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EUDESMANOLIDES AND ENT-PIMARANES FROM *LIATRIS LAEVIGATA*

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Key Word Index—*Liatris laevigata*; Compositae; eudesmanolides; sesquiterpene lactones; ent-pimaranes; diterpenes.

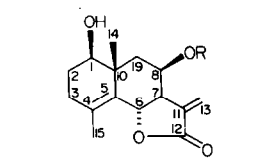
Abstract—*Liatris laevigata* gave five new closely related 1β-hydroxy-8β-tigloxyeudesman-6α,12-olides as well as lupeol, lupeyl acetate and the new diterpenes ent-8,15*R*-epoxy-3-oxopimara-12α,16-diol, ent-8,15*R*-epoxypimara-3β,12α,16-triol and its 15*S* epimer.

INTRODUCTION

In the course of our study of *Liatris* species which produce a variety of cytotoxic and antitumor lactones [1–18], we have examined *Liatris laevigata* Nutt. a taxon endemic to peninsular Florida [19]. In this report, we describe the isolation and structure determination of five new 1β-hydroxy-8β-tiglyloxyeudesman-6α,12-olides (**1a**, **2a**, **3a**, **4** and **5**) as well as three new diterpene ethers, ent-8, 15*R*-epoxy-3-oxopimara-12α, 16-diol (**8**) and the C-15 epimers of ent-8,15-epoxypimara-3β,12α,16-triol (**9a** and **10a**). Lupeol and lupeyl acetate were also found.

RESULTS AND DISCUSSION

The major sesquiterpene lactone constituent was **3a**; lactones **1a**, **2a**, **4** and **5** were present in smaller amounts. Examination of the ^{1}H NMR spectra (Table 1) of the three closely-related compounds **1a**, **2a** and **3a** revealed that they differed from **1b**, **2b** and **3b** recently reported from *Tithonia rotundifolia* [20] only in the nature of the C-8 ester side chain. The sequence H-5 through H-9 was established by spin decoupling and the relative stereochemistry at C-5 (in the case of **2a** and **3a**) and C-6–C-8 was deduced from the coupling constants as was the



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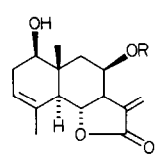
1b R = Ang



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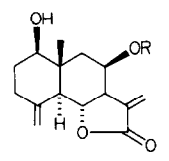

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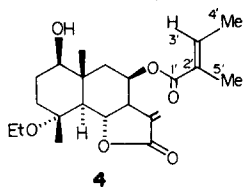
3b R = Ang



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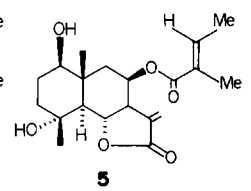

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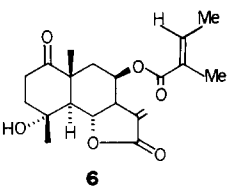

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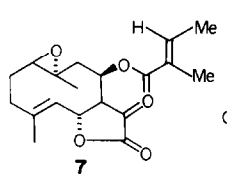

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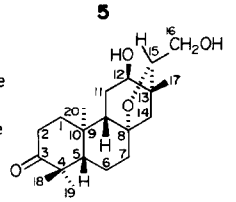

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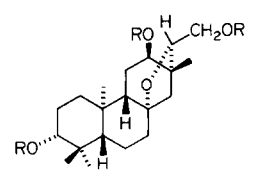
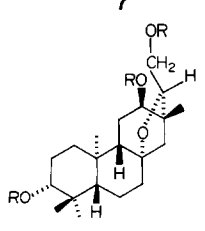


9a R = H
9b R = Ac



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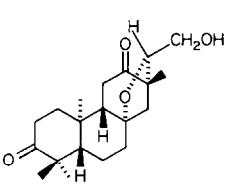
10b R = Ac



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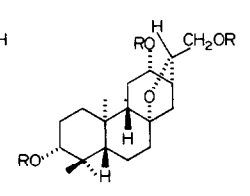


12a R = H
12b R = Ac

Table 1. ^{1}H NMR spectra of **1a–3a**, **4–6** and **5** + TAI (270 MHz, $CDCl_3$, TMS as int. standard)*

