $[C_{19}H_{25}]^+$ (*m/z* 253, ABC ring+C-26, C-27-HOCOPhOMe) and $[C_{15}H_{23}]^+$ (*m/z* 203, DE ring+C-26, C-27-HOAc) [4, 14] supported the proposed structure. Alkaline hydrolysis of **2**-acetate yielded a 3-*p*-methoxybenzoate **2** (*m/z* 574 [M]$^+$ $C_{38}H_{54}O_4$) and a diol **5**.

Table 2 shows the contents of the methanol extracts of the dried seed materials, and of the non-saponifiable lipid and the highly-polar fractions, separated therefrom, in the methanol extracts, which are expressed as weight per cent. Table 2 lists, in addition, the approximate abundances of individual components in the highly-polar fractions of the non-saponifiable lipid. The fractions from all of the Cucurbitaceae seeds examined contained two multiflorane-type tritepenes, karounidiol (**4**) and 7-oxodihydrokarounidol (**7**), as the common constituents. The C-3 benzoyl derivatives of these compounds also occur widely in the Cucurbitaceae seeds. Thus, the *Trichosanthes dioica* seed extract contained 7-oxodihydrokarounidiol-3-benzoate (**1**) as the most predominant component in the highly-polar fraction of the non-saponifiable lipid. This compound was further detected in *Benincasa cerifera*, *Cucumis melo*, *C. sativus*, *Cucurbita moschata* and *Trichosanthes cucumeroides* seed extracts. Karounidiol-3-benzoate (**3**) was present in the extracts of *Cucumis melo* and of six other seed extracts (Table 2). In addition, isokarounidiol-3-*p*-methoxybenzoate (**2**) was present in the *Citrullus battich* and *Momordica charantia* seed extracts. Since the fractions were obtained after alkaline hydrolysis in this study, the native extracts of the Cucurbitaceae seeds are expected to contain these 3-benzoyl and 3-*p*-methoxybenzoyl triterpene diols, **1**–**3**, more widely and abundantly than those shown in Table 2 which constitute the subject of future investigation. The possibility cannot be excluded that 3-*p*-methoxybenzoyl triterpene **2** was an artefact formed from the 3-*p*-hydroxybenzoyl derivative which may have been present in the seed materials during methanol extraction.

Among the 13 known compounds, **3**–**15**, isolated from the Cucurbitaceae seeds in this study, **3**[1], **4**[1], **5**[4], **6**[3], **7**[2] and **9**[6] have so far been isolated only from *Trichosanthes kirilowii* seed extract.

EXPERIMENTAL

Crystallizations were performed in Me_2CO–MeOH. Mp: uncorr. Prep. TLC on silica gel (Kieselgel 60G, Merck; 0.5 mm thick) was developed using *n*-hexane–EtOAc (4:1). HPLC: C_{18} silica columns [HPLC I: Beckman Ultrasphere ODS 5 μm column, 25 cm × 10 mm i.d., temp. 25°; HPLC II: Superiorex ODS S 5 μm column, 25 cm × 10 mm i.d. (Shiseido Co., Tokyo), temp. 25°], 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*$_f$ on HPLC and GC expressed relative to cholesterol acetate. UV spectra were recorded in EtOH. EIMS (70 eV): probe. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) were determined in $CDCl_3$ with TMS (^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as int. standard. Acetylation employed Ac_2O–pyridine at room temp. overnight. Hydrolysis of acetylated triterpenes: 2.5% KOH in MeOH at room temp. for 8 hr. Sources of the seed materials are described in the footnotes to Table 2. The following are the reference compounds used as the acetyl derivatives: **3** and **4**[1], **5**[4], **6**[3], **7**–**9**[2], **11** and **12**[15], **13**[16], and **15**[10]. The NMR signal assignments for the novel compounds **1**, **1**-acetate, **2** and **2**-acetate described below were performed by comparison with the lit. data for **5**[4] and **7**[2], and further with the aid of the following NMR experiments: ^{13}C DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC, NOE spectroscopy.

General isolation procedure. Air-dried and ground seeds were extracted × 3 for 3 days each with MeOH. The nonsaponifiable lipid obtained from the MeOH extract by alkaline hydrolysis (5% KOH in MeOH, reflux 3 hr) were subjected to prep. TLC which yielded a highly-polar (H-P) fr. recovered from the zones with R_f 0.02–0.27. Under the TLC conditions, cholesterol had R_f value of 0.32. The H-P fr., on acetylation, gave the acetate fr. Isolation of individual compounds as the acetyl derivatives was performed by prep. HPLC.

