Tumor-Promoting Diterpene Esters from Latex of *Euphorbia cauducifolia* L.

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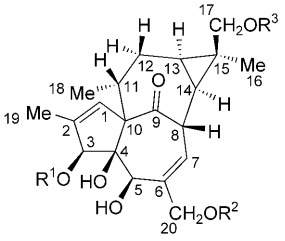
Tumor-promoting characteristics of seven esters, **1**–**7**, obtained from the latex of *Euphorbia cauducifolia* L. was appraised by carrying out NMRI mice back skin. The structures of **1**–**7** were elucidated by spectroscopic techniques like ^{1}H- and ^{13}C-NMR, 2D-NMR (HMQC, HMBC, HOHAHA (homonuclear *Hartmann–Hahn*), NOESY, and NOE), FT-IR, UV, and MS as esters of 17-hydroxyingenol, namely 17-[(2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoyloxy]ingenol (**1**), 3-*O*-angeloyl-17-[(2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoyloxy]ingenol (**2**), 3-*O*-acetyl-20-*O*-angeloyl-17-hydroxyingenol (**3**), 17-(acetyloxy)-3-*O*-angelyl-ingenol (**4**), 20-*O*-acetyl-3-*O*-angeloyl-17-hydroxyingenol (**5**), 3-*O*-angelyl-17-(benzoyloxy)ingenol (**6**) and 20-*O*-acetyl-3-*O*-angelyl-17-(benzoyloxy)ingenol (**7**). Compounds **1**–**4** were isolated for the first time, whereas **5**–**7** are known metabolites but detected for the first time in this plant. Biological investigations revealed that these compounds are tumor promoters.

Introduction. – The Euphorbiaceae family abunds in Pakistan, and is reported to contain tumor-promoting/retarding or antitumor compounds [1–9]. In addition, the extract of latex and roots has been used since long in the indigenous medicines for the treatment of cancer [10]. However, no special attention has been paid to this very important plant. This prompted us to investigate the latex of *Euphorbia cauducifolia,* which had shown tumor-promoting activity. In this report, the biological activity and structure elucidation by spectroscopic data of seven diterpene esters of 17-hydroxy-ingenol obtained from the acetone extract of *E. cauducifolia latex* are presented. Among these, the four compounds **1**–**4** are new and **5**–**7** are known but reported for the first time from this source.

Results and Discussion. – An acetone extract, of *E. cauducifolia* was separated by HPLC into compounds **1**–**7** (see *Exper. Part*). Their ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) showed close similarity to known esters of 17-hydroxyingenol and were identified as esters of 17-hydroxyingenol [11–16]. The relative configuration of all the stereogenic centers of **1**–**7** was determined from NOESY and NOE data [17–19]. Low δ



	R^1	R^2	R^3
1	H	H	deca-2,4,6-trienoyl
2	angeloyl	H	deca-2,4,6-trienoyl
3	Ac	angeloyl	H
4	angeloyl	H	Ac
5	angeloyl	Ac	H
6	angeloyl	H	PhCO
7	angeloyl	Ac	PhCO

values in ^{1}H- and ^{13}C-NMR spectra of **1**–**7** for H–C(3), H–C(5), CH_2(17), and CH_2(20)[1]) indicate the presence of an OH group at these C-atoms, whereas high δ values of these protons, point to an acyloxy residue at these C-atom, such as an angeloyloxy, benzoyloxy, acetyloxy or (2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoyloxy group. Their position of attachment was deduced from HMBC experiments [20–22].

Table 1. *^{1}H-NMR Data of Compounds* **1**–**4**[1]). δ in ppm, *J* in Hz.

