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ANTITUMOR AGENTS, 82.[1] CYTOTOXIC SAPOGENOLS FROM *AESCULUS HIPPOCASTANUM*

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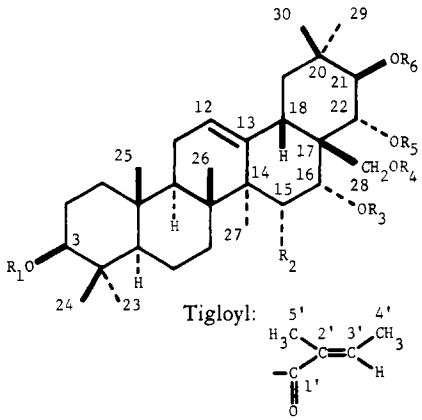
ABSTRACT.—Two cytotoxic sapogenols, the new hippocaesculin (**1**) and the known barringtogenol-C 21-angelate (**2**), were isolated from the acid hydrolysis product of a crude saponin fraction that was obtained from the fruits of *Aesculus hippocastanum*. The structures of **1** and **2** were determined from their chemical transformations and spectral data. Compound **1** is either 21-*O*-angeloyl, 22-*O*-tigloyl R_1-barrigenol, or 21-*O*-tigloyl, 22-*O*-angeloyl R_1-barrigenol.

The fruit of *Aesculus hippocastanum* L. (horse chestnuts, Hippocastanaceae) has been used as an herbal remedy for the treatment of mammary indurations and cancer (1). Prior phytochemical studies (2-5) on this plant have yielded a saponin, aescin, which is a mixture of the acylated glycosides of protoaesigenin and barringtogenol-C, with angelic, tiglic, and acetic acids as the acyl groups. As a result of our continuing searches among medicinal plants for novel, naturally occurring, potential antitumor agents, the acid hydrolyzed product of an *n*-BuOH extract of the fruits of *A. hippocastanum* was found to show significant in vitro cytotoxicity in the 9-KB (human nasopharyngeal carcinoma) cell culture assay (6). Bioassay-directed fractionation of the foregoing active extract led to the isolation and characterization of two cytotoxic sapogenols, the new hip-



	R_1	R_2	R_3	R_4	R_5	R_6	
1	H	OH	H	H	angeloyl*	tigloyl*	
2	H	H	H	H	H	angeloyl	
3	H	OH	H	H	H	H	(R_1-barrigenol)
4	H	H	H	H	H	H	(barringtogenol-C)
5	Ac	OAc	H	Ac	angeloyl*	tigloyl*	
6	Ac	OAc	H	Ac	Ac	Ac	
7	H	$-O(CH_3)_2C-$		$-(CH_3)_2C-$		H	
8	Ac	$-O(CH_3)_2C-$		$-(CH_3)_2C-$		Ac	
9	H	OH	H	H	angeloyl	angeloyl	
10	Ac	H	H	Ac	Ac	angeloyl	
11	Ac	H	H	Ac	Ac	Ac	
12	H	H	H	$-(CH_3)_2C-$		H	

*In these compounds, the angeloyl and the tigloyl moieties are interchangeable.

[1]For part 81, see N. Fukamiya and K.H. Lee, *J. Nat. Prod.*, **49**, 348 (1986).

pocaesculin [**1**, ED_{50} (KB)=3.6 μg/ml] and the known barringtogenol-C 21-angelate [**2**, ED_{50} (KB)=3.0 μg-ml]. Compound **2** was previously isolated from the leaves of *Pittosporum tobira* (7); however, its cytotoxicity is revealed for the first time.

On alkaline hydrolysis, **1** and **2** gave the known sapogenins R_1-barrigenol (**3**) (7) and barringtogenol-C (**4**) (7), respectively. Compounds **3** and **4** were inactive in the KB test system (ED_{50}≧ μg/ml), but the acetonide derivative (**7**) of **3** showed significant cytotoxicity with an ED_{50}=4.0 μg/ml.

