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NEW EUDESMANOLIDE SESQUITERPENES FROM A PHILIPPINES COLLECTION OF *WEDELIA PROSTATA*

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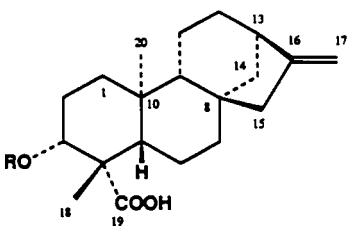
ABSTRACT.—Two known, but previously uncharacterized, *ent*-kaurenic acid derivatives, **1** and **2**, and four new sesquiterpenes of the eudesmanolide type, **3–6**, have been isolated from *Wedelia prostata*. The structures were eludidated by a range of 1D and 2D high field nmr techniques, and the stereochemistry of the new eudesmanolides was defined from a combination of coupling constant analyses and nuclear Overhauser difference spectra.

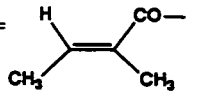
Plants of the family Compositae have been characterized by the production of structurally varied sesquiterpene lactones that include germacranolides (1), eudesmanolides (2,3), guaianolides (4), pseudoguaianolides (5), and xanthanolides (6). These commonly co-occur with diterpenoid *ent*-kaurenic acid derivatives (2,3), which is of particular relevance to our present report.

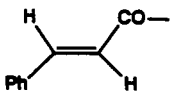
Wedelia prostata Jacquin is a common herbaceous vine growing in thickets along streams and behind sand dune areas in many parts of the Philippines. It was investigated because of its wide use as a hair colorant and hair tonic, and because it is reported to have beneficial properties in the treatment of such diverse maladies as coughs, cephalagic skin diseases, and uterine hemorrhages (7). We here report the isolation and identification of the free acids of the known (2,8) *ent*-kaurenic acid derivatives **1** and **2** and of four new eudesmanolide sesquiterpenes **3–6** from *W. prostata*.

RESULTS AND DISCUSSION

$CHCl_3$ extraction of the ground leaves of *W. prostata* afforded a 5% organic extract. Rapid chromatography (9) of the crude extract on a Si gel column with increasing proportions of EtOAc in petroleum ether as eluent afforded two *ent*-kaurenic free acid derivatives, one of which (**1**) possessed a tiglate ester function while the other (**2**) possessed a cinnamate ester function. The methyl esters of these acids were reported by Bohlmann and co-workers (2,8), who isolated them after methylation of the acid-containing fraction of the extract of *Wedelia trilobata*. The structures **1** and **2** were established on the basis of extensive 2D nmr experimentation. The 3α-ester drawn in



1 R= 

2 R= 

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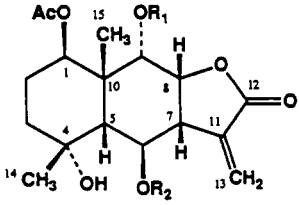
Bohlmann *et al.* (2), rather than the 3β-ester function drawn in Bohlmann and Le Van (8), is consistent with these data.

The more polar components in the extract were shown to be four closely related eudesmanolide sesquiterpenes **3**, **4**, **5**, and **6**. The most polar metabolite (**3**) co-eluted with **4** in the 10–20% MeOH in EtOAc fractions, and the two compounds were clearly very similar. They co-crystallized from Et_2O, and the metabolite **3** was obtained pure only after repeated vacuum liquid chromatography (9) under isocratic conditions.