	1	**2**	**3**	**4**
H–C(1)	6.08 (*d*, *J* = 1.5)	6.02 (*d*, *J* = 1.5)	6.08 (*d*, *J* = 1.5)	6.02 (*d*, *J* = 1.5)
H–C(3)	3.65 (*s*)	5.45 (*s*)	5.95 (*s*)	5.45 (*s*)
H–C(5)	3.85 (br. *s*)	3.77 (br. *s*)	3.80 (br. *s*)	3.98 (br. *s*)
H–C(7)	6.21 (*d*, *J* = 3.7)	6.05 (*d*, *J* = 3.7)	6.12 (*d*, *J* = 3.7)	6.09 (*d*, *J* = 3.7)
H–C(8)	4.2 (*dd*, *J* = 3.7, 15)	4.06 (*dd*, *J* = 3.7, 15)	4.23 (*dd*, *J* = 3.7, 15)	4.06 (*dd*, *J* = 3.7, 15)
H–C(11)	2.60 (*ddq*, *J* = 3.5, 7)	2.59 (*ddq*, *J* = 3.5, 7.0)	2.66 (*ddq*, *J* = 3.5, 7)	2.59 (*ddq*, *J* = 3.5, 7.0)
CH_2(12)	2.25 (*dd*, *J* = 16, 5.5), 2.61 (*dd*, *J* = 16, 3.5)	2.22 (*dd*, *J* = 16, 5.5), 2.71 (*dd*, *J* = 16, 3.5)	2.23 (*dd*, *J* = 16, 5.5), 2.71 (*dd*, *J* = 16, 3.5)	2.23 (*dd*, *J* = 16, 5.5), 2.71 (*dd*, *J* = 16, 3.5)
H–C(13)	1.02 (*ddd*, *J* = 9, 6, 8)	1.08 (*ddd*, *J* = 9, 6, 8)	1.02 (*ddd*, *J* = 9, 6, 8)	1.09 (*ddd*, *J* = 9.6, 8.0)
H–C(14)	1.31 (*dd*, *J* = 11.6, 8.5)	1.26 (*dd*, *J* = 11.6, 8.5)	1.21 (*dd*, *J* = 11.6, 8.5)	1.26 (*dd*, *J* = 11.6, 8.5)
Me(16)	1.07 (*s*)	1.18 (*s*)	1.09 (*s*)	1.28 (*s*)
CH_2(17)	4.23 (*d*, *J* = 12.5), 4.26 (*d*, *J* = 12.5)	4.23 (*d*, *J* = 12.5), 4.26 (*d*, *J* = 12.5)	4.30 (br. *s*)	4.23 (*d*, *J* = 12.5), 4.26 (*d*, *J* = 12.5)
Me(18)	0.94 (*d*, *J* = 7.4)	0.98 (*d*, *J* = 7.4)	0.94 (*d*, *J* = 7.4)	0.98 (*d*, *J* = 7.4)
Me(19)	1.77 (*d*, *J* = 1.5)	1.78 (*d*, *J* = 1.5)	1.77 (*d*, *J* = 1.5)	1.78 (*d*, *J* = 1.5)
CH_2(20)	4.09 (br. *s*)	4.09 (br. *s*)	4.42 (*d*, *J* = 12.5), 4.74 (*d*, *J* = 12.5)	4.09 (br. *s*)
R^1	H	angeloyl	Ac	angeloyl
R^2	H	H	angeloyl	H
R^3	deca-2,4,6-trienoyl	deca-2,4,6-trienoyl	H	Ac

In the HR-EI-MS, compound **1** exhibited a molecular-ion peak at *m/z* 512.2634 corresponding to $C_{30}H_{40}O_7$ and indicated the presence of eleven double-bond equivalents. In the EI-MS, **1** displayed a peak at *m/z* 346 ($[M-166]^+$) typical for a monoester of deca-2,4,6-trienoic acid. The ^{13}C-NMR (APT and DEPT) spectra of **1** showed 30 C-atoms with the multiplicities of 4 Me, 5 CH_2, and 14 CH groups and 7 quaternary C-atoms of which 7 are oxygenated, consistent with the presence of one ketone, one primary, two secondary, and one tertiary alcohol, and one ester group. Further data established the structure of **1** as 17-[(2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoyloxy]ingenol.