RESULTS AND DISCUSSION

On acid hydrolysis, the crude saponin fraction that was obtained from a methanolic extract of fruits of *A. hippocastanum* afforded a mixture of sapogenols. The mixture was chromatographed repeatedly on a silica gel column and purified by preparative tlc to give **1** and **2**.

Compound **1**, $C_{40}H_{62}O_8$, showed ir absorption bands (1690, 1640, and 1260 cm^{-1}) due to an α, β-unsaturated ester. Compound **1** gave a triacetate (**5**) upon acetylation.[2] Upon hydrolysis with 3% KOH in MeOH, **1** afforded a triterpene **3**. The ^{13}C-nmr spectrum of **3** (Table 1) showed the presence of five secondary alcohols at δ78.1, 67.5, 72.4, 78.4, 77.2, and one primary alcohol at δ67.8. Compound **3** was converted to a pentaacetate (**6**), a diacetonide (**7**), and a diacetonide diacetate (**8**) by usual methods. Compounds **3**, **7**, and **8** exhibited prominent mass spectral peaks at *m/z* 298, 378, and 420, respectively, due to a retro Diels-Alder cleavage of ring-C in each compound. The characteristic pairs of fragments corresponding to the release of acetonide moieties from rings D and E of **7** and **8** were also observed at *m/z* 320 and 262 as well as

TABLE 1. ^{13}C-nmr Spectra[a] of **1**, **2**, **3**, and **4**

Carbon No.	Compounds				Carbon No.	Compounds			
	1	**3**	**2**	**4**		**1**	**3**	**2**	**4**
1	39.3[b]	39.3	39.2	39.1	21	79.0	78.4	82.0	78.6
2	28.2	28.2	28.1	28.1	22	73.6	77.2	73.3	77.2
3	78.1	78.1	78.1	78.0	23	28.7	28.8	28.7	28.7
4	39.3[b]	39.4	39.4	39.3	24	16.6	16.6	16.6	16.5
5	55.6	55.7	55.8	55.8	25	15.9	16.0	15.8	15.8
6	19.1	19.2	18.8	18.8	26	17.6	17.7	17.0	17.0
7	36.8	36.8	33.3	33.2	27	21.1	21.1	27.4	27.4
8	41.5	41.5	40.1	40.1	28	63.1	67.8	66.2	68.4
9	47.3	47.5	47.2	47.1	29	29.5	30.6	29.8	30.5
10	37.4	37.4	37.5	37.2	30	20.1	19.4	20.4	19.4
11	24.0	24.1	23.9	23.9	1′	168.1		168.6	
12	125.5	124.5	123.1	122.9	2′	129.4		129.5	
13	143.6	144.7	143.5	143.8	3′	136.7		135.9	
14	47.8	47.5	41.9	42.0	4′	15.6		15.9	
15	67.6	67.5	34.4	34.2	5′	21.1		21.0	
16	73.3	72.4	67.9	67.8	1″	167.9			
17	48.4	48.2	48.2	47.2	2″	129.1			
18	41.0	42.1	40.5	41.2	3″	137.0			
19	46.9	47.9	47.9	48.2	4″	12.4			
20	36.5	36.4	36.1	36.3	5″	13.9			

[a]Recorded in pyridine-d_5. Chemical shifts are in δ (ppm). The signal assignments were referred to DEPT experiments.

[b]These signals were overlapping.

[2]The lack of acetylation of the OH at C-16 is due to its steric hindrance with Me-14, H_α-19, and H_α-21.

362 and 304, respectively. The foregoing evidence suggested that one of the six hydroxyl groups was located at either ring A or B. The remainder were at rings D and E, and among them, four form two pairs of α-glycols. The presence of two pairs of doublets at δ4.39 (H_β-15) and 4.93 (H_β-16) with J=6.7 Hz, and δ3.69 (H_β-22) and 4.12 (H_β-21) with J=10.1 Hz (Table 2) in the ^{1}H-nmr spectrum of **7** confirmed this latter assignment, and led to the placement of these five hydroxyl groups at C-15, C-16, C-21, C-22, and C-28. A comparison of the ^{1}H-nmr, ^{13}C-nmr, and ms data between **3** and R_1-barrigenol (8-10) established their identity.

