New Dammarane Triterpenes from the Aerial Parts of *Ibicella lutea* Grown in Argentina

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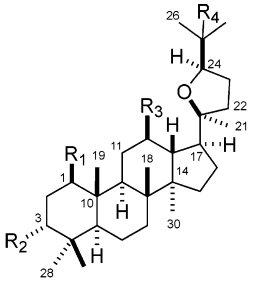
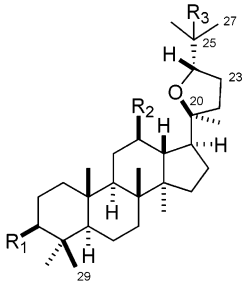
Two new dammarane triterpenes have been isolated from the aerial parts of *Ibicella lutea*, 3-acetyl-24-*epi*-polacandrin (**1**) and 1,3-diacetyl-24-*epi*-polacandrin (**2**), and were obtained along with the known compounds 20*S**,24*S**-epoxy-3β,12β,25-trihydroxydammarane (**3**) and apigenin. The structures of compounds **1** and **2** were deduced by spectroscopic studies and by chemical transformations leading to the preparation of derivatives **4**−**11**. The structure and relative stereochemistry of **1** were verified by single-crystal X-ray analysis. The natural compounds and selected semisynthetic derivatives were evaluated against several tumor cells.

The small family Martyniaceae comprises just three genera (*Martynia*, *Proboscidea*, and *Ibicella*) with 13 reported species.[1] *Ibicella lutea* (Lindl.) Van Eselt (Martynaceae Stampf) is a quasi-carnivorous plant that is native to tropical and subtropical zones of America and grows wild in Argentina, where it is used as a food.[2] This plant, which has an unpleasant smell, grows freely in sandy places, and the body of its seed capsule is covered with short spines. In several places, it is called "cuerno del diablo" (devil's horn), and its infusion is used in Uruguay in popular medicine as an antimicrobial for the treatment of eye and skin infections.[3] In previous studies on the aerial parts of *I. lutea*, a stearic acid glycoside [11-*O*-(6′-*O*-acetyl-β-D-glucopyranosyl)stearic acid] was the only constituent isolated and identified. This compound was also shown to be responsible for the antibacterial activity found in the chloroform extract of this species.[1]

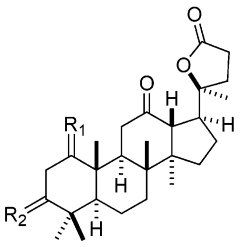
In view of the pharmaceutical and chemotaxonomic interest of this species, we investigated the aerial parts of two samples of *I. lutea* from different locations in Argentina. In this paper we describe the isolation and structure elucidation of two new dammarane triterpenes, namely, 3-acetyl-24-*epi*-polacandrin (**1**) and 1,3-diacetyl-24-*epi*-polacandrin (**2**), both of which are acetylated epimeric derivatives at C-24 of polacandrin, a cytotoxic principle isolated from *Polanisia dodecandra*.[4] The two new compounds were isolated along with the known compound 20*S**,24*S**-epoxy-3β,12β,25-trihydroxydammarane (**3**) and the flavonoid apigenin. Furthermore, the cytotoxicity of compounds **1** and **2** and several derivatives, which were prepared to confirm the proposed structures, was evaluated against a panel of tumor cell lines.



	R_1	R_2	R_3	R_4
1	OH	OAc	OH	OH
2	OAc	OAc	OH	OH
4	OH	OH	OH	OH
6	OAc	OAc	OAc	OH
7	OAc	OAc	OAc	OAc



	R_1	R_2	R_3
3	OH	OH	OH
9	OAc	OH	OH
10	OAc	OAc	OH
11	OAc	OAc	OAc



	R_1	R_2
5	O	α-OAc
8	H_2	O
12	β-OAc	α-OAc

Results and Discussion

An acetone extract of the dried and chopped aerial parts of *I. lutea* (collected in the Province of San Luis, Argentina) was concentrated, dissolved in MeOH−H_2O (7:3), and partitioned between hexane and $CHCl_3$. The $CHCl_3$ extract was chromatographed repeatedly to afford two pure compounds, **1** and **2**. The aerial parts of *I. lutea* (collected in the Province of Mendoza, Argentina) were treated in a similar manner, and compound **3** was isolated along with the flavonoid apigenin[5] and a mixture of common sterols.

Compound **1** was isolated as colorless prisms, and its IR spectrum showed typical absorptions at 3488, 3303, and 1088 cm^{-1} due to hydroxyl groups and an ether moiety. The terpenoid nature of this compound was deduced from the ^{13}C NMR and DEPT-135 spectra, which showed evidence of 32 carbon atoms: nine methyls, eight methylenes, eight methines (four oxygenated), and seven quaternary (two oxygenated and one carbonyl) carbons, which is consistent with a triterpene structure bearing one acetate

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Table 1. ^{1}H NMR (500 MHz) Chemical Shifts (ppm) and Selected HMBC and NOESY Correlations for Compounds **1** and **2** in $CDCl_3$

	1			**2**		
C	δ_H mult. (*J* in Hz)	HMBC (H → C)	NOESY	δ_H mult. (*J* in Hz)	HMBC (H → C)	NOESY
1	3.62 ddd (10.6, 5.4, 5.4)		H-2α, 9	4.75 dd (10.7, 4.9)		
2α	1.85 m,	C-3, 4	H-1	1.76 m		
2*β*	1.75 m		H-3	1.71 m		H-3
3	4.68 t (3.0)	C-1	H-2*β*, 28	4.66 t (3.0)		H-2*β*, 28
5	1.20 m	C-4, 10	H-9, 28	1.22 m	C-10	H-9, 28
6	1.52 m	C-10		1.42 m	C-8, 10	
7α	1.50 m		H-30	1.49 m		H-30
7*β*	1.27 m			1.23 m		
9	1.74 m	C-1, 8, 10	H-1	1.68 m		H-5
11α	2.67 ddd (11.3, 4.2, 4.2)		H-11	1.71 m	C-12	
11*β*	1.28 m			1.15 m		
12	3.55 ddd (11.3, 11.3, 4.2)		H-11α, 17, 30	3.41 ddd (10.0, 10.0, 4.5)		
13	1.63 t (11.3)	C-11, 12, 14, 17, 20	H-18	1.54 t (10.0)	C-12	
15α	1.54 m	C-13, 17		1.49 m		
15*β*	1.08 dd (11.5, 8.0)			1.05 m		
16α	1.95 m			1.89 m		
16*β*	1.23 m			1.23 m		
17	2.16 ddd (10.9, 10.9, 3.0)	C-20	H-21, 30, 12	2.09 br t (10.1)		H-21, 30
18	0.97 s	C-8, 9	H-13, 11*β*	0.92 s	C-7, 8, 14	H-13, 11*β*
19	0.91 s	C-1, 5, 9, 10	H-2*β*	0.97 s	C-1, 9, 10	
21	1.24 s	C-17, 20	H-17, 24	1.19 s	C-17, 20, 22	H-17, 24
22	1.85 m	C-23		1.56 m		
	1.62 m			1.11 m		
23	1.97 m	C- 24, 25		1.95 m		
	1.81 m			1.79 m		
24	3.81 dd (8.6, 6.7)		H-21	3.75 dd (8.8, 6.8)		H-21
26	1.24 s	C-24, 25, 27		1.19 s	C-25	
27	1.06 s	C-24, 25		1.02 s	C-24, 25	
28	0.78 s	C-3, 4, 5, 29	H-3, 5	0.76 s	C-3, 4, 29	H-3, 5
29	0.86 s	C-3, 4, 5, 28	H-5	0.84 s	C-3, 4	
30	0.91 s	C-8, 15	H-7α, 12, 17	0.84 s	C-8, 13, 14, 15	H-7α, 17
Ac	2.05 s			2.03 s, 1.92 s		
OH	5.40 br s			5.23 br s		
	3.88 br s					

