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Tonantzitlolone and other Diterpenes from *Stillingia sanguinolenta*

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Cembrene A (**1**), two novel diterpenes **2** and **3** with the rare flexibilane skeleton, two new pimaranes **4** and **5**, seven kauranes **6**–**12** (**9** and **12** new), three known atisanes **13**–**15**, a new trachylobane **16**, and the new pentacyclic diterpene sanguinolane **17** with a novel skeleton were obtained from *Stillingia sanguinolenta*.

Introduction

The genus *Stillingia* – a mainly American genus of ca. 25 species with a few disjunct taxa in the Mascarene Islands, eastern Malaysia, and Fiji – belongs to the family Euphorbiaceae. The roots of *S. sanguinolenta* are used in poultices after childbirth, and infusions of leaves are used by northern Mexican natives to treat pulmonary ailments. A similar application of *S. sylvatica* by Navajos and Creek Native Americans has been described.[1,2] Only a few reports on the chemistry of members of the genus have appeared so far; toxic and irritant ingenol esters have been reported from the roots of *S. sylvatica*.[3] Here we report our results with *S. sanguinolenta* (collected in Mexico), which had not previously been chemically investigated.

Results and Discussion

An extract of *Stillingia sanguinolenta* Muell. & Arg. afforded, in addition to acetylaleuritolic acid, the diterpenes cembrene A (**1**),[4,5] the new flexibilanes tonantzitlolone (**2**)[6] and tonantzitlolone B (**3**), the new pimaranes **4** and **5**, the kauranes **6**,[7] **7**,[8] **8**,[9] **9**, abbeokutone (**10**),[10,11] **11**,[12,13] and **12**, the atisanes **13**,[14] **14**,[14] and **15**,[13] the new trachylobane **16**, and the pentacyclic sanguinolane **17**, with a novel skeleton.

The structures of **2** and **3**, each possessing a 15-membered carbocyclic flexibilane ring skeleton, were deduced from detailed evaluation of 1D and 2D NMR spectroscopic data. The ^{1}H NMR spectrum of **2** (Table 1 and Figure 1) displays well dispersed signals. We propose the names tonantzitlolone for the new compound **2** and tonantzitlolone B for the derivative **3** (in Aztec mythology Tonantzin was the lunar mother goddess).[15] By spin decoupling, a methyl senecioate unit was deduced from the ^{13}C NMR spectra (Table 1). Further confirmation was obtained from the mass spectrum, particularly from the base peak at *m*/*z* 97 for the acylium ion. Additionally, three short sequences (-CH(CH_3)CH=CH- [I], -CH(OR)CH_2CH(CH_3)CH(OR)- [II], and -CH_2CH_2CH(OR)- [III]) were identified. Other fragments bore a tertiary and a secondary hydroxy group, as indicated by two D_2O-exchangeable signals at δ = 5.66 ppm (*s*) and 3.10 ppm (*d*) and three tertiary methyl groups. In addition to the signals for the ester residue and protonated sites, the ^{13}C NMR spectrum also displayed four singlets for quaternary carbons. Their chemical shifts were diagnostic for a keto group, an acetal, an oxygen-bearing carbon, and a nonfunctionalized carbon. The interrupted sequences and isolated fragments were connected by correlations obtained from an HMBC experiment (Table 1). The ^2J and ^3J correlations of methyl singlets at δ = 0.91 and 1.14 ppm to C-1, C-14, and C-15 required a *gem* dimethyl group at C-15 and placed it between sequences I and III. Correlation between H-20 and C-4 connected the keto group with the other terminus of sequence I. Those between H-18 and C-10, C-11, and C-12 continued from the other end of sequence III, which was extended to sequence II via the acetal carbon (C-9), as was judged from the correlations between OH-10 and C-9 and between OH-9 and C-9 and C-8. The remaining free valences of C-4 and C-5 had to be connected to each other. Finally, the connections of four oxygen-bearing carbon atoms to form two