The presence of one tigloyl and one angeloyl ester group in **1** was suggested by the characteristic ^{1}H-nmr signals for the acid portion of these esters at δ6.80 (1H, m, tigloyl H-3′) and 1.76 (3H, d, J=6.7 Hz, tigloyl Me-3′), as well as δ6.09 (1H, m, angeloyl H-3′) and 1.92 (3H, d, J=7.0 Hz, angeloyl Me-3′). This was further supported by the prominent mass peaks in **1** at *m/z* 462, 362, and 262, which were due to the D/E ring residue and its subsequent release of the acyl moiety as shown in Table 3.

That the two acyl groups were located at C-21 and C-22 was based upon the fact that in the ^{1}H-nmr spectrum of the triacetate **5**, H-21 and H-22 were shown as two pairs of doublets at δ5.87 and 5.54, respectively, whereas in the R_1-barrigenol pentaacetate **6**, the two pairs of doublets were shifted upfield to δ5.66 (H-21) and 5.37 (H-22). The other protons attached to carbons bearing acetyl groups at C-15, C-16, and C-28 were seen at δ5.10, 4.25, and 3.77 and 3.93, respectively, in **5** and at δ5.09, 4.18, and 3.72 and 3.92, respectively, in **6** and were comparable in their chemical shifts (Table 2).

Since the ^{13}C-nmr spectrum of **1** (hippocaesculin) was superimposable with that of 21, 22-di-*O*-angeloyl R_1-barrigenol (**9**) obtained from the fruits of *Xanthoceras sorbifolia* (11), except for the acyl moieties (Table 1), this led to the confirmation of the structure of hippocaesculin as depicted in **1**, in which the angeloyl and the tigloyl groups located at the C-22 and C-21 positions are interchangeable.[3]

Compound **2** possessed four secondary alcoholic groups (δ78.1, 67.9, 82.0, and 73.3) and one primary alcohol (δ66.2) as shown in its ^{13}C-nmr spectrum and gave a triacetate **10** upon acetylation. Alkaline hydrolysis of **2** yielded the known sapogenin **4**(7), which in turn was converted to a tetraacetate (**11**) and a monoacetonide (**12**) by standard methods. A comparison of the ^{1}H-nmr spectra of **11** and **12** with those reported in the literature (12, 13) led to the characterization of **4** as barringtogenol-C.

The presence of an angeloyl group in **2** was revealed by an ir band at 1680 (α,β-unsaturated ester) cm-^{1}; five carbon signals at δ168.6 (C-1′), 129.5 (C-2′), 135.9 (C-3′), 15.9 (C-4′), and 21.0 (C-5′) in the ^{13}C-nmr spectrum (Table 1); and by a diagnostic mass peak at *m/z* 264 [(a)-angeloyl as shown in Table 3] in the mass spectrum. A comparison of the ^{13}C-nmr spectra between **2** and **4** showed that the signals of C-21 and C-22 in **2** were shifted downfield by 3.4 ppm and upfield by 3.9 ppm, respectively, compared to those in **4**, due to an acylation shift (Table 1). Thus, compound **2** was characterized as 21-*O*-angeloyl barringtogenol-C based upon evidence described above.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. Ir spectra were measured on Perkin-Elmer 1320 ir spectrometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker 250 MHz spectrometer using TMS as an internal standard. Mass spectra were determined on a V. G. Micromass 70-70 instrument at 70 eV using a direct inlet system. Silica gel (Merck, type G 60, 70-230 mesh) was used for column chromatography, and precoated silica gel plates were used for analytical (Merck, 60 F-254, 0.25 mm) and preparative (Analtech, G, 1000 m) tlc. Compounds were visualized by uv light or spraying with 15% H_2SO_4 solution followed by heating.