In the ^{1}H-NMR spectrum of **1**, the protons of the deca-2,4,6-trienoyl group displayed the following signals: H–C(4) at δ 7.48 (*dd*, *J* = 15, 11.3 Hz), H–C(5) at δ 6.79 (*dd*, *J* = 15.2, 11.4 Hz), H–C(3) at δ 6.64 (*dd*, *J* ≈ 11, 11 Hz), H–C(6) at δ 6.19 (br. *dd*, *J* ≈ 11.0, 11 Hz), H–C(7) at δ 5.68 (*dt*, *J* = 11.5, 7.5 Hz), H–C(2) at δ 5.63 (*d*, *J* = 11.06 Hz), CH_2(8) at δ 2.21 (br. *q*, *J* = 7.1 Hz), CH_2(9) at δ 1.43 (*sext.*, *J* = 7.3 Hz), and Me(10) at δ 0.93 (*t*, *J* = 7.4 Hz). The ^{13}C-NMR spectrum indicated the conjugated CH=CH moieties of this acyl group in the region δ 128–145 for C(2) to C(7), besides signals at δ 13.7 (C(10)), 22.9 (C(9)), 30.3 (C(8)) and 166.7 (C(1)), consistent with the data of (2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoic acid reported in [23–27]. The CH_2(17) resonated at δ(H) 4.22 and 4.28 as an *AB* system with ^2J = 12.6 Hz and at δ(C) 66.4. Thus, it is concluded that the OH group at C(17) is esterified. This was confirmed by the HMBC spectrum of **1**, which showed a cross-peak at δ(C) 166.93 (C(1)=O of deca-2,4,6-trienoyl) with δ(H) 4.22 (CH_2(17)).

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[1]) Trivial numbering, for systematic names, *see Exper. Part.*

Table 2. *^{13}C-NMR Chemical Shifts of Compounds* **1**–**7**[1]). δ in ppm.

	1	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	131.65 (*d*)	131.25 (*d*)	131.65 (*d*)	131.65 (*d*)	131.65 (*d*)	131.65 (*d*)	131.65 (*d*)
C(2)	136.25 (*s*)	136.8 (*s*)	136.25 (*s*)	136.25 (*s*)	136.65 (*s*)	136.25 (*s*)	136.25 (*s*)
C(3)	80.23 (*d*)	84.74 (*d*)	84.08 (*d*)	84.18 (*d*)	83.62 (*d*)	84.38 (*d*)	83.92 (*d*)
C(4)	74.74 (*s*)	74.16 (*s*)	74.14 (*s*)	74.17 (*s*)	74.28 (*s*)	74.45 (*s*)	74.36 (*s*)
C(5)	74.96 (*d*)	73.96 (*d*)	74.96 (*d*)	74.46 (*d*)	74.32 (*d*)	74.16 (*d*)	74.26 (*d*)
C(6)	136.60 (*s*)	136.83 (*s*)	136.60 (*s*)	136.70 (*s*)	136.74 (*s*)	136.46 (*s*)	136.26 (*s*)
C(7)	128.38 (*d*)	128.5 (*d*)	128.38 (*d*)	128.28 (*d*)	127.99 (*d*)	128.39 (*d*)	128.08 (*d*)
C(8)	42.85 (*d*)	42.05 (*d*)	42.85 (*d*)	42.85 (*d*)	42.38 (*d*)	42.85 (*d*)	42.82 (*d*)
C(9)	205.19 (*d*)	207.19 (*d*)	205.19 (*d*)	205.99 (*d*)	204.95 (*d*)	205.71 (*d*)	205.39 (*d*)
C(10)	71.97 (*s*)	71.67 (*s*)	71.87 (*s*)	71.93 (*s*)	71.69 (*s*)	71.87 (*s*)	71.77 (*s*)
C(11)	37.67 (*d*)	37.15 (*d*)	37.67 (*d*)	37.657 (*d*)	37.55 (*d*)	37.63 (*d*)	37.61 (*d*)
C(12)	35.15 (*t*)	34.9 (*t*)	35.17 (*t*)	35.13 (*t*)	35.17 (*t*)	35.12 (*t*)	35.25 (*t*)
C(13)	29.08 (*d*)	29.39 (*d*)	29.18 (*d*)	29.38 (*d*)	29.45 (*d*)	29.28 (*d*)	29.78 (*d*)
C(14)	29.39 (*d*)	29.08 (*d*)	29.69 (*d*)	29.59 (*d*)	29.78 (*d*)	29.39 (*d*)	29.59 (*d*)
C(15)	30.08 (*s*)	29.71 (*s*)	29.98 (*s*)	30.16 (*s*)	29.95 (*s*)	29.78 (*s*)	29.98 (*s*)
C(16)	24.71 (*q*)	24.8 (*q*)	24.71 (*q*)	24.77 (*q*)	24.69 (*q*)	24.87 (*q*)	24.87 (*q*)
C(17)	66.84 (*t*)	66.19 (*t*)	62.21 (*t*)	66.32 (*t*)	62.47 (*t*)	66.32 (*t*)	66.32 (*t*)
C(18)	18.19 (*q*)	18.52 (*q*)	18.39 (*q*)	18.59 (*q*)	18.80 (*q*)	18.69 (*q*)	18.21 (*q*)
C(19)	15.52 (*q*)	16.13 (*q*)	15.52 (*q*)	15.32 (*q*)	15.99 (*q*)	15.95 (*q*)	15.92 (*q*)
C(20)	62.34 (*t*)	62.23 (*t*)	66.38 (*t*)	62.37 (*t*)	65.52 (*t*)	62.32 (*t*)	66.34 (*t*)