H	1a	2a	3a	4	5	5 + TAI	6
1	3.56 *dd* (11.5, 4)	3.67 *dd* (9.5, 6.5)	3.53 *dd* (11, 4.5)	3.44 *m* —	3.45 *dd* (10.5)	4.87 *dd* (11.5, 4)	— —
2a	1.69–	2.40 *m*	1.50–	1.63–	1.61–	2.09 *m*	2.70–
2b	1.90 *m*	1.99 *m*	1.93 *m*	1.93 *m*	1.83 *m*	1.88 *m*	2.47 *m*
3a	2.21 *m*	5.36 *m*	2.36 *m*			2.57 *dt* (4.5, 14)	2.20–
3b	2.05 *m*	—	1.93 *m*			2.35 *td* (3, 14)	1.96 *m*
5	—	2.44 *br d* (11)	2.27 *br d*	2.00 *d*	1.93 *d*	3.01 *d*	2.45 *d*
6	5.14 *br d* (11)	4.44 *t* (11)	4.54 *t*	4.52 *t*	4.59 *t*	4.52 *t*	4.56 *t*
7	2.93 *m* (11, 3.2, 3, 3)	2.81 *m*	2.85 *m*	2.91 *m*	2.90 *m*	2.98 *m*	2.84 *m*
8	5.81 *m* (3, 4, 2)	5.79 *m*	5.80 *m*	5.76 *m*	5.78 *m*	5.80 *m*	5.83 *m*
9a	2.41 *dd* (15, 2)	2.36 *dd*	2.39 *dd*	2.36 *dd*	2.34 *dd*	2.19 *dd*	2.27 *dd*
9b	1.62 *br dd* (15, 4)	1.56 *br dd*	1.60 *br dd*	1.57 *br dd*	1.57 *br dd*	1.82 *br dd*	1.81 *m*
13a	6.22 *d* (3.2)	6.13 *d*	6.15 *d*	6.13 *d*	6.19 *d*	6.12 *d*	6.21 *d*
13b	5.53 *d* (3)	5.43 *d*	5.47 *d*	5.41 *d*	5.50 *d*	5.48 *d*	5.56 *d*
14†	1.26 *br*	1.06 *br*	1.00 *br*	1.16 *br*	1.11 *br*	1.32 *br*	1.32 *br*
15†	1.91 *br*	1.89 *br*	5.04, 4.97 *br*‡	1.39 *br*	1.43 *br*	1.72 *br*	1.61 *br*
3′	6.82 *br q* (7)	6.81 *br q*	6.81 *br q*	6.81 *br q*	6.81	6.82 *br q*	6.81 *br q*
4′†	1.81 *br d* (7)	1.81 *br d*	1.81 *br d*	1.82 *br d*	1.81 *br d*	1.82 *br d*	1.81 *br*
5′†	1.83 *br*	1.82 *br*	1.83 *br*	1.83 *br*	1.82 *br*	1.83 *br*	1.83 *br*
Misc.				3.44§ 1.16 *t* (7)†			

*Unmarked signals are singlets. Figures in parentheses are coupling constants in Hz and are not listed listed if they correspond to those in preceding column.

†Intensity three protons.

‡Two protons.

§Centre of AB system.

equatorial orientation of the hydroxyl group on C-1. The absolute configurations which tally with those shown in the formulas can be derived from the CD curves which exhibit a negative Cotton effect in the η, π^*-region of the α,β-unsaturated lactone chromophore.

Substances **4** and **5** had ethoxyl and hydroxyl substituents at C-4. This was made evident by the changes in the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), with those of **4** exhibiting extra signals characteristic of the ethoxyl group. The attachment of the latter to C-4 is also clear from the upfield shifts of C-3 and C-5 and the downfield shift of C-4 in comparison with the corresponding signals in the ^{13}C NMR spectrum of **5**. Oxidation of **5** with Jones' reagent gave **6** which was a cyclohexanone (new IR band at 1715 cm^{-1}).

From the coupling constants (Table 1) and the CD curves (see Experimental) the relative and absolute stereochemistry of **4** and **5** is clearly the same as that of **1a–3a**. The C-4 stereochemistry of **5**, shown in the formula, is based on the significant paramagnetic shift of the H-5 signal ($\Delta\delta$ 1.08) on acylation of **5** with trichloroacetylisocyanate which indicates that H-5 and the tertiary hydroxyl are *cis* [21]. The expected paramagnetic shift of the H-15 signal ($\Delta\delta$ 0.28) is also observed. As the chemical shifts of H-14 and C-14 in **4** and **5** do not differ significantly, we assume that the ethoxyl group of **4** is equatorial like the tertiary hydroxyl of **5**.