[3]Structural determination of **1** by single crystal X-ray crystallographic analysis is in progress.

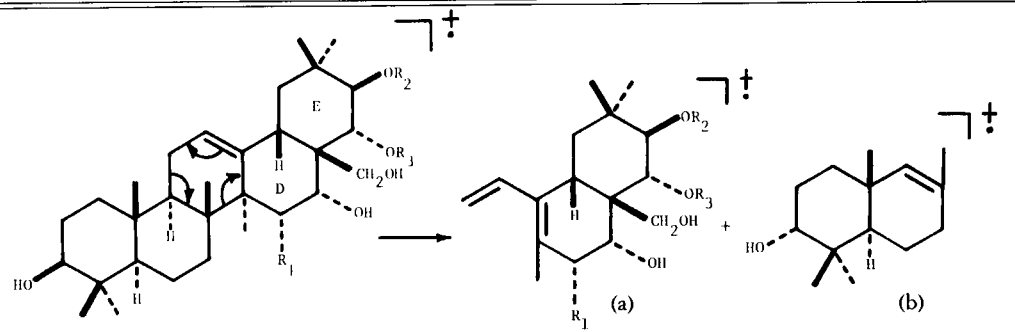
TABLE 2. Nmr Spectral Data of **5, 6, 7, 8, 10, 11,** and **12**[a]

Compounds	H_α-3	H-12	H_β-15	H_β-16	H_α-21[b]	H_β-22[b]	H_2-28	Acyl moieties
5	4.50 t-like	5.49 t-like	5.10 d, J=3.7	4.25 brs	5.87 d, J=10.3	5.54 d, J=10.3	3.77, 3.93 ABd, J=11.7	6.80(1H, m), 5.98 (1H, dq, J=7.0, 1.5) 1.74 (3H, d, J=7.0), 1.91 (3H, d, J=7.0) 1.80 (6H, s), 2.04, 2.07, 2.10 (each 3H, s, $COCH_3\times3$)
6	4.50 t-like	5.50 t-like	5.09 d, J=3.9	4.18 brs	5.66 d, J=10.2	5.37 d, J=10.2	3.71, 3.92 ABd, J=11.7	2.02 (6H, s), 2.05, 2.07, 2.09 (each 3H, s,) ($COCH_3\times5$)
7	3.20 t-like	5.32 t, J=3.4	4.39 d, J=6.7	4.93 d, J=6.7	4.12 d, J=10.1	3.69 d, J=10.1	3.41, 3.48 ABd, J=11.8	
8	4.48 t-like	5.32 t-like	4.38 d, J=7.0	4.93 d, J=7.0	5.59 d, J=10.5	3.82 d, J=10.5	3.40, 3.49 ABd, J=11.8	2.04, 2.05 (each 3H, s, $COCH_3\times2$)
10	4.50 t, J=8.0	5.37 t-like		4.20 brs	5.66 d, J=10.2	5.45 d, J=10.2	3.68 (2H) brs	6.05 (1H, dq, J=9.0, 1.5), 1.94 (3H, d, J=9.0), 1.86 (3H, brs), 2.05 (9H, s, $COCH_3\times3$)
11	4.50 t, J=8.0	5.36 t-like		4.18 brs	5.53 d, J=10.2	5.41 d, J=10.2	3.66 (2H) brs	2.01, 2.02, 2.05, 2.06 (each 3H, s, $COCH_3\times4$)
12	3.21 t-like	5.29 t-like		4.78 brs	4.15 d, J=10.2	3.73 d, J=10.2	3.38, 3.58 Bd, J=11.7	

[a]Chemical shifts are shown in δ-values (ppm) with coupling constants (J) in Hz. The abbreviations brs, d, t, dq, and m refer to broad singlet, doublet, triplet, doublet of quartet, and multiplet, respectively.

[b]H_α-21 is shifted downfield by the adjacent O_α-16 compared to H_β-22.