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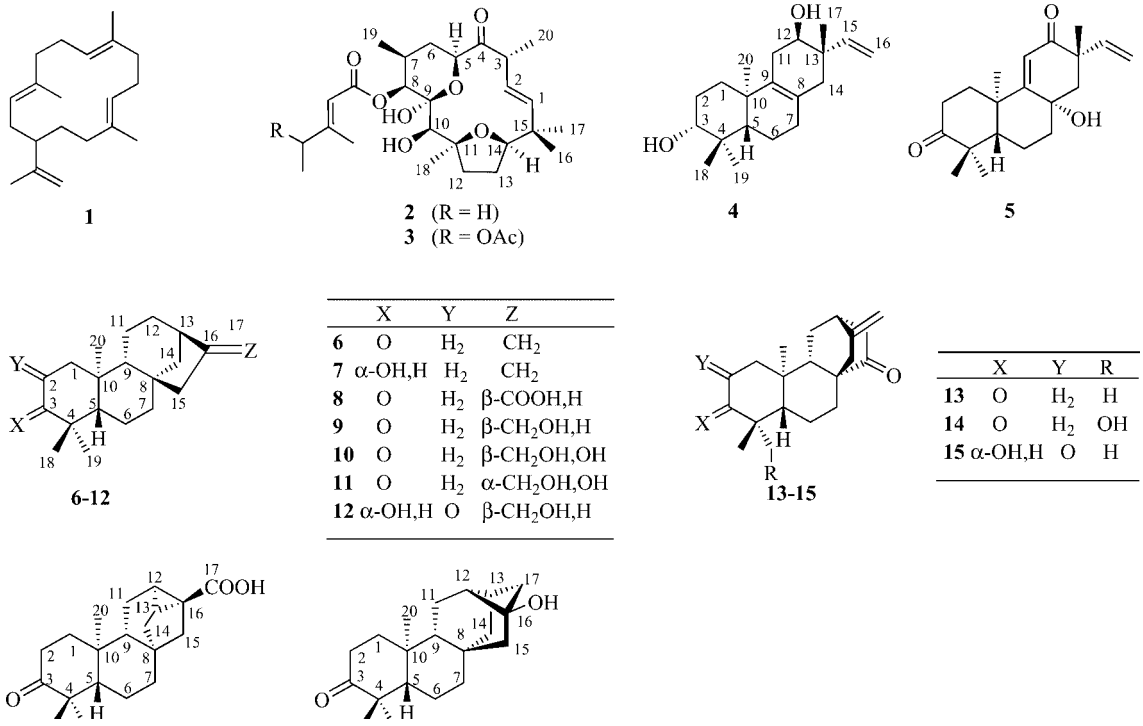
ether rings were established by use of various NMR techniques. Unfortunately, the HMBC spectrum did not reveal any ^3J correlations between H-5 and C-9 and/or H-14 and C-11, which would have located and fixed the ether ring positions. The crucial information was provided by NOE experiments, from which the relative configuration and the preferred conformation could be deduced. In that respect, NOE results obtained by saturation of the methyl groups and both hydroxy groups were particularly important (Table 1). The conformation calculated with the aid of the PCMODEL[16] molecular modeling program fitted excellently with the experimental data. Moreover, the observed and calculated coupling constants matched perfectly. An attempt to derivatize the secondary hydroxy group of **2** as its Mosher esters failed. However, formation of Mosher esters and the assignment of the absolute configuration of **2** was possible after reduction of the keto group with DIBAL-H.[17] The reduction yielded two epimeric alcohols **18** (pseudoequatorial hydroxy group) and **19** (pseudoaxial hydroxy group) (see Supporting Information), which gave smooth access to the corresponding Mosher ester pairs (*R*/*S*)-**20a**/(*R*/*S*)-**20b** and (*R*/*S*)-**21a**/(*R*/*S*)-**21b**, respectively (Figure 2). ^{1}H NMR analysis of the esters (*R*/*S*)-**20a** and (*R*/*S*)-**20b** and comparison of the ^{1}H chemical shifts (δ) of

Table 1. ^{13}C and ^{1}H NMR data for compounds **2** and **3**; NOE and HMBC results with **2**.