The epoxygermacradienolide **7** is a logical precursor of **1–5** (it is possible that **4** is an artifact as one step in the isolation procedure involves the use of ethanolic lead acetate), but was not isolated. Instead three new closely related tetraoxygenated ent-pimaranes were found, one a ketodiol $C_{20}H_{32}O_4$, the other two triols of formula $C_{20}H_{34}O_4$. Analysis of the ^{1}H (see Experimental) and ^{13}C NMR spectra (Table 3) of the new compounds, or their derived acetates, indicated that the triols incorporated one primary and two secondary hydroxyl groups, each of the latter being of type A, where ■ represents a quaternary carbon, that the ketodiol was an oxidation product of one of the triols and that the fourth oxygen atom of the

Table 2. ^{13}C NMR spectra of **1a–3a**, **4** and **5** (67.89 MHz, $CDCl_3$, TMS as int. standard)*

C	1a	2a	3a	4§	5
1	77.78 *d*	75.62 *d*†	75.30 *d*	76.06 *d*	76.85 *d*
2	26.72 *t*	32.77 *t*	30.75 *t*	27.76 *t*	27.85 *t*
3	33.20 *t*	121.58 *d*	33.37 *t*	35.36 *t*	38.14 *t*
4	128.58‡	133.08	142.05	74.89	71.33
5	126.81‡	51.37 *d*†	52.06 *d*	54.67 *d*	57.06 *d*
6	77.96 *d*	77.48 *d*†	78.40 *d*	78.64 *d*	78.55 *d*
7	51.84 *d*	53.55 *d*†	53.39 *d*	53.22 *d*	53.18 *d*
8	66.50 *d*	66.06 *d*†	66.07 *d*	66.30 *d*	66.23 *d*
9	42.85 *t*	39.29 *t*†	40.32 *t*	43.83 *t*	43.52 *t*
10	41.82	40.68	42.69	42.28	41.88
11	134.08	134.22	134.47	134.44	133.25
12	169.47	169.99	169.92	170.32	168.94
13	120.92 *t*	119.25 *t*	119.49 *t*	119.12 *t*	120.56 *t*
14	19.53 *q*	12.77 *q*	13.57 *q*	16.48 *q*‡	15.95 *q*
15	20.81 *q*	23.24 *q*	110.63 *t*	19.77 *q*	24.36 *q*
1′	167.13	167.13	167.11	167.18	167.08
2′	128.17	128.17	128.09	128.12	128.02
3′	138.28 *d*	138.39 *d*	138.42 *t*	138.34 *d*	138.51 *d*
4′	14.49*q*	14.46 *q*	14.49 *q*	14.49 *q*	14.50 *q*
5′	12.17 *q*	12.15 *q*	12.18 *q*	12.19 *q*	12.18 *q*

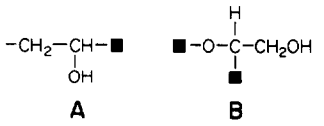
*Unmarked signals are singlets.
†Assignment by selective decoupling.
‡Assignments may be interchanged.
§Ethoxyl carbons resonated at δ 55.65 *t* and δ 16.23 *q*‡.