3 R_1=H, R_2=$COCH(CH_3)_2$
4 R_1=H, R_2=$COC(CH_3)=CH_2$
5 R_1=Ac, R_2=$COCH(CH_3)_2$
6 R_1=Ac, R_2=$COC(CH_3)=CH_2$

The sesquiterpene **3** had a molecular formula $C_{21}H_{30}O_8$ as determined by a combination of hrms on the $[M-HOAc]^+$ peak and ^{13}C-nmr spectroscopy that confirmed the presence of twenty-one carbons and twenty-eight protons attached to carbons. The molecular ion was not observed in the mass spectrum of **3**. The ^{13}C-nmr spectrum of **3** (Table 1) contained three ester carbonyl carbons, one of which was attributed to a lactone function (170.4 ppm), and two other ester groups (170.4, 175.9 ppm). The ^{13}C nmr revealed only one carbon double bond, so the molecule was tricyclic. There were signals for five oxygenated carbons: the two esters and the lactone accounted for three of these carbons (and six oxygens); clearly the molecule contained two hydroxyl groups (ν max 3450 cm^{-1}). The two ester functions accounted for six carbon atoms: one was an acetate (loss of HOAc in ms; Me singlet in ^1H nmr at δ 2.10); the other was shown to be an isobutyrate by the presence of two methyl doublets at δ 1.20 in the ^1H-nmr spectrum of **3** coupled to a methine proton at δ 2.57. The nature of the lactone remained to be determined. Two vinyl proton doublets (δ 6.43, d, 3.8 Hz; 5.87, d, 3.0 Hz) were characteristic of the *exo*-methylene-γ-lactone function commonly encountered in plants of this genus. Two additional methyl groups (δ1.32, 1.09) were present in the molecule. The more deshielded methyl signal was assumed to be on an oxygenated carbon, while the more shielded signal was a bridgehead methyl group.

A COSY spectrum (10) of **3** enabled quite extensive chains of coupling to be delineated. Thus, the two *exo*-methylene protons (δ 6.34, 5.87; H-13, -13′) were coupled to the bridgehead proton (δ 3.25, H-7), which was in turn coupled to the other bridgehead proton (δ 4.93, H-8) and to the proton on the adjacent ester-bearing carbon (δ 5.81, H-6). The latter proton coupled to the methine proton at δ 1.84 (H-5), while the lactonic proton at δ 4.93 (H-8) was coupled to an adjacent proton on an oxygenated carbon (δ 3.84, H-9). The coupling chain was then blocked by non-proton-bearing carbons in all directions. A further short coupling chain linked H-1 (δ 5.12) to H-2, -2′ (δ 2.31, 1.60) and H-2, -2′ to H-3, -3′ (δ 1.75, 1.60). These data could be accommodated by the eudesmanolide-based structure **3** which contained the same skeleton and oxygenation pattern as compound **7** reported from *W. trilobata* (2). The spectroscopic properties of **3** and **7** were quite dissimilar, however, due to stereochemical differences.

TABLE 1. ^{13}C-nmr Data for **3**–**6**. Assignments for **3** and **5** are Unambiguous[a]; Assignments for **4** and **6** are Based on Comparison with **3** and **5**.

Carbon	Compound			
	3	**4**	**5**	**6**
C-1	75.3	74.8	68.4	68.2
C-2	24.4	24.2	21.9	21.8
C-3	34.2	34.3	34.9	34.9
C-4	68.7	68.8	70.4	70.2
C-5	51.2	51.5	54.2	54.9
C-6	71.0	71.5	73.1[b]	73.9
C-7	42.9	42.7	42.8	42.5
C-8	74.3	74.3	73.0[b]	73.0
C-9	71.7	71.6	71.8	71.4
C-10	41.0	41.0	40.5	40.4
C-11	133.9	134.0	135.8	135.6
C-12	170.4	170.5	169.0	169.2
C-13	122.7	122.7	122.6	122.6
C-14	21.8	21.8	22.3	22.4
C-15	29.9	30.0	31.7	31.5
6-ester	175.9	166.4	177.8	168.2
	34.1	136.1	34.6	135.9
	18.8	126.6	18.9	128.1
	18.8	18.2	18.5	18.2
1-OAc	170.4	170.4	170.4	170.4
	21.2	21.2	21.2	21.2
9-OAc			169.0	168.8
			20.5	20.6

[a]Assignments based on XHCORRD, HMBC, and COSY spectroscopy.
[b]These assignments may be interchanged.

The ^{13}C and ^{1}H assignments for **3** (Tables 1 and 2) were verified by the heteronuclear 2D experiment (Bruker XHCORRD program), and connectivity was verified by the inverse long-range heteronuclear experiment HMBC optimized for $J = 8$ Hz. All long-range correlations observed were consistent with the proposed structure. Before discussing the stereochemistry of **3**, it is convenient to mention the other related metabolites isolated.