7-Oxodihydrokarounidiol-3-benzoate-29-acetate (**1**-*acetate*). Mp 94–98° (amorphous solid). UV λ_{max} nm (log ε): 250 (3.91). MS *m/z* (rel. int.): 602.3940 [M]$^+$ (5, $C_{39}H_{54}O_5$, requires 602.3968), 587.3766 (5, $C_{38}H_{51}O_5$), 542.3770 (3, $C_{37}H_{50}O_3$), 527.3484 (3, $C_{36}H_{47}O_3$), 480.3592 (3, $C_{32}H_{48}O_3$), 420.3434 (1, $C_{30}H_{44}O$), 391.2271 (2, $C_{26}H_{31}O_3$), 365.2115 (3, $C_{24}H_{29}O_3$),

Table 2. Cucurbitaceae seeds investigated, contents of methanol (MeOH) extracts, nonsaponifiable lipids (NSL) and highly-polar (H-P) fractions, and compositions (%) of the H-P fractions

Cucurbitaceae seeds	Source†	Contents (wt %) MeOH ext. (dried seeds)	NSL (MeOH ext.)	H-P Fr. (MeOH ext.)	Compositions (%)* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Others (unidentified)
Benincasa cerifera Savi. (wax gourd)	A	28.6	3.0	0.2	12			43	9	1	8	2	2	11						12
Citrullus battich Forskal (watermelon)	B	3.5	5.1	0.2		8	1	19	10	3	11	2		7			13		1	25
Coccinea grandis Voigt (ivy gourd)	C	n.d.‡	5.7	0.1				21			3				48	4	16		3	5
Cucumis melo L. (melon)	B	4.4	6.1	0.5	19		23	25	1	1	3	7		2						19
Cucumis sativus L. (cucumber)	D	4.8	8.9	0.9	14		9	43	6	1	8	4						2		13
Cucurbita moschata Dutch. (pumpkin)	B	2.4	2.0	0.4	1			87	2	1	1	1						2		5
Lagenaria leucantha Rusby. var. *Gourda* Makino (bottle gourd)	D	3.2	5.3	1.3			8	40	17		2			14						19
Momordica charantia L. (balsam pear)	D	4.0	3.9	0.9		7	17	19	6		8						1	7		35
Trichosanthes dioica Roxb. (painted gourd)	C	n.d.	8.4	0.1	56		6	12			4				10	6				6
Trichosanthes cucumeroides Maxim. (snake gourd)	A	20.1	1.1	0.2	4		13	43	4	1	17	2	1		6	2	3	1		3

*Determined based on the HPLC and GC data.

†A: purchased form Kinokuniya Kan-yaku Kyoku Co., Tokyo, Japan; B: cultivated locally at Yamagata, Japan; C: collected locally at West Bengal, India; D: purchased from Sakata Seeds, Co., Yokohama, Japan.

‡n.d. = not determined.

340.2034 (23, $C_{22}H_{28}O_3$), 263.1997 (17, $C_{17}H_{27}O_2$), 255.1729 (5, $C_{18}H_{23}O$), 243.1707 (20, $C_{17}H_{23}O$), 218.1686 (5, $C_{15}H_{22}O$), 203.1675 (13, $C_{11}H_{23}O_3$), 105.0354 (100, C_7H_5O). NMR δ_C and δ_H: C-1 [29.7; 1.62 (2H)], C-2 [23.0; 1.93, 2.04], C-3 [77.7; 4.95 (*dd*, $J = 2.5, 2.5$ Hz)], C-4 [37.0], C-5 [43.3; 2.28], C-6 [36.4; 2.40 (2H)], C-7 [198.3], C-8 [142.9], C-9 [163.3], C-10 [39.1], C-11 [22.1; 2.16, 2.35], C-12 [29.9; 1.42, 1.57], C-13 [38.1], C-14 [39.3], C-15 [29.7; 1.76, 2.45], C-16 [35.8; 1.38, 1.62], C-17 [31.2], C-18 [41.3; 1.62], C-19 [30.3; 1.46, 1.64], C-20 [32.0], C-21 [28.2; 1.42 (2H)], C-22 [38.5; 1.01, 1.45], C-23 [27.0; 0.96 (*s*)], C-24 [18.2; 1.09 (*s*)], C-25 [21.4; 1.08 (*s*)], C-26 [27.0; 1.40 (*s*)], C-27 [18.0; 0.79 (*s*)], C-28 [30.5; 1.20 (*s*)], C-29 [74.7; 3.78 (2H, *s*)], C-30 [26.1; 1.04 (*s*)], C-1′ [165.7], C-2′ [130.7], C-3′ and C-7′ [129.5; 8.02 (2H, *dt*, $J = 8.0, 1.4$ Hz], C-4′ and C-6′ [128.5; 7.46 (2H, *ddt*, $J = 7.4, 8.0, 1.4$ Hz)], C-5′ [133.0; 7.58 (*ddt*, $J = 7.4, 7.4, 1.4$ Hz)], 29-OCOMe [171.5], 29-OCOMe [21.0; 2.08 (*s*)]. On alkaline hydrolysis, **1**-acetate (7 mg) yielded **1** (2.5 mg) and **7** (2.0 mg).