TABLE 3. Mass Spectral Fragmentation of **1**, **2**, **3**, and **4**



Compounds					M^+-H_2O					(a)-H_2O				
	R_1	R_2	R_3	M^+	M^+-$2H_2O$	M^+-acyl	(a)	(a)-H_2O	(a)-acyl	-acyl	(b)	(b)-H_2O	base peak
1	OH	Angeloyl[a] tigloyl		670[b]	652 634	570 470	462	444	362 262	344 244	207	189	83
3	OH	H	H	506	488 470 452		298	280 262			207	189	43
2	H	Angeloyl	H	572	554 536	472	364	346	264	246	207	189	83
4	H	H	H	490	472 454		282	264 246			207	189	215

[a]Angelic acid or tiglic acid: $CH_3CH=(CH_3)COOH$ *m/z* 100 and $CH_3CH=C(CH_3)CO$- *m/z* 83.
[b]*m/z*.

PLANT MATERIAL.—The fruits of *A. hippocastanum* were purchased in January 1985, from Wilcox Drug Company, Inc., Boone, North Carolina. A voucher specimen was placed on file at Wilcox Drug Co.

EXTRACTION OF CRUDE SAPONIN FRACTION.—The crushed fruit of *A. hippocastanum* (7.5 kg) was defatted with *n*-hexane (5 liters×2) at room temperature and extracted with hot MeOH (5 liters×3). The MeOH solution was concentrated in vacuo to yield a residue that was then suspended in H_2O (1.2 liters). The suspended aqueous layer was washed with Et_2O and EtOAc, and extracted with *n*-BuOH. Evaporation of the *n*-BuOH extract under reduced pressure gave the crude saponin fraction as a yellowish gum (230 g).

ACID HYDROLYSIS OF THE CRUDE SAPONIN FRACTION.—A solution of *n*-BuOH extract (6 g) in 1 N HCl-EtOH (1:1, 110 ml) was refluxed for 2 h. The reaction mixture was diluted with 30 ml of H_2O and concentrated to the half volume in vacuo to afford a brown precipitate. The precipitate was washed with H_2O and dried to yield white powder (2 g). Repeated column chromatography of this white powder on silica gel [MeOH-$CHCl_3$ (5:95)], followed by recrystallization of the resulting major fraction from MeOH/H_2O furnished colorless needles of **1** (23 mg, 0.00031% yield) and **2** (35 mg, 0.00047% yield).

Compound **1**.—Mp 254-256°; $[\alpha]_D$=+25° (c, 0.53, MeOH); ir (KBr) cm^{-1} 3400 (OH), 1690, 1640, and 1260; ^{1}H-nmr δ0.79, 0.91, 0.94 (3H each, s, Me×3), 0.99 (6H, Me×2), 1.08, 1.38 (3H each, s, Me×2), 1.76 (3H, d, J=6.7 Hz, tigloyl Me-3′), 1.92 (3H, d, J=7.0 Hz, angeloyl Me-3′), 1.80 (6H, br s, tigloyl, and angeloyl Me-2′×2), 6.09 (1H, m, angeloyl H-3′), and 6.80 (1H, m, tigloyl H-3′); ^{13}C-nmr (see Table 1); ms *m/z* 670 (M^1, weak), 652.4337 [calcd for $C_{40}H_{60}O_7$ (M^+-H_2O): 652.4369]; other peaks, see Table 3.

Compound **2**.—Mp 242-244°; $[\alpha]_D$=+31° (c, 0.62, MeOH); lit (7) reported mp 252-254° and $[\alpha]_D$=+31.7° (MeOH) for 21-*O*-angeloylbarringtogenol-C; ir (KBr) cm^{-1} 3400 (OH), 1680, 1260; ^{1}H-nmr δ0.79, 0.90, 0.94, 1.02, 1.01, 1.43 (21 H, s each, Me x 7), 1.95 (3H, br s, angeloyl Me-2′), 2.01 (3H, d, J=7.1 Hz, angeloyl Me-3′), and 6.09 (1H, dq, J=7.1 and 1.5 Hz, angeloyl H-3′); ^{13}C-nmr (see Table 1); ms, see Table 3.