group. This information, along with the pseudomolecular ion $[M + H]^+$ at *m/z* 535 in the (+)-LRFABMS ($[M - H]^-$ at *m/z* 533 in the negative FABMS mode), suggested a molecular formula of $C_{32}H_{54}O_6$ and, consequently, six degrees of unsaturation. This molecular formula was corroborated by (+)-HRFABMS, which showed the $[M + H]^+$ peak at *m/z* 535.3999 (calcd 535.3999). Furthermore, the characteristic peaks at *m/z* 235, 223, 218, 205, 203, and 189 in the LREIMS of **1** suggested a pentacyclic or tetracyclic triterpenoid structure.[6]

The presence of nine singlet methyl groups was corroborated by the ^{1}H NMR spectrum of **1**, including the acetate methyl group at δ_H 2.05. The spectrum also contained four downfield signals corresponding to four oxymethine protons in the molecule. ^{1}H−^{1}H gCOSY and gHMQC experiments were used for the assignment of all proton resonances, as shown in Tables 1 and 2. Thus, the triplet at δ_H 4.68 (J = 3.0 Hz), the doublet of doublets at δ_H 3.81 (J = 8.6 and 6.7 Hz), and two doublets of doublets of doublets at δ_H 3.62 (J = 10.6, 5.4, and 5.4 Hz) and δ_H 3.55 (J = 11.3, 11.3, and 4.2 Hz) were assigned to H-3, H-24, H-1, and H-12, respectively. The presence in **1** of hydroxyl groups at C-1 and C-12 and an acetoxy group at C-3 was deduced from the ^{13}C NMR signals at δ_C 78.8 (d, C-3), 75.5 (d, C-1), and 71.1 (d, C-12). A gHMBC experiment allowed the gross planar structure shown to be assembled, with the complete proton and carbon assignments given in Tables 1 and 2. The proton data, along with a comparison of the carbon resonances of **1** with those of related triterpenes, showed that this compound possesses a dammarane-type skeleton.[7] In addition, the presence of the oxymethine carbon C-24 at δ_C 85.3 (d) and the oxygenated quaternary carbon resonance at δ_C 86.4 (C-20, s) in the ^{13}C NMR spectrum of **1**, along with the base peak at *m/z* 143 (see ion **A** in Scheme S1, Supporting Information)[6] in the EIMS and (+)-LRFABMS, indicated the existence of a 20,-24-epoxy-25-hydroxydammarane-type derivative.

The relative stereochemistry of **1** was elucidated by a NOESY experiment (see Table 1 and Figure 1), coupling constant analysis, and comparison of the spectroscopic data with those of a known analogue.[8,9] The observation of cross-peaks in the NOESY spectrum between H-1 and the protons H-2α (1.85 m) and H-9 at 1.74 ppm provided evidence for the *β*-configuration of the hydroxyl group at C-1. Furthermore, NOESY correlations between H-3 and the protons H-2*β* at δ_H 1.75 and H-28 at δ_H 0.78 indicated an α-configuration of the acetoxy group at C-3. The *β*-orientation of the hydroxyl group at C-12 was deduced by the NOESY correlations from H-12 to the protons Me-30 and H-11α and by the two large axial−axial coupling constants exhibited by the H-12 proton (1H, $J_{11ax-12}$ = 11.3 Hz, $J_{13ax-12}$ = 11.3 Hz, $J_{11eq-12}$ = 4.2 Hz). Interestingly, a sharp broad singlet at δ_H 5.40 in the ^{1}H NMR spectrum is indicative of an intramolecular hydrogen bond between the hydroxyl group at C-12 and the oxygen atom of the tetrahydrofuran ring, a situation that is very characteristic of a 20,24-epoxy-12*β*,25-dihydroxydammarane-type derivative.[9] On the other hand, the ^{13}C NMR resonance for C-24 in **1** appeared at δ 85.3 (d), and this is consistent with the data reported for 24*R** isomers.[7,10] This fact was substantiated by the NOESY cross-peak between the signal of H-24 and the protons of Me-21. In an analogous fashion, a 20*S** relative stereochemistry was established by the strong NOESY correlation between the protons of Me-21 and H-17 and this, in turn, with the protons of Me-30. Comparison of all of these spectroscopic data with those of known triterpene analogues suggested a structure similar to polacandrin, a cytotoxic principle isolated from *Polanisia*

Table 2. ^{13}C NMR Chemical Shifts (δ_C mult. in ppm) for Compounds **1**−**10** in $CDCl_3$ (multiplicity by DEPT)