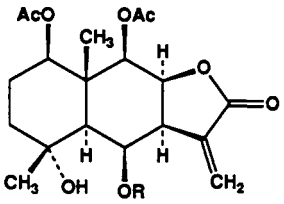
A small quantity of the sesquiterpene **4** was eventually isolated free of **3** by repeated hplc with CH_2Cl_2-hexane-Me_2CO (5:3.5:1.5). Inspection of the ^{1}H data for **3** and **4** revealed that all the spectral differences could be explained by replacement of the isobutyrate function in **3** by a methacrylate function in **4** (vinyl protons δ 6.15, 5.65, br s; vinyl methyl δ 1.95). Nmr spectral data only are presented for this compound.

The less polar eudesmanolides **5** and **6** were present in the fractions eluted from the original column with EtOAc. Rechromatography permitted separation of **5** and **6**. Although some chemical shifts and couplings observed in the ^{1}H-nmr spectra of **5** and **6** (Table 2) were significantly different from those observed for **3** and **4**, acetylation of a mixture of the hard to separate diols **3** and **4** yielded the diacetates **5** and **6**, which could now be more readily separated. The ^{13}C and ^{1}H assignments for **5** (Tables 1 and 2) were also verified by the heteronuclear 2D experiment XHCORRD, and connectivity was verified by the inverse long-range heteronuclear experiment HMBC optimized for $J = 8$ Hz. All long-range correlations observed were consistent with the proposed structure.

TABLE 2. ^{1}H-nmr Data[a] for **3–8**. Assignments for **3–6** are Unambiguous.

Proton	Compound					
	3	**4**	**5**	**6**	**7**[b]	**8**[b]
H-1	5.12 dd 3.6, 3.6	5.22 dd 3.8, 3.8	5.51 dd 7.8, 8.9	5.60 dd 8.0, 9.0	4.61 dd 11.0, 4.5	4.62 dd 11.0, 4.5
H-2	2.31 m	2.30 m	2.20 m	2.25 m		
H-2′	1.60 m	1.60 m	1.44 m	1.45 m		
H-3	1.75 m	1.76 m	1.78 m	1.80 m		
H-3′	1.60 m	1.60 m	1.25 m	1.25 m		
H-5	1.84 d 2.3	1.89 d 3.2	1.73 d 10.6	1.75 d 11.0	1.93 d 3.0	1.94 d 3.0
H-6	5.81 dd 2.3, 2.3	5.92 dd 3.2, 3.1	5.77 dd 10.6, 8.3	5.84 dd 11.0, 8.9	5.98 dd 3.0, 2.0	6.04 dd 3.0, 2.0
H-7	3.25 dddd 9.0, 3.8, 3.3, 2.3	3.35 dddd 9.0, 3.6, 3.3, 3.1	3.40 dddd 9.7, 8.3, 3.2, 2.7	3.45 dddd 9.9, 8.9, 3.5, 3.1	3.19 dddd 8.0, 3.5, 3.0, 2.0	3.30 dddd 8.0, 3.5, 3.0, 2.0
H-8	4.93 dd 9.1, 3.8	4.95 dd 9.0, 3.6	4.96 dd 9.7, 3.1	4.98 dd 9.9, 3.5	4.90 dd 8.0, 4.5	4.93 dd 8.0, 4.5
H-9	3.84 d 3.8	3.84 d 3.6	5.30 d 3.1	5.35 d 3.5	5.24 d 4.5	5.26 d 4.5
H-13	6.34 d 3.8	6.33 d 3.6	6.25 d 3.2	6.25 d 3.5	6.26 d 3.5	6.28 d 3.5
H-13′	5.87 d 3.3	5.83 d 3.3	5.57 d 2.7	5.55 d 3.1	5.68 d 3.0	5.72 d 3.0
H-14	1.09 s	1.13 s	1.32 s	1.34 s	1.37 s	1.40 s
H-15	1.32 s	1.32 s	1.25 s	1.20 s	1.38 s	1.36 s
6-ester	2.57 sept 7.0	6.14 brs	2.63 sept 7.0	6.25 brs	2.61 sept 7.0	6.14 brs
	1.20 d 7.0	5.67 t 1.5	1.23 d 7.0	5.81 brs	1.23 d 7.0	5.71 brs
	1.20 d 7.0	1.97 s	1.26 d 7.0	2.00 s	1.21 d 7.0	2.00 s
1-OAc	2.10 s	2.07 s	1.99 s	1.98 s	2.02 s	2.02 s
9-OAc			1.98 s	1.97 s	1.96 s	1.96 s