Table 3. ^{13}C NMR spectra of diterpenes

C	8*	9b*	10b*	12b*	13*	15a†	15b†	16a‡	16b‡
1	38.13 *t*	37.67 *t*	37.66 *t*	37.62 *t*	38.57 *t*	37.8	38.0	39.2	38.3
2	34.24 *t*	23.36 *t*	23.22 *t*	23.38 *t*	18.42 *t*	17.5	17.6	18.8	18.4
3	217.05	80.87 *d*	80.76 *d*	80.80 *d*	42.09 *t*	35.2	35.2	36.6	36.5
4	47.62	37.75	37.44	37.73	33.18	37.3	37.4	47.3	47.2
5	55.35 *d*	54.62 *d*	54.60 *d*	54.71 *d*	55.28 *d*	47.8	47.9	49.6	49.6
6	20.29 *t*	19.01 *t*	18.92 *t*	18.99 *t*	19.34 *t*	19.4	19.1	23.2	23.3
7	37.67 *t*	37.51 *t*	37.58 *t*	36.97 *t*	40.15 *t*	39.2	39.5	39.8	39.8
8	82.43	81.86	81.88	80.80	82.76	81.9	82.4	84.3	84.2
9	50.01 *d*	51.43 *d*	51.74 *d*	54.43 *d*	55.41 *d*	54.6	54.9	55.1	54.8
10	36.26	36.36	36.44	36.83	37.10	36.8	36.7	38.1	37.9
11	28.32 *t*	25.40 *t*	26.11 *t*	25.77 *t*	19.51 *t*	19.0	19.2	17.5	17.4
12	73.75 *d*	75.73 *d*	73.10 *d*	78.03 *d*	39.28 *t*	33.0	39.1	32.3	39.0
13	46.51	45.81	45.02	45.50	41.06	40.4	40.9	41.6	41.9
14	44.32 *t*	45.21 *t*	48.26 *t*	49.47 *t*	52.22 *t*	55.0	52.0	47.6	44.6
15	83.77 *d*	80.87 *d*	84.65 *d*	78.57 *d*	84.72 *d*	88.0	84.7	85.3	82.0
16	64.02 *t*	64.94 *t*	64.42 *t*	65.44 *t*	64.38 *t*	61.2	64.2	61.7	64.1
17	16.15 *q*	17.02 *q*	18.81 *q*	17.05 *q*	19.96 *q*	22.6	19.9	23.2	20.4
18	26.37 *q*	28.60 *q*	28.53 *q*	28.61 *q*	33.89 *q*	71.7	71.7	178.8	178.6
19	22.28 *q*	16.55 *q*	16.93 *q*	16.76 *q*	22.21 *q*	18.0	18.0	17.2	17.1
20	14.36 *q*	14.62 *q*	14.56 *q*	14.77 *q*	14.85 *q*	15.4	15.4	16.1	16.1
OAc	—	170.89, 170.64	170.77(2)	170.87(2)	—	—	—	—	—
		170.56	170.20	170.57					
		21.27 *q*(2)	21.18 *q*(2)	21.25 *q*, 21.14 *q*					
		20.97 *q*	20.85 *q*	21.06 *q*					

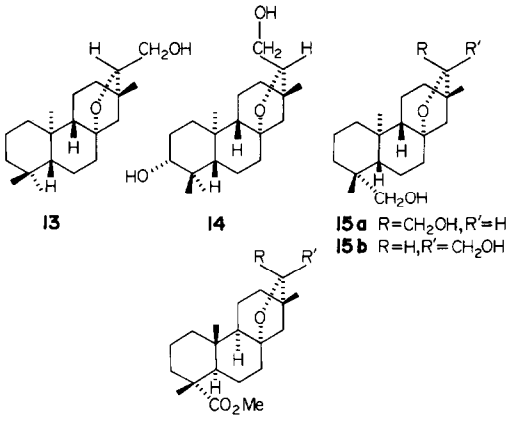
*Spectra in first five columns run in $CDCl_3$ at 67.89 MHz using TMS as int. standard.
†Data taken from ref. [23].
‡Data taken from ref. [24].

empirical formulae was part of an ether function of type **B**. The coupling constants of the signals representing the two protons geminal to the hydroxyl groups required that one hydroxyl (the one oxidized to a carbonyl in the ketodiol) be equatorial (J = 9, 6 Hz) and the other axial (J = 5, < 2 Hz).



A **B**

Plausible structures incorporating these features were **8**, **9a** and **10a**, i.e. analogues of isodarutigenol B(**14**) [22, 23], but without commitment as to the stereochemistry at C-10, C-13 and C-15. The equatorial hydroxyl groups of the triols, and the keto group of the ketodiol, were placed at C-3 to accommodate the carbon shifts of ring A and its attachments; similarly comparison with the ^{13}C NMR spectra of ring C-unsubstituted 8,15-tetrahydrofurans possessing pimarane [23] and isopimarane [24] stereochemistry required placement of the axial hydroxyl on C-12.



13 **14** **15a** R=CH_2OH, R'=H
15b R=H, R'=CH_2OH

16a R=CH_2OH, R'=H
16b R=H, R'=CH_2OH

Wenkert *et al.* [23, 24] have shown that carbon shifts in ring C and its attachments can be used to differentiate pimarane-based 8,15*R*- and 8,15*S*-tetrahydrofurans, such as **15a** and **15b**, and that similar criteria can be applied to isopimarane-based C-15 epimeric 8,15-tetrahydrofurans, such as **16a** and **16b** (see Table 3). In theory, it should also be possible to classify an unknown 8,15-tetrahydrofuran as a pimarane or isopimarane by inspection of the ^{13}C NMR spectrum (cf. in Table 3, C-8, C-10, C-11, C-13 and C-14 shifts of **15a**, **15b** vs C-8, C-10, C-11, C-13 and C-14 shifts of **16a**, **16b**). However, while it appeared obvious from the data of Table 3 that **9a** and **10a** were C-15 epimers and that **8** was related to **9a**, the complicating effect of the C-12 hydroxyl on the carbon shifts in ring C hampered an attempt at simultaneous assignment of C-15 as well as pimarane or isopimarane stereochemistry.