C	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**9**[b]	**10**[b]	**11**[b]
1	75.5 d	78.4 d	38.8 t	74.8 d	211.1 s	78.8 d	78.8 d	39.2 t	38.6 t	39.5 t	39.1 t
2	34.1 t	29.2 t	27.3 t	36.3 t	39.2 t	29.1 t	29.0 t	33.7 t	23.6 t	23.5 t	23.5 t
3	78.8 d	77.9 d	78.3 d	76.4 d	80.9 d	78.0 d	77.9 d	216.7 s	80.7 d	80.5 d	80.6 d
4	36.5 s	36.5 s	38.8 s	37.3 s	37.3 s	38.9 s	36.5 s	47.3 s	37.8 s	37.8 s	37.8 s
5	49.8 d	49.9 d	55.8 d	48.3 d	45.6 d	49.9 d	49.8 d	56.0 s	56.0 d	55.8 d	55.8 d
6	18.2 t	18.1 t	18.2 t	18.4 t	18.5 t	17.9 t	17.8 t	19.6 t	18.1 t	18.0 t	18.0 t
7	34.5 t	34.3 t	34.6 t	34.6 t	33.4 t	36.6 t	38.9 t	33.4 t	34.7 t	34.3 t	34.4 t
8	40.6 s	40.4 s	39.6 s	40.6 s	40.9 s	40.4 s	40.3 s	40.3 s	39.7 s	39.7 s	39.5 s
9	51.1 d	50.6 d	50.1 d	51.9 d	52.9 d	50.8 d	49.4 d	53.5 d	50.1 d	50.1 d	49.7 d
10	43.5 s	42.2 s	37.0 s	43.5 s	42.6 s	42.1 s	42.0 s	37.2 s	37.0 s	37.0 s	37.0 s
11	33.4 t	33.1 t	31.5 t	34.1 t	40.6 t	30.2 t	31.0 t	39.7 t	31.6 t	28.3 t	28.3 t
12	71.1 d	70.6 d	70.4 d	71.2 d	209.6 s	75.7 d	75.7 d	210.0 s	70.4 d	75.4 d	75.5 d
13	48.8 d	48.6 d	48.7 d	48.0 d	56.8 d	45.7 d	45.5 d	56.9 d	48.8 d	46.3 d	46.3 d
14	51.8 s	51.7 s	52.1 s	51.2 s	55.8 s	51.8 s	51.8 s	55.1 s	52.1 s	52.3 s	52.3 s
15	32.8 t	32.8 t	32.1 t	32.8 t	31.5 t	34.1 t	34.0 t	31.5 t	32.1 t	30.8 t	30.9 t
16	28.4 t	28.3 t	28.4 t	28.3 t	24.9 t	22.2 t	26.9 t	24.9 t	28.5 t	25.6 t	27.1 t
17	48.2 d	48.2 d	48.8 d	48.8 d	42.8 d	49.6 d	51.0 d	42.7 d	48.9 d	49.6 d	49.6 d
18	15.6 q	15.6 q	15.2 q	15.6 q	16.2 q	15.9 q	15.8 q	15.4 q	15.4 q	15.5 q	15.5 q
19	11.9 q	13.2 s	16.2 q	12.2 q	15.1 q	12.7 q	12.6 q	15.7 q	16.3 q	16.0 q	16.0 q
20	86.4 s	86.3 s	87.3 s	86.4 s	88.8 s	85.6 d	85.9 d	88.6 s	87.3 s	85.4 s	85.5 s
21	28.0 q	28.1 q	28.8 q	27.8 q	24.8 q	25.8 q	21.7 q	24.2 q	28.8 q	27.9 q	27.9 q
22	31.2 t	31.3 t	31.5 t	31.2 t	32.5 t	31.3 t	30.0 t	28.9 t	31.6 t	38.4 t	38.4 t
23	25.0 t	25.1 t	25.0 t	25.0 t	28.9 t	24.2 t	25.6 t	32.4 t	25.0 t	26.8 t	25.5 t
24	85.3 d	85.4 d	87.0 d	85.3 d	177.0 s	83.4 d	82.5 d	176.9 s	87.1 d	84.5 d	82.7 d
25	70.1 s	69.9 s	70.0 s	70.2 s		71.0 s	83.1 s		70.0 s	70.4 s	82.7 s
26	27.6 q	27.7 q	27.9 q	27.5 q		27.5 q	21.7 q		28.0 q	27.6 q	22.4 q
27	26.1 q	26.1 q	24.1 q	26.1 q		26.8 q	22.6 q		24.2 q	24.0 q	22.5 q
28	27.5 q	27.3 q	27.9 q	27.9 q	27.1 q	27.4 q	27.4 q	26.5 q	27.9 q	27.6 q	27.9 q
29	21.5 q	21.4 q	15.3 q	21.8 q	21.0 q	21.6 q	21.6 q	21.0 q	16.3 q	16.4 q	16.4 q
30	18.2 q	18.0 q	17.7 q	18.4 q	16.5 q	17.5 q	17.3 q	16.3 q	17.7 q	17.3 q	17.3 q
$COCH_3$	21.3 q	21.9 q			21.8 q	21.6 q	22.5 q		21.2 q	21.8 q	21.8 q
		21.2 q				21.4 q	21.5 q			21.2 q	21.5 q
						21.3 q	21.4 q				21.2 q
							21.2 q				
$COCH_3$	170.6s	170.5 s			170.3 s	170.6 s	170.6 s		170.9 s	170.8 s	170.8 s
		170.4 s				170.1 s	170.4 s			170.4 s	170.4 s
						170.0 s	170.1 s				
							170.0 s				

[a] Acquired at 125 MHz. [b] Acquired at 50.2 MHz.

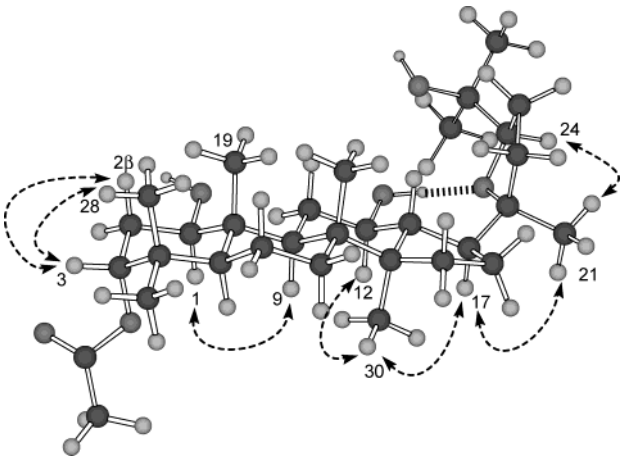

Figure 1. Selected NOE effects observed in compound **1**.

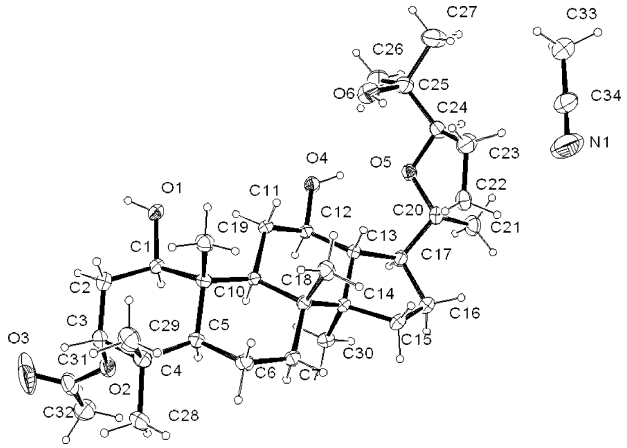

Figure 2. ORTEP projection of **1** with a molecule of acetonitrile.

dodecandra,[4] but differing in the number of acetyl groups and the stereochemistry at C-24.