[a]The following abbreviations are used for signal multiplicities: s, singlet; brs, broad singlet; d, doublet; m, multiplet; sept, septet; t, triplet.
[b]Data for these compounds are from Bohlmann *et al.* (2).



7 R=$COCH(CH_3)_2$
8 R=$COC(CH_3)=CH_2$

The following discussion of stereochemistry and conformation for the diol **3** and the diacetate **5** can also be applied to **4** and **6**, as they differ only in the nature of one ester group.

The stereochemistry of **3** was deduced by consideration of coupling constants and by nuclear Overhauser difference spectra. The lactonic methine proton (δ 4.93, H-8) coupled to the other bridgehead proton (δ 3.25, H-7) with a large coupling constant (J = 9.1 Hz). The magnitude of the coupling is consistent with cis stereochemistry; compound **7**, where $J_{7,8}$ = 8 Hz, was also assigned cis geometry (2). (See also the following discussion on the conformation of **5** for verification of cis stereochemistry.) The protons H-6 and H-9 were both equatorial ($J_{6,7}$ = 2.3 Hz, $J_{8,9}$ = 3.8 Hz). The oxygen functions are thus axially disposed to the six-membered ring. The H-1 proton is also equatorial ($J_{1,2}$ = 3.6, $J_{1,2'}$ = 3.6 Hz). On chemical shift grounds the ester functional groups are at C-1 (δ 5.12) and C-6 (δ 5.81), while C-4 and C-9 (δ 3.84) bear the hydroxyl groups. NOe experiments confirmed the relative positions of the acetate and isobutyrate functions and established the relative stereochemistry of **3**. Irradiation of the bridgehead methyl (δ 1.09) showed an nOe at H-5 (4%), which confirmed the cis nature of the decalin ring fusion, and smaller enhancements at H-1 (1.8%), H-9 (1.2%), and the 1,3-diaxial proton H-8 (2.2%). Irradiation of the other methyl signal (δ 1.32) showed enhancement of the signals at δ 1.84 (H-5, 3.7%) and 5.81 (H-6, 4%). Irradiation at δ 4.93 (H-8) showed enhancement (4.7%) at δ 2.57 to the isobutyrate methine proton and to the methyl group at C-4 (δ 1.09). This suggests placement of the isobutyrate at C-6, on the same face of the molecule as the bridgehead methyl on C-10 and the lactonic methine (H-8). An all-chair conformation for the *cis*-decalin (Figure 1) is consistent with all the observed nOe's and coupling constants.

The conformation of the decalin system changes when going from the monoacetates to the diacetates. Thus, in **3** and **4**, both H-1 (**3** $J_{1,2}$ = 3.6, $J_{1,2'}$ = 3.6 Hz; **4** $J_{1,2}$ = 3.8, $J_{1,2'}$ = 3.8 Hz) and H-6 (**3** $J_{5,6}$ = 2.3, $J_{6,7}$ = 2.3; **4** $J_{5,6}$ = 3.2, $J_{6,7}$ = 3.1 Hz) are equatorial. However, when the hydroxyl group at C-9 is replaced by the bulkier