7-Oxodihydrokarounidiol-3-benzoate (**1**). Mp 135–140° (amorphous solid). RR_t (HPLC II) 0.20. λ_{max} nm (log ε): 250 (4.03). MS *m/z* (rel. int.): 560.3831 $[M]^+$ (28, $C_{37}H_{52}O_4$, requires 560.3862), 545.3577 (11, $C_{36}H_{49}O_4$), 530.3738 (8, $C_{36}H_{50}O_3$), 529.3694 (5, $C_{36}H_{49}O_3$), 438.3486 (3, $C_{30}H_{46}O_2$), 423.3277 (3, $C_{29}H_{43}O_2$), 407.3275 (2, $C_{29}H_{43}O$), 365.2064 (5, $C_{24}H_{29}O_3$), 340.2053 (29, $C_{22}H_{28}O_3$), 271.2080 (3, $C_{19}H_{27}O$), 265.1947 (9, $C_{20}H_{25}$), 243.1746 (30, $C_{17}H_{23}O$), 221.1924 (45, $C_{15}H_{25}O$), 218.1715 (4, $C_{15}H_{22}O$), 105.0363 (100, C_7H_5O). NMR δ_C and δ_H: C-1 [29.8; 1.62 (2H)], C-2 [23.0; 1.93, 2.05], C-3 [77.7; 4.95 (*dd*, $J = 2.4, 2.4$ Hz)], C-4 [37.0], C-5 [43.3; 2.28], C-6 [36.4; 2.42 (2H)], C-7 [198.3], C-8 [142.9], C-9 [163.4], C-10 [39.2], C-11 [22.1; 2.15, 2.33], C-12 [29.8; 1.41, 1.62], C-13 [38.2], C-14 [39.3], C-15 [29.8; 1.76, 2.43], C-16 [35.9; 1.38, 1.62], C-17 [31.3], C-18 [41.4; 1.64], C-19 [29.8; 1.15, 1.50], C-20 [33.5], C-21 [28.0; 1.39 (2H)], C-22 [38.8; 1.10, 1.43], C-23 [27.0; 0.96 (*s*)], C-24 [21.4; 1.09 (*s*)], C-25 [18.2; 1.08 (*s*)], C-26 [27.0; 1.40], C-27 [18.0; 1.02], C-28 [30.5; 1.20], C-29 [74.2; 3.27 (*d*, $J = 10.3$ Hz), 3.32 (*d*, $J = 10.3$ Hz)], C-30 [25.8; 1.01 (*s*)], C-1′ [165.7], C-2′ [130.7], C-3′ and C-7′ [129.5; 8.02 (2H, *dt*, $J = 7.7, 1.1$ Hz)], C-4′ and C-6′ [128.5; 7.45 (2H, *ddt*, $J = 7.7, 7.3, 1.1$ Hz)], C-5′ [133.0; 7.57 (*ddt*, $J = 7.3, 7.3, 1.1$ Hz)].