An X-ray structure analysis established unequivocally the complete structure and relative stereochemistry of **1** and corroborated the above-mentioned hydrogen bond between the hydroxyl at C-12 and the oxygen of the tetrahydrofuran ring (see Figure 2). Consequently, the chemical structure of compound **1**, which was named 1-acetyl-24-*epi*-polacandrin, was determined as 20*S**,24*R**-epoxy-3α-acetoxy-1*β*,12*β*,25-trihydroxydammarane.

To prepare different derivatives and to compare their cytotoxicity against a small tumor panel cell, compound **1** was subjected to basic hydrolysis with KOH/MeOH to afford tetraol **4**. The LREIMS of this compound showed the molecular ion at *m*/*z* 492 and the (+)-LRFABMS displayed the $[M + Na]^+$ peak at *m*/*z* 515, indicating a molecular formula of $C_{30}H_{52}O_5$ for this compound. Comparison of the ^{13}C NMR and DEPT spectroscopic data of **4** (Table 2) with those of polacandrin[4] indicated similar carbon resonances with the exception of signals for nuclei around C-24. Indeed, the carbon resonance corresponding to C-24 was shifted upfield (δ_C 85.3 in **4** versus 87.5 in polacandrin), signifying that **4** must be the epimer of polacandrin at C-24. Additionally, **1** was oxidized with Jones' reagent to give the keto derivative **5**. The LREIMS (*m*/*z* 486 $[M]^+$) and (+)-

LRFABMS (*m/z* 509 $[M + Na]^+$ and 487 $[M + H]^+$) of this compound were in good agreement with the molecular formula $C_{29}H_{42}O_6$. The ^{13}C NMR and DEPT-135 spectra (Table 2) showed the presence of four oxygenated quaternary carbon signals (two of which, at δ_C 211.1 and 209.6 ppm, were assigned to two ketone groups) and the absence of the oxymethine carbon resonances at C-1 and C-12, which were present in **1**, suggesting the oxidation of these positions in **5**. The remaining quaternary carbon signals at δ_C 177.0 and 170.3 were assigned to one lactone and one ester carbonyl group, respectively. Furthermore, instead of the nine methyl signals observed in the ^1H NMR spectrum of **1**, the spectrum of **5** (Table 1) contained only seven such signals, indicating the degradation of part of the molecule. The absence of the corresponding proton signals due to H-1, H-12, and H-24 confirmed the oxidation of these positions in **5**. The final identity of this compound was established by direct comparison of its NMR spectroscopic data with those reported for **12**, which was obtained by oxidation of the diacetylated derivative of polacandrin under the same conditions.[4]

Compound **2** was isolated as colorless crystals. The molecular formula of **2** was established as $C_{34}H_{56}O_7$ on the basis of (+)-HRFABMS, which showed the pseudomolecular ion $[M + H]^+$ at *m/z* 577.4105 (calcd 577.4104). The difference of C_2H_2O between **2** and **1** suggests that compound **2** must be an acetylated derivative of compound **1**. This was confirmed by the ^1H NMR (δ_H 2.03 s/1.92 s) and ^{13}C NMR and DEPT-135 (δ_C 170.5 s/170.4 s and δ_C 21.9 q/21.2 q) spectra of **2**, which showed signals corresponding to two acetyl groups (Tables 1 and 2).

The location of the additional acetyl group at C-1 was suggested by the downfield shift of the signal for H-1 at δ_H 4.75 (dd $J = 10.7$ and 4.9 Hz) in the ^1H NMR spectrum of **2** in comparison to the corresponding signal in **1**. The H-12 and H-24 proton resonances remained essentially unchanged. On the other hand, the resonance at δ_C 78.4, assigned to C-1 in the ^{13}C NMR spectrum of **2**, was shifted downfield by 2.9 ppm in relation of that in **1**, while C-2 (δ_C 29.2) and C-10 (δ_C 42.2) resonances were shifted upfield by 4.9 and 1.3 ppm, respectively. These changes are characteristic of α- and *β*-acetylation effects and indicate that the hydroxyl group at C-1 in **1** is acetylated in **2**. Furthermore, acetylation of **1** at room temperature gave a compound, together with **6**, that was identical in all respects to the natural product **2**, thus confirming the location of the acetyl groups in that compound.

The structure of **6** was determined by comparison of its spectroscopic data with those of **2**. Thus, the ^1H NMR data of **6** showed a downfield shift of the H-12 signal by 1.41 ppm with respect to the H-12 signal for **2**. In the ^{13}C NMR spectra, on the other hand, the characteristic α- and *β*-effects of an acetoxy group in C-12 were observed, thus indicating that the hydroxyl group at C-12 in **2** is acetylated in **6**. On the basis of these data, the structure of compound **2**, named 1,3-diacetyl-24-*epi*-polacandrin, was established as 1*β*,3α-diacetoxy-12*β*,25-dihydroxy-20*S**,24*R**-epoxydammarane, and that of compound **6** as 20*S**,24*R**-epoxy-1*β*,3α,12*β*-triacetoxy-25-hydroxydammarane.

Acetylation of **1** under reflux gave compound **7** along with the triacetate **6**. The peracetylated structure of **7** was deduced by comparison of its NMR data to those of **1**, **2**, and **6**. The downfield shift of the nonprotonated carbon signal C-25 (from $\delta_C \sim 70$ in **1**, **2**, **6** to δ_C 83.1 in **7**) and the upfield shift of the two methyl signals CH_3-26 and CH_3-27 (from $\delta_C \sim$27/26 in **1**, **2**, **6** to δ_C 21.7/22.6 in **7**) and the methine carbon signal C-24 (from $\delta_C \sim$85 in **1** and **2** and δ_C 83.4 in **6** to δ_C 82.5 in **7**) in the ^{13}C NMR spectrum of **7** showed that the tertiary hydroxyl group at C-25 in **1**, **2**, and **6** is acetylated in **7**.[11] This situation was corroborated by the ion fragments at *m/z* 143 (**A**), 185 (**B**), and 125 (**C**) in the (+)-LRFABMS spectrum of **7** (see Scheme S1, Supporting Information).