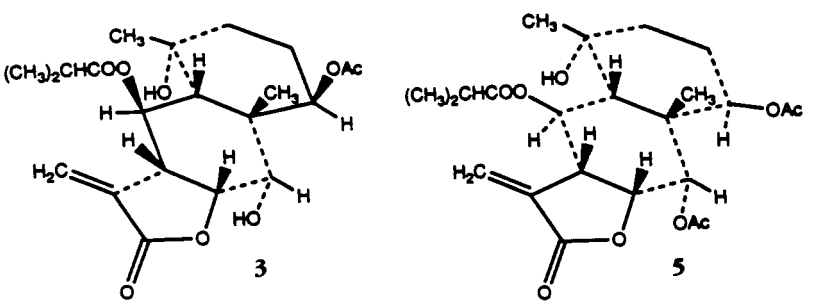


FIGURE 1. Proposed conformations and stereochemistries of **3** and **5**.

acetoxyl group in **5** and **6**, the conformation changes so that both H-1 and H-6 are axial. The dihedral angles made by H-1 with H-2 and H-2′ in compounds **5** and **6** must be approximately 140° and 20°, respectively, to account for the unusual magnitude of the coupling constants observed for H-1 (**5** H-1 7.8, 8.9 Hz; H-6 8.3, 10.6 Hz). We believe this indicates that the ring is in a boat conformation (in $CDCl_3$), as the magnitude of these couplings is not normal for a chair conformation. The nOed's observed for **5** were most helpful in the assignment of the conformation and in verification of the stereochemistry. In particular, irradiation of the signal from the decalin bridgehead proton (δ 1.73, H-5) gave a 3.5% nOe to the bridgehead proton (δ 3.40, H-7), a 1.2% nOe to the lactone proton (δ 4.96, H-8) and nOe's to both the bridgehead methyl group (1.2%) and the methyl at C-4 (1.6%). Irradiation of the bridgehead methyl group (δ 1.32) gave a 9% nOe to the bridgehead proton (H-5), a 9% nOe to the lactone proton (δ 4.96, H-8), and a 7.5% nOe to H-9 (δ 5.30). Irradiation of H-9 (δ 5.30) gave a 3.6% nOe to the lactone proton (δ 4.96, H-8), a 1.1% nOe to the bridgehead methyl group (δ 1.32), and a 1% nOe to H-1 (δ 5.77). These results can only be accommodated if the central ring is also in a boat conformation (see Figure 1). The stereochemistry at C-1 was defined by the observation of a 2% nOe to H-6. The proton at C-1 must be "axial" to exhibit couplings of 7.8 and 8.9 Hz, and it must be on the α face to have an nOe to H-6. The stereochemistry at C-4 was indicated by a 6% nOe from the methyl at C-4 to H-5. Assuming this ring is in a boat conformation, the methyl group at C-4 and H-5 would be in a trans diaxial orientation if the methyl were on the α face. No nOe to H-5 would be expected, but the methyl group at C-4 might be expected to exhibit an nOe to H-1, as these groups would both be in axial orientations on the boat apexes. The methyl group on C-4 also exhibited nOe's to H-6 (3%) and H-13′ (1%). These observations are consistent with the methyl group occupying an equatorial position (i.e., β orientation). It is noted that if the ring were to take up a chair conformation, an unfavorable 1,3-diaxial interaction would exist between the two methyl group substituents (C-14 and C-15).

It would appear that the molecules **3** and **4** may be held in this all chair conformation by H-bonding between the hydroxyl groups at C-4 and C-9. This possibility is removed on acetylation of the 9-OH, and for this reason or because of steric effects, the central ring adopts a boat conformation in **5** and **6**. We propose the semi-systematic name "prostatolide" for the oxygenated sesquiterpene skeleton with stereochemistry as specified in this study. Metabolite **3** is thus 1β-acetoxy-4α,9α-dihydroxy-6β-isobutyroxyprostatolide, and **4** is 1β-acetoxy-4α,9α-dihydroxy-6β-methacryloxyprostatolide.

^{13}C-nmr data for **3**, **4**, **5**, and **6** appear in Table 1, while ^{1}H-nmr data for **3**, **4**, **5**, and **6** from *W. prostata* and **7** and **8** from *W. trilobata* (2) appear in Table 2. It is obvious from the comparison of **5** with **7** and **6** with **8** that the relative stereochemistry of the metabolites from the two plants is significantly different.