To overcome this problem and to verify the stereochemistry assigned to the secondary hydroxyl groups, the following procedure was adopted. Selective oxidation of the secondary hydroxyl groups of **9a** with sodium hypochlorite [25] afforded a dione **11** and a small amount of **8**, thus confirming the previously postulated relationship between ketol **8** and triol **9a**. Reduction of **11** with sodium borohydride gave **9a** and a new triol **12a** in the ratio 1:3. While H-3 of **9b** and **12b** exhibited identical coupling constants, H-12 of **9b** and **12b** did not, the hydroxyl group now being equatorial ($J_{11,12}$ = 10.5 and 6.5 Hz). In the ^{13}C NMR spectra the change from axial hydroxyl in **9b** to equatorial hydroxyl in **12b** was accompanied by a predictable, though relatively small, paramagnetic shift of C-12 ($\Delta\delta$2.3); other significant shifts were associated with C-9 ($\Delta\delta$3), C-14 ($\Delta\delta$ 4.2) and C-15 ($\Delta\delta$ −2.3) which, inspection of models suggested, could be associated equally well with pimarane or isopimarane stereochemistry. However, the constancy of C-10 which should be affected by the nature of the B/C ring fusion rather than by the hydroxyl on C-12 and whose shift was essentially identical with that of C-10 in **15a**, **15b** suggested that the diterpenes from *L. laevigata* possess pimarane stereochemistry.

This was proved as follows. Conversion of **11** to the dithioketal and subsequent Raney nickel desulphurization produced the 3,12-dideoxygenated derivative, **13**. Comparison of the ^{13}C NMR spectrum of this substance with the spectra of **15a**, **15b** and **16a**, **16b** [23, 24] which are also listed in Table 3 demonstrates that **13** has the same relative stereochemistry as **15b**. Consequently, the precursor of **13**, its C-15 epimer and the ketodiol are the ent-pimaranes **9a**, **10a**, **8** or their respective mirror images. As the CD curve of the ketodiol exhibited a relatively weak positive Cotton effect, comparable in magnitude but opposite in sign to that of 4,4-dimethyl-3-ketosteroids and similar substances [26]*, its absolute configuration is that shown in **8** and that of the triols is as shown in **9a** and **10a**.

L. laevigata is unique among the *Liatris* species studied so far in elaborating neither heliangolides nor guaianolides [1–17] but a series of closely related eudesmanolides derivable from the epoxygermacradienolide, **7**. It has been frequently treated as a variety of *L. tenuifolia* (e.g. ref. [28]) with which it intergrades where the ranges overlap [18]†; however, the chemistry of *L. tenuifolia* is distinctly different [12].

EXPERIMENTAL

Extraction of Liatris laevigata. Above-ground parts of *L. laevigata* Nutt. (4.5 kg), collected by Dr. R. K. Godfrey near Alexander Springs, Lake Co., Florida (Godfrey voucher No. 79204 on deposit in the Herbarium of Florida State University) were extracted with $CHCl_3$ and worked-up in the usual manner [30]. The crude gum (47 g) was preadsorbed on 60 g silicic acid (Mallinckrodt, 100 mesh) and chromatographed over 700 g of the same adsorbent packed in C_6H_6. Fractions (500 ml each) were collected as follows: 1–8 (C_6H_6); 9–14 (C_6H_6–$CHCl_3$, 1:1); 15–20 ($CHCl_3$); 21–28 ($CHCl_3$–MeOH, 99:1); 29–34 ($CHCl_3$–MeOH, 49:1); and 35–42 ($CHCl_3$–MeOH, 19:1).

The major compounds from fractions 2 (150 mg) and 6 (110 mg) were identified as lupeyl acetate and lupeol, respectively. Rechromatography of fraction 16 (1.7 g) over Si gel (40 g) gave, in the initial fractions, predominantly a single substance which on further purification by TLC ($CHCl_3$–MeOH–EtOAc,

*See also ref. [27] for ORD curves of enantiomeric isopimara-8(14),15-dien-3-ones.

†Presumably var. *quadriflora* Chapm. listed in ref. [29] is a synonym.