The spectroscopic and physical data of compound **3** were found to be practically identical to those reported for the aglycon moiety of the dammarane glycosides neoalsoside D1[12] and neoalsoside II,[13] both of which were isolated from the aerial parts of *Neoalsomitra integrifoliola*. The same compound, though differing in the sign and value of the optical rotation, was reported previously by Appendino et al. from the frond exudate of the fern *Notholaena greggi* (collected in Mexico) and named 3*β*,12*β*,20*S**,24*S**-epoxydammarane-3,12,25-triol.[14]

Several derivatives of **3** were obtained following a strategy similar to that used for **1**. Oxidation of **3** with Jones' reagent gave the diketone *γ*-lactone **8** in 70% yield. The physical properties and spectroscopic data of **8** were in good agreement with the published data for the reported compound, which was obtained in low yield (6%) by PDC oxidation of 3*β*,12*β*,20*S**,24*S**-epoxydammarane-3,12,25-triol for 11 days at room temperature.[14] Acetylation of **3** at room temperature yielded the monoacetate **9** and the diacetate **10**, while acetylation of the tertiary hydroxyl function of **3**, which gave rise to the triacetate **11**, had to be carried out under refluxing conditions. The structures of **9**−**11** have not been reported before and were established on the basis of their spectroscopic data and by comparison of the corresponding data for **3**, taking into account the characteristic shifts due to α- and *β*-acetylation effects. Furthermore, the proton and carbon assignments for compound **10** were also made by comparison of its spectroscopic data with those of its reported diasteroisomer, namely, (20*R*,24*R*)-20,24-epoxydammarane-3*β*,12*β*,25-trioldiacetate.[15] The chemical shift assignments for **9** and **11** were also deduced by comparison with those of their reported epimers at C-20, the acetylated pyxinols.[16]

A weak cytotoxic activity ($IC_{50} = 5$ *μ*g/mL) was found for the 20*S**,24*S** triol **3** against A-549 (human lung carcinoma) and H-116 (human colon carcinoma) cells, and for the peracetylated derivative **7** against H-116 cells, as well as the diacetylated derivative **10** against A-549 cells. The 20*S**,24*R** compounds **1** and **2**, their derivatives **4** and **6**, and the acetylated 20*S**,24*S** compounds **9** and **11** were devoid of cytotoxic activity: **1**, **2**, and **4** ($IC_{50} > 10$ *μ*g/mL) against A-549, H-116, and HT-29 (human colon carcinoma) cells, and **6**, **9**, and **11** ($IC_{50} > 5$ *μ*g/mL) against A-549, H-116, PSN1 (human pancreatic adrenocarcinoma), and T98G (human caucasian glioblastoma) cells. Compound **4** differs only in the configuration at C-24 from the reported polacandrin,[4] which showed potent cytoxicity. These observations seem to indicate the crucial role of the 20*S**,24*S** stereochemistry for the presence of cytotoxic activity in this type of compound.

The work described here involved the isolation of triterpenes from *I. lutea* for the first time and is taxonomically relevant. Although dammarane triterpenes are widespread in seed plants, the 1,3,12-oxygenated-20,24-epoxydammarane, of which compounds **1** and **2** are examples, are very rare, with polacandrin being the only previously reported compound of this class. On the other hand, compound **3** has the same structure as one of the metabolites formed by incubation of certain ginseng sapogenins with microsomes from rat liver.[17] In addition, this compound was more recently identified and quantified in human urine

with the treatment of alkaline cleavage after the ingestion of Sanchi Ginseng, the roots of *Panax notoginseng*.[18] The pharmacological activities reported for ginsenosides, along with the fact that some authors assume that the absorbed sapogenins or prosapogenins of ginseng from the digestive tract are transformed to their 20,24-epoxides, emphasize the importance of the isolation of **3** from *I. lutea*. Indeed, these aspects could well be related to some of the pharmacological uses of this plant.

Experimental Section

General Experimental Procedures. Melting points were taken on a Stuart Scientific melting point apparatus (SMP3) and are uncorrected. Optical rotations were measured in $CHCl_3$ using a JASCO DIP-1000 polarimeter with a sodium lamp operating at 589 nm. IR (neat) spectra were recorded on a Matson FT-IR spectrometer. X-ray data was collected on a Nonius-Mach3 diffractometer. Intensity data were collected using graphite-monochromated copper radiation and an $\omega-2\theta$ variable scan speed technique. Reflections were used in the solution and refinement, and the structure was solved using WinGX software (SIR-92 for solution and SHELXL-97 for refinement).[19] NMR spectra were recorded at 500/125 MHz ($^1H/^{13}C$) (AMX-Bruker spectrometer) and 200/50 MHz ($^1H/^{13}C$) (Bruker AC-200 NMR spectrometer) using $CDCl_3$ as solvent and internal standard. Carbon multiplicities were determined using DEPT-135. Atom connectivities were determined using gHMQC, gHMBC, and gCOSY data. NOESY experiments were carried out using a mixing time of 0.8 s. (+)-LRFABMS were measured on a VG-Quattro spectrometer, while (+)-HRFABMS were measured on a Trisector EBE spectrometer from Micromass Instruments using thioglycerol with 1% NaI as matrix. Semipreparative HPLC was performed using C_{18} and NP columns (250 × 10 mm) with RI detection.

Biological Material. The first sample of *I. lutea* was collected at El Volcan, Dpto. La Capital, Province of San Luis, Argentina, in March 2001. A herbarium sample was deposited at the Herbario of the Universidad Nacional de San Luis [Luis A. Del Vitto et al. voucher 9226-(UNSL)]. The second sample of *I. lutea* was collected at La Dormida, Dpto. Santa Rosa, Province of Mendoza, Argentina, in April 2002. A herbarium sample was deposited at the same institution [Luis A. Del Vitto et al. voucher 9232-(UNSL)].

Extraction and Isolation. The air-dried aerial parts of *I. lutea* from the Province of San Luis (2.5 kg, without fruits) were extracted with Me_2CO at room temperature for 2 days. After evaporation of the solvent, the residue (55 g) was dissolved in a mixture of MeOH−H_2O (9:1), filtered, and extracted with *n*-hexane in order to remove pigments and fatty materials. Water was added to the aqueous alcohol fraction until the mixture became MeOH−H_2O (7:3) and was then extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated under vacuum, and the resulting dark brown syrup (30 g) was fractionated by vacuum-liquid chromatography (VLC) over silica gel 60 H (Merck, Darmstadt), using mixtures of *n*-hexane−EtOAc of increasing polarity as eluent. The fraction eluted with 50% EtOAc was then chromatographed on reversed-phase silica gel with MeOH−H_2O (7:3), followed by HPLC purification, and recrystallization from CH_3CN, to afford compounds **1** (2.3 g, 0.092%) and **2** (50 mg, 0.002%). The acetone extract (37 g) of the air-dried aerial parts of *I. lutea* (2 kg, without fruits) from the Province of Mendoza was extracted and partitioned as described above to afford 17 g of a dark brown $CHCl_3$ extract, which was fractionated as above to give three fractions. Normal-phase column chromatographic separation of the first fraction eluted with hexane−EtOAc (9:1) afforded a sterol mixture, which GC−MS analysis showed to be mainly composed of stigmasterol, campesterol, and sitosterol. The second fraction, eluted with hexane−EtOAc (1:1), was chromatographed on reversed-phase silica gel (eluent: MeOH−H_2O, 7:3), followed by HPLC purification with MeOH−H_2O (7:3) and recrystallization from CH_3CN, to give compound **3** (160 mg, 0.008%). Finally, fraction 3 was eluted with pure EtOAc and afforded, after purification on a Sephadex LH-20 column eluted with MeOH, 50 mg (0.0025%) of apigenin.