Compound **2** is an angelic acid (=(2*Z*)-2-methylbut-2-enoic acid) derivative of **1**, displaying a peak at *m/z* 594.3224 ($C_{35}H_{46}O_8^+$) in the HR-EI-MS. The EI-MS of **2** displayed peaks at *m/z* 494 ($[M-100]^+$) and 428 ($[M-166]^+$) disclosing the presence of both an ester of angelic acid and an ester of (2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoic acid [27], the former being located at C(3). The spectral data showed that **2** is isomeric with a compound isolated previously from *E. ingens* [14].

The ^{13}C-NMR (APT and DEPT) spectra of **2** showed 35 C-atoms, namely 6 Me, 5 CH_2, 15 CH, and 9 quaternary C-atoms, of which 8 are oxygenated (one ketone, one primary, one secondary, and one tertiary alcohol, and two ester groups). The HMBC spectrum displayed a cross-peak correlation between the C(1)=O of the angeloyl moiety and H–C(3), consistent with the proposed structure.

Compounds **3**–**5** are constitutional isomers with the composition $C_{27}H_{36}O_8$ (HR-MS: *m/z* 472.2459). The EI-MS of these compounds showed peaks at *m/z* 412 ($[M-60]^+$) and 372 ($[M-100]^+$) indicating an acetic acid and an angelic acid derived ester unit in each case. The location of these ester groups was deduced by HMBC correlations between their carbonyl C-atom and the H-atom at the oxygenated ring C-atom bearing the ester groups. The spectral data established that **3** and **4** are novel compounds, while **5** has been isolated before from a different source [15].

The physical and UV, IR, mass, and NMR data of compounds **6** and **7** are identical to those reported for 3-*O*-angeloyl-17-(benzoyloxy)ingenol and 20-*O*-acetyl-3-*O*-angeloyl-17(benzoyloxy)ingenol, respectively [16].

Biological Activity of Compounds **1**–**7**. All the compounds **1**–**7** were highly toxic on the back skin of mice, with $ID_{50} < 10^{-10}$ μg/ml for **1**, $< 10^{-9}$ μg/ml for **2**, $< 10^{-6}$ μg/ml for **3**, 10^{-4} μg/ml for **4**, 10^{-6} μg/ml for **5**, $< 10^{-6}$ μg/ml for **6**, and 12.02 μg/ml for **7**.

Compounds **1**–**7** were evaluated for co-carcinogenic and tumor-promoting activity on the back skin of NMRI mice, and all exhibited a weak tumor-promoting activity. The results are presented in *Table 3*. Pure compounds **1**–**7**, with unsaturated acids such as (2*Z*,4*E*,6*Z*)-deca-2,4,6-trienoic acid, angelic acid, and benzoic acid tested without initiation possessed week tumor-promoting activity in comparison to TPA. After 24 weeks, an average tumor rate of 7% and an average tumor yield of 0.07 tumors/mouse were noticed. After 36 weeks, an average tumor rate of 36% was observed and the average tumor yield was 0.45 tumors/mouse.