9.25:0.25:0.5) and crystallization from Et_2O–hexane afforded 1β-hydroxy-8β-tigloxy-4,11(13)-eudesmadien-6α,12-olide (**1a**), yield 135 mg, mp 156–159°, IR ν_{max}^{KBr} cm^{-1}: 3500, 1780, 1755, 1705 and 1650; CD curve (MeOH) $[\theta]_{249} - 2220$. (Calc. for $C_{20}H_{26}O_5$: MW, 346.1778. Found: MW(MS), 346.1776.) Other significant ions in the low resolution MS were at *m/z* (rel. int.) 263 (1), 246 (77), 228 (14), 213 (19), 83 (100) and 55 (98). ^1H and ^{13}C NMR spectra are listed in Tables 1 and 2. Continuation of the CC yielded, in the later fractions, gummy 1β-hydroxy-8β-tigloxy-3,11(13)-eudesmadien-6α,12-olide (**2a**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1770, 1710 and 1650; CD curve (MeOH) $[\theta]_{260} - 1420$. (Calc. for $C_{20}H_{26}O_5$: MW, 346.1778. Found: MW(MS), 346.1786.) Other significant peaks in the low resolution MS were at *m/z* (rel. int.) 263 (1), 246 (13), 228 (4), 83 (100) and 55 (66). ^1H and ^{13}C NMR spectra are listed in Tables 1 and 2.

Fraction 18 (5.2 g) contained one major constituent. Purification by CC gave 3.9 g of slightly impure 1β-hydroxy-8β-tigloxy-4(15),11(13)-eudesmadien-6α,12-olide (**3a**) which was further purified by TLC and then crystallized from Et_2O mp 154–156°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1770, 1710 and 1650; CD curve (MeOH) $[\theta]_{258} - 1200$. (Calc. for $C_{20}H_{26}O_5$: MW, 346.1778. Found: MW(MS), 346.1779.) Other significant peaks in the low resolution MS were at *m/z* (rel. int.) 263 (1), 246 (6), 228 (11), 213 (5), 83 (100) and 55 (71). ^1H and ^{13}C NMR spectra are listed in Tables 1 and 2.

Fraction 36 (2.3 g) was rechromatographed over a column of Si gel. Repurification of each fraction by TLC ($CHCl_3$–MeOH–EtOAc, 8:1:1) gave a total of 75 mg ent-8,15*R*-epoxy-3-oxopimara-12α,16-diol (**8**) and 420 mg 1β,4α-dihydroxy-8β-tigloxy-11(13)-eudesmen-6α,12-olide (**5**). Lactone **5** remained a gum which had IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1775, 1710 and 1650; CD curve (MeOH) $[\theta]_{258} - 1210$. (Calc. for $C_{20}H_{28}O_6$: MW, 364.1883. Found: MS(MS), 364.1878.) Other important peaks in the low resolution MS were at *m/z* (rel. int.) 349 (5), 264 (1), 246 (6), 83 (100) and 55 (72). ^1H and ^{13}C NMR spectra are listed in Tables 1 and 2. Oxidation of 70 mg **5** in 15 ml Me_2CO with 0.3 ml Jones' reagent at 0° for 45 min followed by the usual work-up and purification by TLC ($CHCl_3$–MeOH–EtOAc, 8.5:0.5:1) gave 44 mg **6** which had mp 143–144°; IR ν_{max}^{KBr} cm^{-1}: 3520, 1770, 1715 and 1650. The ^1H NMR spectrum is listed in Table 1. (Calc. for $C_{20}H_{26}O_6$: MW, 362.1729. Found. MW(MS), 362.1733.) Other significant peaks in the low resolution MS were at *m/z* (rel. int.) 344 (0.5) 262 (15), 244 (13), 226 (8), 216 (7), 205 (16), 189 (10), 99 (35), 83 (100) and 55 (49).

Ketodiol **8** also was a gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 and 1700; CD curve (MeOH) $[\theta]_{302} + 628$; NMR ($CDCl_3$, 270 MHz): δ 3.66 (narrow multiplet of H-12 partially superimposed on H-15), 3.64 (*dd*, *J* = 7.5, 3.5 Hz, H-15), 3.48 (centre of AB system, H-16), 2.59 (*ddd*, *J* = 15.5, 13.5, 6.5 Hz, H-2a), 2.29 (*ddd*, *J* = 15.5, 5, 3 Hz, H-2b), 1.18 (3H), 1.09 (6H) and 1.03 (each 3H, H-17–H-20). The ^{13}C NMR spectrum is listed in Table 3. (Calc. for $C_{20}H_{32}O_4$: MW, 336.2300. Found: MW (MS), 336.2306.) Other significant peaks in the low resolution MS were at *m/z* (rel. int.) 305 (73), 287 (47), and 269 (20).