1-Acetyl-24-*epi*-polacandrin (1): colorless prisms (CH_3CN); mp 164−165 °C; $[\alpha]^{25}_D$ −13.9° (*c* 0.225, $CHCl_3$); IR (neat) ν_{max} 3488, 3303, 2970, 1738, 1468, 1374, 1184, 1088, 1044, 948, 873 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; (+)-LRFABMS *m/z* 535 (17) $[M + H]^+$, 439 (15), 143 (100) [side chain, $C_8H_{15}O_2$]; (−)-LRFABMS *m/z* 533 (10) $[M - H]^-$, 275 (10), 183 (100), 151 (20); LREIMS (70 eV) *m/z* 534 (1), 392 (4), 380 (30), 235 (5), 223 (7), 218 (19), 205 (22), 203 (25), 189 (50), 143 (100) [$C_8H_{15}O_2$], 125 (65) [$C_8H_{15}O_2$ − 18], 107 (96); (+)-HRFABMS *m/z* 535.3999 $[M + H]^+$ (calcd for $C_{32}H_{55}O_6$ 535.3999, Δ 0.0 mmu).

1,3-Diacetyl-24-*epi*-polacandrin (2): colorless prisms (CH_3CN); mp 212−213 °C; $[\alpha]^{27}_D$ −47.5° (*c* 0.060, $CHCl_3$); IR (neat) ν_{max} 3528, 3374, 1736, 1466, 1375, 1247, 1184, 1089, 1045 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; LREIMS (70 eV) *m/z* 576 (1), 517 (2), 381 (75), 314 (15), 235 (15), 223 (6), 217 (12), 205 (10), 201 (50), 189 (65), 143 [side chain, $C_8H_{15}O_2$] (100), 125 [$C_8H_{15}O_2$ − H_2O] (60), 107 (96), 85 (35); (−)-LRFABMS *m/z* 575 $[M - H]^-$ (10), 533 (20), 325 (35), 183 (100), 151 (25); (+)-LRFABMS *m/z* 577 (10) $[M + H]^+$, 535 (30), 421 (15), 185 (35), 143 (100), [side chain, $C_8H_{15}O_2$]; (+)-HRFABMS *m/z* 577.4105 $[M + H]^+$ (calcd for $C_{34}H_{57}O_7$ 577.4104, Δ 0.1 mmu).

20*S,24*S**-Epoxy-3β,12β,25-trihydroxydammarane (3):** colorless prisms (CH_3CN); mp 147 °C; $[\alpha]^{20}_D$ +16.5° (*c* 0.200, $CHCl_3$); IR (neat) ν_{max} 3395, 2970, 1454, 1377, 1248, 1044, 1079 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ_H 3.89 (1H, dd, *J* = 10.5, 4.0 Hz, H-24), 3.54 (1H, ddd, *J* = 10.4, 10.4, 4.3 Hz, H-12), 3.17 (1H, dd, *J* = 9.8, 5.4 Hz, H-3), 2.25 (1H, ddd, *J* = 10.4, 10.4, 4.3 Hz, H-17), 1.28 (3H, s, H-21), 1.22 (3H, s, H-18), 1.12 (3H, s, H-27), 0.97 (3H, s, H-26), 0.92 (3H, s, H-29), 0.88 (3H, s, H-30), 0.85 (3H, s, H-28), 0.78 (3H, s, H-19); ^{13}C NMR, see Table 2; (+)-LRFABMS *m/z* 499 $[M + Na]^+$ (100), 385 (5), 279 (18), 237 (18), 143 [side chain, $C_8H_{15}O_2$] (42).

Hydrolysis of Compound 1. Compound **1** (10 mg) was added to a solution of 10% KOH in MeOH (5 mL) under argon, and the mixture was stirred at room temperature for 8 h. Then, 2 mL of the solvent was evaporated and the remaining solution was poured into a mixture of 5 g of ice and 10 mL of 1 M HCl. The mixture was extracted with EtOAc (2 × 10 mL), and the combined extracts were washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a residue, which was purified by HPLC [NP column, 300 × 8 mm, EtOAc−hexane (9:1), flow rate: 1.0 mL] to afford **4** (7 mg, 70% yield, R_f 0.35 in EtOAc).

Compound 4: colorless prisms; mp 149−150 °C; $[\alpha]^{26.6}_D$ −12.6° (*c* 0.075, $CHCl_3$); IR (neat) ν_{max} 3400, 2964, 1454, 1383, 1307, 1085, 994 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.85 (1H, dd, *J* = 8.0, 5.6 Hz, H-24), 3.81 (1H, dd, *J* = 4.6, 10.5 Hz, H-1), 3.58 (1H, ddd, *J* = 5.0, 10.6, 10.6 Hz, H-12), 3.51 (1H, t, *J* = 4.2 Hz, H-3), 2.64 (1H, ddd, *J* = 3.8, 3.8, 13.3 Hz, H-11α), 1.24 (3H, s, H-21), 1.23 (3H, s, H-26), 1.07 (3H, s, H-27), 0.97 (3H, s, H-18), 0.90 (3H, s, H-19), 0.90 (3H, s, H-28), 0.89 (3H, s, H-30), 0.80 (3H, s, H-29); ^{13}C NMR, see Table 2; LREIMS (70 eV) *m/z* 492 (2), 575 (10), 457 (13), 439 (15), 379 (40), 349 (16), 189 (62), 143 (50), 107 (100), 91 (80), 79 (51); (+)-LRFABMS *m/z* 515 $[M + Na]^+$ (96), 439 (5), 159 (7), 143 (100).

Oxidation of 1. To a solution of **1** (50 mg) in Me_2CO (10 mL) was added Jones' reagent dropwise with stirring at 0 °C until a permanent orange color persisted. The reaction mixture was evaporated under reduced pressure to remove Me_2CO. The residue was dissolved in ice/water (15 mL), and the mixture was extracted with $CHCl_3$. The extract was evaporated to dryness, and the residue was purified by HPLC [RP-18 column, 250 × 10 mm, MeOH−H_2O (9.5:0.5), flow rate: 1.0 mL] to afford 34 mg (64% yield, R_f 0.45, EtOAc−hexane, 1:1) of pure **5**.