	2				**3**	
No.	δ_C	δ_H (J in Hz)	NOE[a]	HMBC[b]	δ_C	δ_H (J in Hz)
1	140.1 (CH)	5.86 (d, 1 H, 15.5)	3(3), 13β(1), 16(w), 10-OH(<1)	3, 16	140.1 (CH)	5.85 (d, 1 H, 15.5)
2	126.8 (CH)	5.23 (dd, 1 H, 15.5, 9.5)	5(2.5), 17(s), 20(m)	3	126.8 (CH)	5.24 (dd, 1 H, 15.5, 9.5)
3	49.5 (CH)	3.33 (dq, 1 H, 9.5, 7.0)	1(5), 10-OH(1.5)		49.5 (CH)	3.32 (dq, 1 H, 9.5, 7.0)
4	211.4 (C)				211.2 (C)	
5	74.1 (CH)	4.62 (dd, 1 H, 12.0, 3.0)	2(2), 7(3), 9-OH(2)		74.1 (CH)	4.63 (dd, 1 H, 12.0, 3.0)
6α	28.8 (CH_2)	1.85 (ddd, 1 H, 13.5, 12.0, 4.0)			28.7 (CH_2)	1.85 (ddd, 1 H, 13.5, 12.0, 4.0)
6β		1.38 (ddd, 1 H, 13.5, 12.5, 3.0)				1.38 (ddd, 1 H, 13.5, 12.5, 3.0)
7	29.0 (CH)	2.33 (dddq, 1 H, 12.5, 7.0, 4.0, 2.5)	5(4), 8(5)		29.0 (CH)	2.31 (dddq, 1 H, 12.5, 7.0, 4.0, 2.5)
8	73.1 (CH)	4.90 (d, 1 H, 2.5)	7(6), 10(7), 19(s), 9-OH(1.5), 2_{OR}(<1)	7, 1_{OR}	73.7 (CH)	4.90 (d, 1 H, 2.5)
9	97.1 (C)				97.0 (C)	
10	78.2 (CH)	3.43 (d, 1 H, 6.0)	8(5), 12β(1.5), 18(s), 9-OH(1.5), 2_{OR}(<1)		78.1 (CH)	3.41 (d, 1 H, 6.5)
11	87.6 (C)				87.6 (C)	
12α	37.3 (CH_2)	1.54 (ddd, 1 H, 12.5, 12.5, 7.5)		14	37.3 (CH_2)	1.53 (ddd, 1 H, 12.5, 12.5, 7.5)
12β		2.44 (br. dd, 1 H, 12.5, 7.5)	10(2)			2.43 (br. dd, 1 H, 12.5, 7.5)
13α	28.0 (CH_2)	1.76 (br. ddd, 1 H, 12.5, 7.5, 5.0)			28.0 (CH_2)	1.76 (br. ddd, 1 H, 12.5, 7.5, 5.0)
13β		2.04 (dddd, 1 H, 12.5, 12.5, 12.5, 7.5)	1(5), 16(w)			2.04 (dddd, 1 H, 12.5, 12.5, 11.5, 7.5)
14	88.9 (CH)	3.77 (dd, 1 H, 12.5, 5.0)	16(m), 17(m)		88.9 (CH)	3.77 (dd, 1 H, 11.5, 5.0)
15	38.7 (C)				38.7 (C)	
16	25.4 (CH_3)	0.91 (s, 3 H)	1(5), 13α(4), 13β(1.5), 14(5), 17(s)	1, 14, 15, 17	25.4 (CH_3)	0.91 (s, 3 H)
17	25.1 (CH_3)	1.14 (s, 3 H)	2(12), 14(7), 16(s), 9-OH(5)	1, 14, 15, 16	25.1 (CH_3)	1.14 (s, 3 H)
18	28.1 (CH_3)	1.37 (s, 3 H)	8(1.5), 10(8), 12α(5), 14(2), 9-OH(7)	10, 11, 12	28.1 (CH_3)	1.37 (s, 3 H)
19	17.0 (CH_3)	0.84 (d, 3 H, 7.0)	6α(2), 6β(2), 8(3)	7, 8	17.0 (CH_3)	0.84 (d, 3 H, 7.0)
20	16.0 (CH_3)	1.12 (d, 3 H, 7.0)	2(4)	2, 3, 4	16.0 (CH_3)	1.14 (d, 3 H, 7.0)
OH-9		5.66 (s, 1 H)	5(5). 8(1.5), 10(1.5), 17(m), 18(s)	8, 9		5.70 (s, 1 H)
OH-10		3.10 (d, 1 H, 6.0)	1(2), 3(1.5), 2_{OR}(1)	9		3.06 (d, 1 H, 6.5)
OR 1	166.6 (C)				166.3 (C)	
2	113.8 (CH)	5.69 (br. q, 1 H, 1.5)		4_{OR}, 6_{OR}	115.1 (CH)	5.91 (br. q, 1 H, 1.5)
3	163.0 (C)				157.6 (C)	
4	33.9 (CH_2)	2.17 (br. q, 2 H, 7.0)		2_{OR}, 3_{OR}, 5_{OR}	73.8 (CH)	5.24 (br. q, 1 H, 7.0)
5	11.8 (CH_3)	1.07 (t, 3 H, 7.0)		3_{OR}, 4_{OR}	19.1 (CH_3)	1.35 (d, 3 H, 7.0)
6	19.0 (CH_3)	2.16 (d, 3 H, 1.5)		2_{OR}, 3_{OR}, 4_{OR}	16.0 (CH_3)	2.13 (d, 3 H, 1.5)
OAc					169.9 (C), 21.2 (CH_3)	2.09 (s, 3 H)