Fraction 35 was purified by TLC ($CHCl_3$–MeOH–EtOAc, 18.5:0.5:1, developed twice) to give 145 mg of non-crystalline **4** which had IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1770, 1710 and 1650; CD curve (MeOH) $[\theta]_{258} - 1100$. (Calc. for $C_{22}H_{32}O_6$: MW, 392.2196. Found: MW(MS), 392.2157.) Other significant peaks in the low resolution MS were at *m/z* (rel.int.) 262 (5), 99 (100), 83 (73) and 55 (72). ^1H and ^{13}C NMR spectra are listed in Tables 1 and 2.

Fraction 37 on trituration with $CHCl_3$ gave 730 mg ent-8,15*R*-epoxypimara-3β,12α,16-triol (**9a**) which was recrystallized from MeOH–EtOAc, mp 218–218°; ^1H NMR signals ($CDCl_3$–3 drops DMSO-d_6–D_2O, 270 MHz): δ 3.59 (*m*, H-12 and H-15), 3.45 (centre of AB system, H-16), 3.17 (*m*, H-3), 1.85 (*d*, *J* = 12 Hz, H-14b) and 1.01, 1.00, 0.99, 0.83 (each 3H, H-17–H-20). (Calc. for $C_{20}H_{30}O_4$: MW, 338.2457. Found: MW(MS), 338.2443.) Acetylation of 100 mg **9a** with 1 ml Ac_2O and 1 ml pyridine at room temp. overnight followed by the usual work-up, TLC purification (C_6H_6–EtOAc, 9:1) and crystallization from $CHCl_3$–hexane gave 85 mg **9b**, mp 133–134°; ^1H NMR ($CDCl_3$, 270 MHz): δ 4.85 (*m*, $W_{1/2}$ = 8 Hz, H-12), 4.43 (*dd*, *J* = 9, 6Hz, H-3), 4.01 (centre of AB system, H-16), 3.77 (*t*, *J* = 5 Hz, H-15), 1.81 (*d*, *J* = 12 Hz, H-14a), 1.46 (*br d*, *J* = 12Hz, H-14b), 2.08, 2.06, 2.04 (Ac) and 1.01, 0.94, 0.91, 0.88 (each 3H, H-17–H-20). The ^{13}C NMR spectrum is listed in Table 3.

Fraction 40 was rechromatographed over Si gel. Fractions eluted with $CHCl_3$–MeOH (19:1) were combined and crystallized from MeOH–EtOAc to yield 155 mg ent-8,15*S*-epoxypimara-3β,12α,16-triol (**10a**), mp 213–217°; ^1H NMR ($CDCl_3$–3 drops DMSO-d_6–D_2O, 270 MHz): δ 3.81 *m* (H-15), 3.69 (*br d*, *J* = 5 Hz, H-12), 3.72 (centre of AB system, H-16), 3.18 (*dd*, *J* = 9, 6 Hz, H-3), 1.98 (*d*, *J* = 12 Hz, H-14a), 1.34 (*br d*, *J* = 12 Hz, H-14b) and 1.06, 1.00, 0.98, 0.83 (each 3H, H-17–H-20). (Calc. for $C_{20}H_{34}O_4$: MW, 338.2457. Found: MW(MS), 338.2450.) Other significant ions in the low resolution MS were at *m/z* (rel.int.) 307 (68), 289 (87) and 271 (48). Acetylation of 50 mg **10a** overnight at room temp., purification of the crude product by TLC (EtOAc–C_6H_6, 1:4) and crystallization from $CHCl_3$–hexane gave 42 mg **10b**, mp 181–184°, ^1H NMR ($CDCl_3$, 270 MHz): δ 4.99 (*br d*, *J* = 5 Hz, H-12), 4.47 (*dd*, *J* = 9, 6 Hz, H-3), 4.25 (centre of AB system, H-16), 3.88 (*dd*, *J* = 7.5, 4 Hz, H-15), 1.96 (*d*) and 1.48 (*br d*, *J* = 12 Hz, H-14a), 2.08, 2.07, 2.04 (Ac), 0.98 (6H), 0.91 and 0.88 (each 3H, H-17–H-20). The ^{13}C NMR spectrum is listed in Table 3.