Compound 5: colorless needles; mp 253−254 °C; $[\alpha]^{25}_D$ −67.2° (*c* 0.040, $CHCl_3$); IR (neat) ν_{max} 2967, 1737, 1709, 1462, 1374, 1239 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.00 (1H, t, *J* = 3.4 Hz, H-3), 3.30 (1H, dd, *J* = 3.4, 13.5 Hz, H-2α), 2.90 (1H, brd, *J* = 9.8 Hz, H-13), 2.84 (1H, dd, *J* = 3.4, 13.5 Hz,

H-2β), 2.61 (2H, m, H-23), 2.44 (1H, dd, J = 3.5, 13.0 Hz, H-11α), 2.25 (1H, dd, J = 3.5, 13.0 Hz, H-11β), 2.18 (1H, m, H-22a), 2.07 (3H, s, OCO*CH*$_3$), 1.95 (3H, m, H-22b), 1.32 (3H, s, H-21), 1.23 (3H, s, H-18), 1.23 (3H, s, H-19), 1.20 (3H, s, H-28), 0.92 (3H, s, H-29), 0.83 (3H, s, H-30); ^{13}C NMR, see Table 2; LREIMS (70 eV) *m/z* 487 (1), 412 (100), 394 (50), 316 (65), 203 (55), 160 (50), 99 (13), 81 (42); (+)-LRFABMS *m/z* 509 (100) [M + Na]$^+$, 487 (10) [M + H]$^+$, 318 (20), 317 (84), 289 (100), 214 (10), 181 (30).

Acetylation of 1 at Room Temperature. Compound **1** (50 mg) was dissolved in a mixture of pyridine (1 mL) and Ac_2O (5 mL) with a catalytic amount of DMAP, and the mixture was allowed to stand overnight at room temperature and in darkness. The reaction mixture was concentrated under reduced pressure and, after the usual workup procedure and purification by HPLC [NP column, 300 × 8 mm, EtOAc−hexane (7:3), flow rate: 1.0 mL], gave compounds **2** (30 mg, 60% yield, R_f 0.55 EtOAc−hexane, 7:3) and **6** (10 mg, 20% yield R_f 0.72, EtOAc−hexane, 7:3).

Acetylation of 1 Under Reflux. Compound **1** (25 mg) was dissolved in a mixture of pyridine (1 mL) and Ac_2O (3 mL) with a catalytic amount of DMAP, and the mixture was heated under reflux in a glycerin bath for 3 h. The reaction mixture was then concentrated under reduced pressure and, after the usual workup procedure and purification by HPLC [NP column, 300 × 8 mm, EtOAc−hexane (7:3), flow rate: 1.0 mL], gave the peracetylated compound **7** (17 mg, 68% yield, R_f 0.91 EtOAc−hexane, 7:3) and compound **6** (4 mg, 20% yield R_f 0.72, EtOAc−hexane, 7:3).

Compound 6: white powder; mp 218−219 °C; $[\alpha]^{22}_D$ −7.02° (*c* 0.035, $CHCl_3$); IR (neat) ν_{max} 2966, 1735, 1465, 1373, 1250 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.85 (1H, dd, J = 10.0, 5.0 Hz, H-1), 4.82 (1H, ddd, J = 10.0, 10.0, 5.0 Hz, H-12), 4.70 (1H, t, J = 3.0 Hz, H-3), 3.66 (1H, t, J = 6.5 Hz, H-24), 2.13 (3H, s, OCO*CH*$_3$), 1.99 (3H, s, OCO*CH*$_3$), 1.92 (3H, s, OCO*CH*$_3$), 1.18 (3H, s, H-26), 1.16 (3H, s, H-21), 1.12 (3H, s, H-27), 1.07 (3H, s, H-18), 1.05 (3H, s, H-30),0.98 (3H, s, H-19), 0.92 (3H, s, H-29), 0.85 (3H, s, H-28); ^{13}C NMR, see Table 2; LREIMS (70 eV) *m/z* 618 (5), 500 (16), 440 (35), 381 (100), 294 (45), 227 (65), 143 (57), 107 (85), 79 (40); (+)-LRFABMS *m/z* 641 (32) [M + Na]$^+$, 581 (5), 421 (12), 173 (10), 143 (100).

Compound 7: colorless gum; $[\alpha]^{20}_D$ −30.98° (*c* 0.297, $CHCl_3$); IR (neat) ν_{max} 2962, 1737, 1465, 1371, 1249, 1043, 1079 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.83 (1H, ddd, J = 10.3, 10.3, 5.5 Hz, H-12), 4.80 (1H, dd, J = 10.0, 5.0 Hz, H-1), 4.73 (1H, t, J = 3.0 Hz, H-3), 3.89 (1H, t, J = 6.9 Hz, H-24), 1.43 (3H, s, H-27), 1.42 (3H, s, H-26), 1.16 (3H, s, H-21), 1.03 (3H, s, H-19), 1.01 (3H, s, H-18), 0.95 (3H, s, H-30), 0.91 (3H, s, H-29), 0.84 (3H, s, H-28), 2.12 (3H, s, OCO*CH*$_3$), 1.98 (3H, s, OCO*CH*$_3$), 1.96 (3H, s, OCO*CH*$_3$), 1.92 (3H, s, OCO*CH*$_3$); ^{13}C NMR, see Table 2; (+)-LRFABMS *m/z* 683 (5) [M + Na]$^+$, 421 (27), 185 (63), 143 (65), 125 (100).

Oxidation of 3. Compound **3** (10 mg) was oxidized as described for **1** and purified by HPLC [RP-18 column, 250 × 10 mm, MeOH−H_2O (9.5:0.5), flow rate: 1.0 mL] to give 7 mg of **8** (70% yield, R_f 0.60, EtOAc−hexane, 1:1).

Compound 8: colorless needles; mp 214−216 °C; $[\alpha]^{25}_D$ +77.5° (*c* 0.200, $CHCl_3$); IR (neat) ν_{max} 2971, 1770, 1707, 1461, 1386, 1274, 966, 935 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.95 (1H, brd, J = 10.0 Hz, H-13), 2.63 (1H, m, H-17), 2.66−2.54 (2H, m, H-22), 2.60 (2H, m, H-23), 2.47 (2H, m, H-2), 2.33−2.17 (2H, m, H-11), 1.9 (1H, m, H-9), 1.6 (2H, m, H-6), 1.47 (2H, m, H-7), 1.38 (1H, m, H-5), 1.18−1.31 (2H, m, H-15), 1.27 (3H, s, H-21), 1.27 (3H, s, H-18), 1.11 (3H, s, H-28), 1.08 (3H, s, H-29), 1.05 (3H, m, H-19), 0.79 (3H, s, H-30); ^{13}C NMR, see Table 2; (+)-LRFABMS *m/z* 451 (100) [M + Na]$^+$, 382 (5), 328 (4) [M − $C_5H_8O_2$]$^+$, 237 (12), 163 (23), 149 (50), 121 (62).