Our investigation of *W. prostata* thus afforded two *ent*-kaurenic acid derivatives, identical to those presumed to be present in the extracts of *W. trilobata*, and four eudesmanolide sesquiterpenes whose substitution patterns were similar to those isolated from *W. trilobata* but whose stereochemical properties are significantly different.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Reichert microscopic hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions with a Perkin-Elmer 141 polarimeter. Ir spectra were determined on KBr plates as films or Nujol mulls with a Perkin-Elmer 297 ir spectrometer. Uv spectra were recorded in EtOH solutions with a Varian series 634 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ solutions on a Bruker AM300 nmr spectrometer with $CHCl_3$ (δ 7.26, 77.0 ppm) as reference. Hreims and eims were carried out on a JEOL D

100 mass spectrometer with peak matching unit. Perfluorokerosene was used as reference. Si gel type 60 (Merck) was used for cc, and plastic-backed plates coated with Si gel F_{254} (Merck) were used for tlc. Plates were visualized by spraying with vanillin/H_2SO_4 and warming. Hplc was carried out with a Waters 4500A solvent delivery system connected to a Waters U6K injector and a Waters R401 differential refractometer. Hplc columns were from Techsil (250 × 8 mm, filled with Techsil 5 mm silica) and Hewlett-Packard (250 × 8 mm, filled with Si-100 7 mm). These hplc columns were used in series.

BIOLOGICAL MATERIAL.—*W. prostata* was collected from the campus of the University of the Philippines at Los Banos in May 1987, and a voucher specimen is held in the Chemistry Department at U.P.L.B.

EXTRACTION AND ISOLATION.—The air-dried leaves (300 g) were ground in a Wiley Mill and soaked in $CHCl_3$ (1.2 l) for 3 days. The extract was filtered and the filtrate concentrated in vacuo to give a crude extract (15 g).

The crude extract was chromatographed on a Si gel rapid column with increasing percentages of EtOAc in petroleum ether followed by increasing percentages of MeOH in EtOAc as eluents. The fraction eluted with 20% EtOAc in petroleum ether was rechromatographed with the same step gradient elution system to 20% EtOAc in petroleum ether to afford the tigloyl **1** (39 mg, 0.020%) and cinnamoyl **2** (35 mg, 0.13%) derivatives of *ent*-kaurenic acid. The fraction eluted with EtOAc was rechromatographed with CH_2Cl_2-Et_2O-MeCN (9:0.5:0.5) as eluent to afford the acetylated isobutyrate **5** (3 mg) and acetylated methyl acrylate **6** (2 mg). The fractions eluted with 10% and 20% MeOH in EtOAc were rechromatogrphed with CH_2Cl_2-Et_2O-MeCN (8:1:1) as eluent. The isobutyrate **3** (16.8 mg) was obtained in pure form. In order to obtain the pure methacrylate **4** (15 mg) it was necessary to subject the mixture to repeated hplc with CH_2Cl_2-petroleum ether-Me_2CO (5:3.5:1.5) as eluent. (Although less pure **4** than **3** was obtained, nmr analysis of the crude mixture revealed that significantly more **4** was present in the mixture than **3** prior to separation.) A mixture of **3** and **4** (30 mg) was dissolved in pyridine (1 ml), Ac_2O (1 ml) was added, and the mixture was left overnight. The solvent was evaporated in vacuo and the residue was chromatographed on Si gel to yield the pure acetylated derivatives **5** and **6**.