ISOLATION OF COMPOUNDS **3** AND **4**.—The white powder (500 mg) obtained by acid hydrolysis of the crude saponin fraction was hydrolyzed with 3% KOH in MeOH (50 ml) under reflux for 1 h. The reaction mixture was concentrated and extracted with EtOAc. The organic layer was washed with H_2O and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo to dryness to afford 200 mg of yellow powder. This powder was chromatographed on silica gel [$CHCl_3$-MeOH-H-$_2O$ (9:1:0.1), lower layer] to afford two major fractions. These fractions were purified by recrystallization from MeOH/H_2O to afford compound **3** (R_1-barrigenol) (10) and compound **4** (barringtogenol-C) (13) as colorless needles.

Compound **3**.—Mp >300°; $[\alpha]_D$=+38° (c, 0.54, MeOH); lit (7) reported mp 310-312° and $[\alpha]_D$=+40.7° (dioxane); ir (KBr) cm^{-1} 3400 (OH); ^{1}H-nmr (pyridine-d_5)[4] δ0.99, 1.07, 1.12, 1.24, 1.36, 1.41, 1.88 (3H each, s, Me×7), 3.49 (1H, br s, H_α-3), 3.79, 4.13 (2H, ABq, J=10.4 Hz, H_2-28), 4.45 (1H, d, J=4.5 Hz, H_β-15), 4.63 (1H, d, J=9.6 Hz, H_β-22), 4.84 (1H, d, J=9.6 Hz, H_α-21), 4.98 (1H, d, J=4.5 Hz, H_β-16), and 5.54 (1H, t-like, H-12); ^{13}C-nmr (see Table 1); ms *m/z* 506.3621 [calcd for $C_{30}H_{50}O_6$ (M^+): 506.3605]; other peaks, see Table 3.

Compound **4**.—Mp 275-280°; $[\alpha]_D$=+27° (c, 0.51, MeOH); ir (KBr) cm^{-1} 3400 (OH); ^{1}H-nmr (pyridine-d_5) δ0.97, 0.98, 1.06, 1.24, 1.34. 1.40, 1.86 (3H each, s, Me×7), 3.49 (1H, br s, H_α-3), 3.75, 4.05 (2H, ABd, J=10.0 Hz, H_2-28), 4.64 (1H, d, J=9.5 Hz, H_β-22), 4.80 (1H, d, J=9.5 Hz, H_α-21), and 5.45 (1H, t-like, H-12); ^{13}C-nmr see Table 1; ms *m/z* 490.3654 [calcd for $C_{30}H_{50}O_5$ (M^+): 490. 3655], other peaks, see Table 3.

GENERAL PROCEDURE FOR ACETYLATION.—The acetates described below were prepared by acetylation of compounds **1-4** and **7** in Ac_2O in pyridine at room temperature for 12-18 h followed by usual work up and purification by either preparative tlc or column chromatography to yield the pure compounds.

Triacetate **5**.—Compound **5** (34 mg) was prepared from **1** (60 mg) as colorless needles: mp 263-265°; ir (KBr) cm^{-1} 1730 (acetyl CO), 1690, 1650 (α,β-unsaturated ester), 1250 (RCOOC); ^{1}H-nmr, see Table 2. *Anal.* Found: C 69.64; H, 8.62; calcd for $C_{46}H_{68}O_{11}$: C. 69. 32; H. 8.60%.

Pentaacetate (**6**, *R_1-barrigenol pentaacetate*).—Compound **6** (20 mg) was prepared from **3** (54 mg) as white amorphous powder: ir ($CHCl_3$) cm^{-1} 3660 (OH, weak), 1740 (acetyl CO), 1260 (RCOOC); ^{1}H-nmr, see Table 2, (10). *Anal.* Found: C, 66.77; 8.32; calcd for $C_{40}H_{60}O_{11}$: C, 67.01%; H, 8.44%.