Table 3. *Tumor-Promoting and Solitary Carcinogenic Activities of Compounds* **1**–**7**

	Duration [h]	Single dose [nmol]	Tumor rate[a])	Tumor rate[b])	Tumor yield[c])
1	48	20	0/23	4/23	5/23
		5[d])	0/27	1/27	1/26
2	42	80	0/27	1/26	1/26
3	44	10	5/28	20/28	85/27
4	40	2	5/28	20/28	84/28
5	40	5	5/28	20/28	84/28
6	45	10	0/27	1/27	1/26
7	40	7	3/27	5/27	1/26
TPA	48	5	3/27	20/27	112/27

[a]) After initiation: $i = 100$ nmol of DMBA (=7,12-dimethylbenz[*a*]anthracene) for promotion; twice weekly dose ρ of the promoter. [b]) Average tumor rate: number of tumor-bearing animals/number of the survivors of the group. [c]) Average tumor yield: number of tumors/numbers of survivors of the group. [d]) Number of initiation with DMBA; $i = 0.1$ ml of acetone.

Experimental Part

General: HPLC: *Perkin-Elmer* HPLC equipment, *RP-18* column. M.p.: *Gallenkamp* melting point apparatus. Optical rotation: digital polarimeter supplied by *OSK OGAWA Seiki Co. Ltd*, Japan. UV Spectra: in abs. MeOH; *CE-5501* double-beam spectrophotometer from *Cecil Company*; λ_{max}(log ε) in nm. IR Spectra: nujol mulls; *Perkin-Elmer-1605* FT-IR spectrophotometer; in cm^{-1}. ^{1}H-and ^{13}C-NMR (300 and 75 MHz) Spectra: *Bruker-Biospin-AMX* 300-MHz FT NMR spectrometer; δ in ppm, *J* in Hz.. Mass spectra: double-focusing *Finnigan-MAT-112* spectrometer; in *m/z* (rel. %). HR-MS: *Jeol-HX-110* spectrometer; computerized mass measurements or peak-matching experiments with PFK as internal standard.

Plant Material. Euphorbia cauducifolia latex was collected from Karachi University Campus, Karachi, Pakistan. A voucher specimen was deposited in the Herbarium of Karachi University, Department of Botany, Karachi, Pakistan.

Extraction and Isolation. The latex was shaken with MeOH (4 times) under N_2 yielding MeOH-soluble material. After evaporation of the extract, the semi-solid material (199 g) was suspended in MeOH/H_2O 9:1 and extracted with acetone. The acetone-soluble material was subjected to *Craig*'s distribution in petroleum ether/MeOH/H_2O 30:20:1). The later was further distributed by a second *Craig*'s distribution in CCl_4/MeOH/H_2O (40:20:3) ($z = 30$, $V = 100$ ml/100 ml, $n = 33$) into a hydrophilic fraction ($r = 24$–30, $\sigma = 1$–3, (4.6%) $LD_{50}^{25} = 1.9$ nmol/ear. A third *Craig*'s distribution in petroleum ether/MeOH/H_2O 30:20:1 ($z = 1020$, $V = 10$ ml/10 ml, $n = 2300$, single withdrawal procedure) yielded 63.65 g of a hydrophilic fraction.

The hydrophilic fraction (60.5 g) was subjected to open-air column chromatography (silica gel, $CHCl_3$/MeOH). The obtained highly active fraction (2.3 g) was separated by HPLC (*RP-18*, MeCN/H_2O gradient ($A = H_2O$; $B =$ MeCN/H_2O 88:12; 5 min *A*, linear gradient to *B* in 20 min, 5 min *B*, linear gradient back to *A* in 2 min), flow 1 ml/min): **1**–**7**.