3α-Tiglinoyloxy-ent-*kaur-16-enic acid* [**1**].—Colorless crystals: mp 153–155°; $[\alpha]_D$ −74° (*c* = 0.2, $CHCl_3$); uv λ max (EtOH) 208 (ε 2300), 290 (600) nm; ir ν max 3500–2920, 2850, 1695, 1450, 1375, 1260 cm^{-1}; ^1H nmr δ 6.87 (qq, 1, 7 Hz), 4.80 (br s), 4.74 (br s), 4.59 (dd, 4.7, 12 Hz), 2.62 (br s), 2.36 (dddd, 4, 12, 12, 12 Hz), 2.06 (m, 2H), 1.94 (m), 1.90 (m), 1.82 (dq, 3H, 1, 1 Hz), 1.74 (dq, 3H, 7, 1), 1.73 (m), 1.65 (m), 1.58 (m), 1.53 (m), 1.48 (m), 1.27 (s, 3H), 1.13 (m), 1.04 (s, 3H); ^{13}C nmr 180.9 (s), 167.7 (s), 155.3 (s), 137.4 (d), 128.8 (s), 103.3 (t), 78.8 (d), 56.4 (d), 55.1 (d), 48.7 (t), 43.9 (s), 48.0 (s), 43.7 (d), 41.0 (t), 39.5 (t), 39.4 (s), 38.7 (t), 33.0 (t), 24.0 (t), 23.9 (q), 21.5 (t), 18.5 (t), 15.3 (q), 14.5 (q), 12.0 (q) ppm; eims *m/z* 400 (15%), 300 (19), 285 (15), 83 (83); hreims *m/z* 400.265 ($C_{25}H_{36}O_4$ requires 400.261).

3α-Cinnamoyloxy-ent-*kaur-16-enic acid* [**2**].—Colorless crystals: mp 53–55°; $[\alpha]_D$ −129° (*c* = 0.02, $CHCl_3$); uv λ max (EtOH) 202 (ε 1700), 275 (2250) nm; ir ν max 3450–2920, 2840, 1710, 1695, 1450, 1375, 1240, 1120 cm^{-1}; ^1H nmr δ 7.70 (d, 16 Hz), 7.53 (m, 2H), 7.37 (m, 3H), 6.47 (d, 16 Hz), 4.83 (br s), 4.70 (br s), 2.65 (m), 2.45 (dddd, 4, 12, 12, 12 Hz), 1.31 (s, 3H), 1.05 (s, 3H).

1β-Acetoxy-4α,9α-dihydroxy-6β-isobutyroxyprostatolide [**3**].—Colorless crystals: mp 195–196°; $[\alpha]_D$ +31° (*c* = 0.02, $CHCl_3$); uv λ max (EtOH) 205 nm (ε 3070); ir (Nujol) ν max 3450, 2900, 1740, 1450, 1375, 1250, 1120, 1050 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* 350 (69%), 262 (49), 244 (91), 226 (27), 162 (57), 71 (88), 43 (93), 18 (42); hreims *m/z* 350.179 ($C_{21}H_{30}O_8$ − HOAc requires 350.173).

1β-Acetoxy-4α,9α-dihydroxy-6β-methacryloxyprostatolide [**4**].—^1H nmr see Table 2; ^{13}C nmr see Table 1.

1β,9α-Diacetoxy-4α-hydroxy-6β-isobutyroxyprostatolide [**5**].—Colorless crystals: mp 222–223°; $[\alpha]_D$ +112° (*c* = 0.02, $CHCl_3$); uv λ max (EtOH) 210 nm (ε 3160); ir (Nujol) ν max 3500, 2920, 1750, 1725, 1450, 1375, 1240, 1200, 1020 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* 392 (22%), 304 (22), 246 (13), 228 (6), 162 (17), 71 (27), 43 (77), 18 (73); hreims *m/z* 392.182 ($C_{23}H_{32}O_9$ − HOAc requires 392.182).

1β,9α-Diacetoxy-4α-hydroxy-6β-methacryloxyprostatolide [**6**].—Colorless crystals: mp 213–214°; $[\alpha]_D$ +90° (*c* = 0.2, $CHCl_3$); uv λ max (EtOH) 215 nm (ε 3160); ir (Nujol) ν max 3500, 2920, 1750, 1725, 1450, 1375, 1240, 1200, 1020 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* 390 (22%), 304 (21), 246 (15), 228 (12), 162 (42), 69 (46), 43 (77), 18 (87); hreims *m/z* 390.170 ($C_{23}H_{30}O_9$ − HOAc requires 390.168).

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