Ent-8, 15*R-epoxy*-16-*hydroxy*-3,12-*pimaradione* (**11**). A soln of 275 mg **9a** in 4 ml HOAc was stirred with 400 mg commercial $CaOCl_2$ in 4 ml H_2O for 30 min at 15–25°. Excess reagent was destroyed with satd $NaHSO_3$ soln and the mixture poured over ice–brine. Extraction with Et_2O, evaporation of the washed and dried extract and purification by TLC ($CHCl_3$–MeOH–EtOAc, 18.5:0.5:1) of the residue gave 14 mg **8** and 223 mg **11** which exhibited ^1H NMR signals ($CDCl_3$, 270 MHz) at δ 3.94 (*dd*, *J* = 6, 4 Hz, H-15), 3.55 (centre of AB system, H-16), 2.61 (*ddd*, *J* = 15.5, 13.5, 6.5 Hz, H-2a), 2.56 (*dd*, *J* = 16, 11 Hz, H-11a), 2.41 (*dd*, *J* = 16, 6.5 Hz, H-11b), 2.32 (*ddd*, *J* = 15.5, 5, 3 Hz, H-2b), 2.02 (*d*) and 1.64 (*d*, *J* = 12 Hz, H-14a,b), 1.27, 1.13 (3 H each) and 1.12 (6H, H-17–H-20). (Calc. for $C_{20}H_{30}O_4$: MW, 334.2142. Found: MW(MS), 334.2130.)

Ent-8,15*R-Epoxypimara*-3β,12β,16-*triol* (**12a**). Reduction of 82 mg **11** in 3 ml MeOH with 35 mg $NaBH_4$ for 30 min at room temp. followed by the usual work-up and purification by TLC ($CHCl_3$–MeOH–EtOAc, 8:1:1) yielded **9a** and **12a** in the ratio 1:3. The new triol exhibited ^1H NMR signals ($CDCl_3$–3 drops DMSO-d_6–D_2O, 270 MHz) at δ 3.97 (*dd*, *J* = 6, 4 Hz, H-15), 3.44 (*m*, H-16, H-12), 3.16 (*m*, H-3), 1.06, 1.03, 1.00, 0.84 (3H each, H-17–H-20). Acetylation of the triol in the usual way and crystallization of the crude product from $CHCl_3$–MeOH gave 35 mg **12b**, mp 186–188° which had ^1H NMR signals ($CDCl_3$, 270 MHz) at δ 4.75 (*dd*, *J* = 10.5, 6.5 Hz, H-12), 4.47 (*dd*, *J* = 9, 6 Hz, H-3), 4.11 (*m*, H-15), 4.02 (centre of AB system, H-16), 2.07, 2.05, 2.03 (Ac), 1.76 (*d*) and 1.28 (*d*, *J* = 12 Hz, H-14a, b), 1.04, 0.98, 0.91 and 0.88 (3H each, H-17–H-20). The ^{13}C NMR spectrum is listed in Table 3. The low resolution MS exhibited a very weak $[M]^+$ ion at *m/z* 465 (0.2%) corresponding to $C_{26}H_{40}O_7 + H^+$; other significant peaks were at *m/z* (rel.int.) 404 (38), 391 (23), 344 (56), 302 (72) and 284 (6).

Ent-8,15*R-Epoxypimaran*-16-*ol* (**13**). A mixture of 100 mg **10**, 0.3 ml ethanedithiol and 0.3 ml BF_3 etherate was stirred for 15 min, diluted with 5 ml MeOH, stirred well, cooled and filtered. The ppt was dried and refluxed with *ca* 1 g Raney Ni(W-2) in 5 ml

EtOH for 2 hr, filtered and concd. Purification of the residue by TLC ($CHCl_3$–MeOH–EtOAc, 18.5:0.5:1) and crystallization from EtOAc–hexane afforded 37 mg **13**, mp 86–87°, which had ^1H NMR signals ($CDCl_3$, 270 MHz) at δ 3.71 (*dd*, $J = 7, 3.5$ Hz, H-15), 3.43 (centre of AB system, H-16), 1.02, 0.94 (3H each) and 0.87 (6H, H-17–H-20). The ^{13}C NMR spectrum is listed in Table 3. (Calcd. for $C_{20}H_{34}O_2$: MW, 306.2557. Found: MW(MS), 306.2567.) Other significant peaks in the low resolution MS were at *m/z* (rel. int.) 291 (2) and 275 (100).

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