17-[(2Z,4E,6Z)-Deca-2,4,6-trienoyloxy]ingenol (=*[(1*R,*1a*R,*2*S,*5*R,*5a*R,*6*S,*8a*S,*9*R,*10a*R*)-1a,2,5,5a,6,9,10,10a-Octahydro-5,5a,6-trihydroxy-4-(hydroxymethyl)-1,7,9-trimethyl-11-oxo-1*H*-2,8a-methanocyclopenta[*a*]cyclopropa[*e*]cyclodecen-1-yl]methyl (2*Z*,4*E*,6*Z*)-Deca-2,4,6-trienoate;* **1**): Resin. $[\alpha]_D^{25} = +16.8$ ($c = 0.17$, $CHCl_3$). UV (MeOH): 306.5 (4.26). IR ($CHCl_3$): 3534, 3514, 3523, 3480, 2950, 2914, 1740, 1725, 1552. ^{1}H- and ^{13}C-

NMR: *Tables 1* and *2*. EI-MS: 512 (0.3, M^+), 346 (0.6), 363 (0.9), 450 (1.8), 432 (4.2), 414 (2), 381 (11), 364 (7), 328 (6), 310 (10), 292 (10), 187 (17), 166 (23), 160 (15), 151 (22), 149 (34), 133 (13), 123 (14), 83 (100). HR-EI-MS: 512.2634 ($C_{30}H_{40}O_7^+$; calc. 512.2589).

3-O-*Angeloyl-17-[(2*Z*,4*E*,6*Z*)-Deca-2,4,6-trienoyloxy]ingenol* (=*[(1*R*,1a*R*,2*S*,5*R*,5a*S*,6*S*,8a*S*,9*R*,10a*R*)-1a,2,5,5a,6,9,10,10a-Octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,7,9-trimethyl-6-[(2*Z*)-2-methyl-1-oxobutoxy]-11-oxo-1*H*-2,8a-methanoyclopenta[a]cyclopropa[e]cyclodecen-1-yl]methyl (2*Z*,4*E*,6*Z*)-Deca-2,4,6-trienoate;* **2**): Resin. $[\alpha]_D^{25} = +17.8$ ($c = 0.17$, $CHCl_3$). UV (MeOH): 308.5 (4.28), 214 (4.26). IR ($CHCl_3$): 3534, 3514, 3523, 2950, 2914, 1740, 1725, 1552. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2.* EI-MS: 594 (0.3, M^+), 494 (10), 454 (13), 412 (1.2), 428 (15), 394 (16), 369 (12), 372 (8), 371 (6), 354 (3), 323 (23), 330 (3), 312 (23), 306 (18), 294 (19), 284 (17), 251 (13), 221 (13), 188 (19), 162 (25), 153 (27), 151 (23), 135 (33), 122 (84), 121 (52), 83 (100). HR-EI-MS: 594.2463 ($C_{35}H_{46}O_8^+$;calc. 594.3223).

3-O-*Acetyl-20*-O-*angeloyl-17-hydroxyingenol* (=*[(1*R*,1a*R*,2*S*,5*R*,5a*S*,6*S*,8a*S*,9*R*,10a*R*)-6-(Acetyloxy)-1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-1-(hydroxymethyl)-1,7,9-trimethyl-11-oxo-1*H*-2,8a-methanoyclopenta[a]cyclopropa[e]cyclodecen-4-yl]methyl (2*Z*)-2-Methylbut-2-enoate*; **3**): Resin. $[\alpha]_D^{25} = +17.8$ ($c = 0.17$, $CHCl_3$). UV (MeOH): 218.5 (4.26). IR ($CHCl_3$): 3534, 3514, 3523, 2950, 2914, 1740, 1725, 1552. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2.* EI-MS: 472 (0.3, M^+), 454 (1.3), 429 (31), 412 (1.2), 394 (1.6), 387 (11), 372 (22), 371 (6), 354 (3), 330 (3), 312 (23), 294 (19), 284 (17), 251 (13), 221 (13), 188 (19), 162 (25), 153 (27), 151 (23), 135 (33), 122 (84), 121 (52), 83 (100). HR-EI-MS: 472.2463 ($C_{27}H_{36}O_8^+$; calc. 472.2559).