Isokarounidiol-3-p-methoxybenzoate-29-acetate (**2**-*acetate*). UV λ_{max} nm (log ε): 263 (3.66). MS *m/z* (rel. int.): 616.4079 $[M]^+$ (1, $C_{40}H_{56}O_5$, requires 616.4124), 464.3608 (2, $C_{32}H_{48}O_2$), 449.3414 (4, $C_{31}H_{45}O_2$), 253.1904 (10, $C_{19}H_{25}$), 239.1832 (2, $C_{18}H_{23}$), 227.1845 (2, $C_{17}H_{23}$), 211.1530 (3, $C_{16}H_{19}$), 203.1802 (2, $C_{15}H_{23}$), 197.1313 (3, $C_{15}H_{17}$), 185.1349 (3, $C_{14}H_{17}$), 159.1139 (14, $C_{12}H_{15}$), 152.0542 (4, $C_8H_8O_3$), 149.1266 (8, $C_{11}H_{17}$), 135.0729 (16, $C_8H_7O_2$), 43.0538 (C_3H_7) and 43.0170 (C_2H_3O) (100). NMR δ_C and δ_H: C-1 [28.6; 1.52, 1.62], C-2 [23.3; 1.87, 1.98], C-3 [77.4; 4.90 (*dd*, $J = 3.0, 3.0$ Hz)], C-4 [36.8], C-5 [46.9; 2.52 (*dd*, $J = 3.0, 3.0$ Hz)], C-6 [125.1; 5.76 (*br dd*, $J = 3.0, 9.6$ Hz)], C-7 [125.6; 6.10 (*dd*, $J = 3.0, 9.6$ Hz)], C-8 [136.5], C-9 [137.0], C-10 [38.2], C-11 [19.9; 1.86 (2H)], C-12 [30.6; 1.35, 1.55], C-13 [37.4], C-14 [38.5], C-15 [29.0; 1.69, 1.74], C-16 [35.8; 1.42, 1.66], C-17 [31.2], C-18 [42.1; 1.60], C-19 [29.9; 1.26, 1.57], C-20 [31.8], C-21 [28.5; 1.42 (2H)], C-22 [38.0; 0.98, 1.52], C-23 [26.9; 1.00 (*s*)], C-24 [23.0; 1.10 (*s*)], C-25 [13.4; 0.81 (*s*)], C-26 [27.6; 1.20 (*s*)], C-27 [18.3; 1.05 (*s*)], C-28 [31.0; 1.18 (*s*)], C-29 [74.4; 3.80 (2H *s*)], C-30 [26.8; 1.04 (*s*)], C-1′ [165.7], C-2′ [123.3], C-3′ and C-7′ [131.5; 8.00 (2H, *d*, $J = 8.8$ Hz)], C-4′ and C-6′ [113.7; 6.93 (2H, *d*, $J = 8.8$ Hz)], C-5′ [163.3], 5′-OMe [55.5; 3.86 (*s*)], 29-OCOMe [171.6], 29-OCOMe [21.1; 2.08 (*s*)]. On alkaline hydrolysis, **2**-acetate (4 mg) yielded **2** (1.5 mg) and **5** (0.6 mg).

Isokarounidiol-3-p-methoxybenzoate (**2**). RR_t (HPLC II) 0.21. MS *m/z* (rel. int.): 574.3987 $[M]^+$ (6, $C_{38}H_{54}O_4$, requires 574.4019), 422.3489 (7, $C_{30}H_{46}O$), 253.2000 (3, $C_{19}H_{25}$), 239.1800 (8, $C_{18}H_{23}$), 227.1814 (7, $C_{17}H_{23}$), 211.1469 (6, $C_{16}H_{19}$), 197.1345 (7, $C_{15}H_{17}$), 185.1328 (22, $C_{14}H_{17}$), 171.1181 (16, $C_{13}H_{15}$), 152.0491 (8, $C_8H_8O_3$), 135.0497 (100, $C_8H_7O_2$). ^{1}H NMR δ_H: H-3 [4.90 (*dd*, $J = 3.0, 3.0$ Hz)], H-6 [5.75 (*br*, *dd*, $J = 3.3, 10.5$ Hz)], H-7 [6.10 (*dd*, $J = 3.3, 10.5$ Hz)], H-23 [1.00 (*s*)], H-24 [1.10 (*s*)], H-25 [0.81 (*s*)], H-26 [1.20 (*s*)], H-27 [1.08 (*s*)], H-28 [1.19 (*s*)], H-29 [3.27 (1H, *d*, $J = 10.9$ Hz), 3.37 (1H, *d*, $J = 10.9$ Hz)], H-30 [1.02 (*s*)], H-3′ and H-7′ [8.00 (2H, *d*, $J = 8.8$ Hz)], H-4′ and H-6′ [6.92 (2H, *d*, $J = 8.8$ Hz)], 5′-OMe [3.86 (*s*)].

Acknowledgements—We thank Mr Y Inoue for technical assistance.

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