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[4]Lit (10) reported ^{1}H-nmr (pyridine-d_5, 100 MHz) spectrum of R_1-barrigenol at δ0.99, 1.06, 1.12, 1.24, 1.35, 1.39, 1.86 (3H, each s), 3.44 (1H, t-like, H_α-3), 3.80, 4.12 (2H, ABq, J=11.0 Hz, H_2-28), 4.44 (1H, d, J=4.0 Hz, H_β-15), 4.96 (1H, d, J=4.0 Hz, H_β-16), 4.61 (1H, d, J=10.0 Hz, H_β-22), and 4.81 (1H, d, J=10.0 Hz, H_α-21).

Triacetate **10**.—Acetylation of **2** (60 mg) yielded **10** (24 mg) as colorless needles: mp 276-278°; ir (KBr) cm^{-1} 1740, 1720 (acetyl CO), 1690 (α,β-unsaturated ester), 1240 (RCOOC); ^{1}H-nmr, see Table 2. *Anal.* Found: C, 70.15%; H, 8.69%; calcd for $C_{41}H_{62}O_9$: C, 70.45; H, 8.94%.

Tetraacetate (**11**, *barringtogenol tetraacetate*) (*12, 13*).—Acetylation of **4** (45 mg) gave **11** (22 mg) as colorless needles: mp 223-225°; ir ($CHCl_3$) cm^{-1} 3660 (OH, weak), 1730 (acetyl CO), 1250 (RCOOC); ^{1}H-nmr, see Table 1). *Anal.* Found: C, 69.53; H, 8.68; calcd for $C_{38}H_{58}P_9$: C, 69.27; H, 8.87%.

Diacetonide diacetate (**8**) (7).—Compound **8** (25 mg) was obtained from the diacetonide (**7**, 50 mg) as white amorphous powder: ir ($CHCl_3$) cm^{-1} 1730 (acetyl CO), 1250 (RCOOC), 1030 (C-O-C); ^{1}H-nmr, see Table 2; ms *m/z* 655 (M^+-Me), 612 (M^+-Me_2CO), 554 (M^+-2×Me_2CO), 610 (M^+-HOAc), 420 (DE ring residue), 362 (420-Me_2CO), 304 (420-2×Me_2CO), 250 (AB ring residue), 190 (AB-HOAc).

Acetonide of **3**.—To a solution of **3** (100 mg) in anhydrous Me_2CO (50 ml) was added 2,2-dimethoxypropene (1 ml) and *p*-toluenesulfonic acid (15 mg). The mixture was stirred at room temperature for 2 h; then 3 drops of pyridine was added and evaporated in vacuo to yield a residue. The residue was dissolved in a trace of MeOH and poured into ice H_2O. The resulting precipitate was washed with H_2O, purified by column chromatography on neutral alumina, and recrystallized from Me_2CO to afford the diacetonide (**7**, 55 mg) as colorless prisms: ir ($CHCl_3$) cm^{-1} 3600 (OH, weak) and 1030 (C-O-C), ^{1}H-nmr, see Table 2; ms *m/z* 586 (M^+, weak), 571 (M^+-Me), 528 (M^+-Me_2CO), 470 (M^+-2×Me_2CO), 378 (DE ring residue), 320 (378-Me_2CO), 262 (378-2×Me_2CO), 207 (AB ring residue).

Acetonide of **4**.—Treatment of **4** (100 mg) in an analogous manner to that described above for the preparation of **7**, afforded a monoacetonide (**12**, 40 mg) as amorphous powder: ir ($CHCl_3$) cm^{-1} 3500 (OH, weak) and 1030 (C-O-C); ^{1}H-nmr, see Table 2. *Anal.* Found: C, 74.56; H, 10.35; calcd for $C_{33}H_{54}O_5$: C, 74. 67; H, 10.26%.

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