17-(Acetyloxy)-3-O-*angeloyl-ingenol* (=*[(1*R*,1a*R*,2*S*,5*R*,5a*S*,6*S*,8a*S*,9*R*,10a*R*)-1-(Acetyloxy)-1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,7,9-trimethyl-11-oxo-1*H*-2,8a-methanoyclopenta[a]cyclopropa[e]cyclodecen-6-yl]methyl (2*Z*)-2-Methylbut-2-enoate*; **4**): Resin. $[\alpha]_D^{25} = +16.8$ ($c = 0.27$, $CHCl_3$). UV (MeOH): 216.5 (4.26). IR ($CHCl_3$): 3534, 3514, 3523, 3480, 2950, 2914, 1740, 1725, 1552. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2.* EI-MS: 472 (3, M^+), 454 (2), 414 (2), 413 (9), 412 (9), 387 (23), 372 (6), 354 (14), 340 (12), 330 (1), 329 (2), 312 (21), 294 (11), 252 (7), 221 (8.2), 189 (18), 162 (42), 151 (34), 135 (34), 122 (82), 121 (55), 95 (93), 83 (100). HR-EI-MS: 472.2465 ($C_{27}H_{36}O_8^+$; calc. 472.2559).

20-O-*acetyl-3*-O-*angeloyl-17-hydroxyingenol* (**5**): Resin. $[\alpha]_D^{25} = +24.8$ ($c = 0.17$, $CHCl_3$). UV (MeOH): 218.5 (4.26). IR ($CHCl_3$): 3534, 3514, 3523 3480, 2950, 2914, 1740, 1725, 1552. ^{13}C-NMR: *Table 2.* EI-MS: 472 (3, M^+), 454 (2), 414 (5), 413 (10), 412 (14), 389 (18), 372 (16), 354 (14) 330 (19), 329 (23), 312 (21), 294 (11), 252 (37), 221 (18), 189 (38), 162 (42), 151 (34), 135 (34), 122 (82), 121 (55), 95 (93), 83 (100). HR-EI-MS: 472.2463 ($C_{27}H_{36}O_8^+$; calc. 472.2559).

3-O-*Angeloyl-17-(benzoyloxy)ingenol* (**6**): Resin. $[\alpha]_D^{25} = +1.33$ ($c = 0.07$, $CHCl_3$). UV (MeOH): 224.5 (4.26) 270.5 (2.99) 284.5 (2.82). IR ($CHCl_3$): 3534, 3514, 3523, 3480, 2950, 2914, 1740, 1725, 1552. ^{13}C-NMR: *Table 2.* EI-MS: 550 (3, M^+), 532 (6), 472 (9), 467 (12), 445 (23), 450 (7), 432 (14), 414 (12), 410 (12), 328 (16), 310 (10), 292 (10), 187 (17), 160 (15), 151 (22), 133 (13), 123 (14), 122 (20), 105 (54), 83 (100). HR-EI-MS: 550.2561 ($C_{32}H_{38}O_8^+$; calc. 472.2559).

20-O-*Acetyl-3*-O-*angeloyl-17-(benzoyloxy)ingenol* (**7**): Resin. $[\alpha]_D^{25} = +14.8$ ($c = 0.07$, $CHCl_3$). UV (MeOH): 224.5 (4.26), 272.5 (2.99), 282.5 (2.78). IR ($CHCl_3$): 3534, 2950, 2914, 1740, 1725, 1440, 1373, 1263, 1552. ^{13}C-NMR: *Table 2.* EI-MS: 592 (3, M^+), 532 (8), 490 (7), 492 (9), 487 (34), 470 (15), 467 (11), 445 (17), 432 (6), 428 (19), 414 (9), 370 (14), 328 (16), 310 (21), 292 (17), 282 (18), 264 (12), 189 (17), 151 (40), 133 (17), 123 (24), 122 (26), 105 (75), 83 (100). HR-EI-MS: 592.2675 ($C_{34}H_{40}O_9^+$; calc. 592.6744).

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