Acetylation of Triol 3 at Room Temperature. Compound **3** (25 mg) was acetylated at room temperature in a similar way to **1** and was purified by HPLC [NP column, 300 × 8 mm, EtOAc−hexane (7:3), flow rate: 1.0 mL] to give 14 mg of **9** (56% yield, R_f 0.33 EtOAc−hexane, 7:3) and 6 mg of **10** (24% yield R_f 0.62, EtOAc−hexane, 7:3).

Acetylation of Triol 3 under Reflux. Compound **3** (25 mg) was acetylated under the same conditions as described for **1**, and the product was purified by HPLC as above to give 4 mg of **10** (16% yield, R_f 0.62 EtOAc−hexane, 7:3) and 16 mg of **11** (64% yield, R_f 0.85, EtOAc−hexane, 7:3).

Compound 9: white prisms; mp 232−233 °C; $[\alpha]^{20}_D$ +10.5° (*c* 0.493, $CHCl_3$); IR (neat) ν_{max} 3373, 2969, 1731, 1457, 1373, 1246, 1078, 1030 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.53 (1H, ddd, J = 10.2, 10.2, 5.6 Hz, H-12), 4.48 (1H, dd, J = 8.5, 5.5 Hz, H-3), 3.88 (1H, dd, J = 10.8, 5.6 Hz, H-24), 2.04 (3H, s, OCO*CH*$_3$), 1.28 (3H, s, H-21), 1.26 (3H, s, H-28), 1.10 (3H, s, H-27), 1.00 (3H, s, H-18), 0.91 (3H, s, H-26), 0.91 (3H, s, H-29), 0.85 (3H, s, H-30), 0.85 (3H, s, H-19); ^{13}C NMR, see Table 2; (+)-LRFABMS *m/z* 541 (14) [M + Na]$^+$, 355 (7), 281 (35), 221 (53), 207 (40), 147 (97), 133 (100).

Compound 10: white crystals; mp 185−187 °C; $[\alpha]^{20}_D$ +1.00° (*c* 0.497, $CHCl_3$); IR (neat) ν_{max} 2967, 1732, 1463, 1369, 1244, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.83 (1H, ddd, J = 10.6, 10.6, 5.7 Hz, H-12), 4.48 (1H, dd, J = 10.4, 5.4 Hz, H-3), 3.88 (1H, t, J = 3.64 Hz, H-24), 2.04 (3H, s, OCO*CH*$_3$), 2.00 (3H, s, OCO*CH*$_3$), 1.19 (3H, s, H-21), 1.18 (3H, s, H-28), 1.09 (3H, s, H-27), 0.99 (3H, s, H-18), 0.93 (3H, s, H-26), 0.87 (3H, s, H-29), 0.85 (3H, s, H-30), 0.85 (3H, s, H-19); ^{13}C NMR, see Table 2; (+)-LRFABMS *m/z* 583 (93) [M + Na]$^+$, 523 (14), 279 (18), 237 (20), 189 (12), 153 (19), 143 (100).

Compound 11: colorless gum; $[\alpha]^{20}_D$ +6.7° (*c* 0.535, $CHCl_3$); IR (neat) ν_{max} 2967, 1732, 1464, 1368, 1244, 1092, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.83 (1H, ddd, J = 10.0, 10.0, 5.0 Hz, H-12), 4.49 (1H, dd J = 9.6, 4.3 Hz, H-3), 3.93 (1H, t, J = 6.9 Hz, H-24), 2.04 (3H, s, OCO*CH*$_3$), 2.01 (3H, s, OCO*CH*$_3$), 1.97 (3H, s, OCO*CH*$_3$), 1.44 (3H, s, H-26), 1.42 (3H, s, H-27), 1.20 (3H, s, H-21), 0.99 (3H, s, H-18), 0.93 (3H, s, H-28), 0.87 (3H, s, H-29), 0.85 (3H, s, H-30), 0.85 (3H, s, H-19); ^{13}C NMR, see Table 2; (+)-LRFABMS *m/z* 625 (17) [M + Na]$^+$, 483 (6), 423 (10), 189 (20), 185 (66), 143 (66), 125 (100).

Crystallographic Data and X-ray Structure Analysis of 1. A suitable colorless plate, 0.50 × 0.35 × 0.30 mm, of **1** was grown by the slow evaporation of a CH_3CN solution. Crystal data: $C_{34}H_{54}O_6$•CH_3CN, orthorhombic; space group $P2_12_12_1$; Z = 4, unit cell parameters a = 10.0320(8) Å, b = 12.4660(11) Å, c = 26.2620(2) Å, V = 3284.2 Å^3, d_{calcd} = 1.081 Mg m^{-3}, T = 293(2) K, F(000) = 736, λ = 71073 Å, μ(Mo Kα) = 0.076 mm^{-1}. Final refinement with 7783 reflections led to $R(F)$, $R(F > 2\sigma)$, and GOF of 0.0984, 0.0606, and 1.046. Crystallographic data have been deposited in the Cambridge Crystallographic Data Center with the deposit number CCDC 219432. Copies of the data can be obtained, free of charge, on application to CDCC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Assay for Cytotoxic Activity. This form of assay employs 24-well multidishes of 16 mm diameter. The tumor cell lines employed were A-549 (human lung carcinoma), HT-29 (human colon carcinoma), H-116 (human colon carcinoma), PSN1 (human pancreatic adrenocarcinoma), and T98G (human caucasian glioblastoma). Cells were maintained in their logarithmic phase of growth in Eagle's minimum essential medium, with Earle's balanced salts, with nonessential amino acids, with 2.0 mM L-glutamine, without sodium bicarbonate (EMEM/neaa), supplemented with 5% fetal calf serum (FCS), 10^{-2} M sodium bicarbonate and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate. For each experiment the cells were harvested from subconfluent cultures using trypsin and resuspended in fresh medium before plating. Tumor cells were seeded into wells of 16 mm diameter at 1 × 10^4 cells per well in 1 mL aliquots of EMEM 5%FCS containing different concentrations of the sample to be tested. A separate set of cultures (without drug) was seeded as a growth control to ensure that cells remained in the exponential phase of growth. All determinations were carried out in duplicate. After 3 days of incubation at 37 °C and 5% CO_2 in an atmosphere of 98% humidity, the cells were stained with 0.1% crystal violet. An approximate IC_{50} value was determined by comparing the growth in wells with drug to the growth in the control well.

To quantify the activity after completion of the incubation time, the cells were trypsinized and counted in a Coulter Counter ZM. All counts (net cells per well) represent the

average of duplicate wells. The percentage growth (% G) is relative to cultures without drug. The results of these assays were used to generate dose−response curves from which more precise IC_{50} values were determined (sample concentration that produces 50% cell growth inhibition).

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Supporting Information Available: Scheme showing mass spectral fragments. This information is available free of charge via the Internet at http//pubs.acs.org.

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