[a] NOE correlations are from proton(s) stated to the proton(s) indicated. The percentage intensity increase is listed in parentheses. [b] HMBC correlations are from proton(s) stated to the carbon indicated.



	X	Y	Z
6	O	H_2	CH_2
7	α-OH,H	H_2	CH_2
8	O	H_2	β-COOH,H
9	O	H_2	β-CH_2OH,H
10	O	H_2	β-CH_2OH,OH
11	O	H_2	α-CH_2OH,OH
12	α-OH,H	O	β-CH_2OH,H

	X	Y	R
13	O	H_2	H
14	O	H_2	OH
15	α-OH,H	O	H

Figure 1. Overview of structures of compounds isolated from *Stillingia Sanguinolenta*.

the two epimers showed a disturbed distribution of positive and negative shift differences due to steric congestion in the vicinity. However, chemical shift analysis of the pseudoaxial esters (*R*/*S*)-**21a** and (*R*/*S*)-**21b** demonstrated an even distribution of chemical shift differences and allowed the configurations of the stereogenic centers at C-4 in (*R*/*S*)-**21a** and (*R*/*S*)-**21b** to be assigned as (*R*). Therefore, the absolute configuration of **2** was assigned as depicted in Figure 1.[6,18]

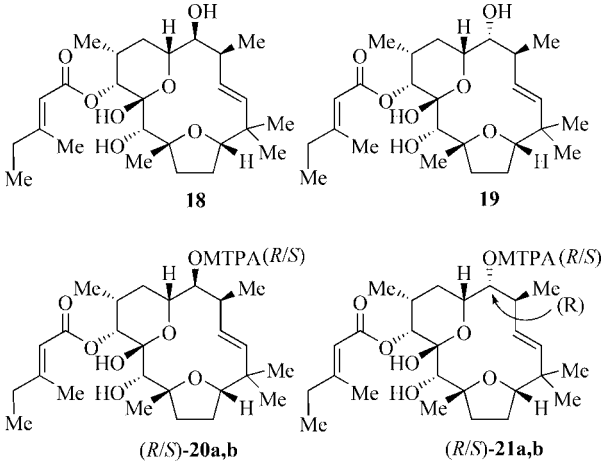


Figure 2. Determination of the absolute configuration of **2** after reduction of the keto group and formation of all possible Mosher esters (for details see electronic supporting information); MTPA = methoxy(trifluoromethyl)phenylacetyl.

The ^{1}H NMR spectrum of **3** (Table 1) differed from **2** only in selected signals of the ester residue. In fact, compound **3** contains a 5-acetoxy-methylsenecioate unit instead of the methylsenecioate present in **2**. Nevertheless, the configuration of the side chain stereogenic center could not be determined.

It has to be pointed out that the only known natural product with this diterpene skeleton is flexibilene, which was isolated from the soft coral *Sinularia flexibilis*.[19–21]

The ^{1}H NMR spectra of compounds **4** and **5** (Table 2) each show four methyl groups and one vinyl group, which points to the pimaranes. In compound **5**, the presence of a keto group at C-3 was deduced from the chemical shifts of H-2, which were similar to those of all other 3-oxo diterpenes discussed in this paper. A second conjugated keto group had to be placed at C-12, as the signal of H-11 appeared as a downfield-shifted singlet. The sequence H-5 through H-7 followed from spin decoupling experiments. The two fragments (C-5–C-7 and C-9–C-12) are interchangeable, but a strong NOE effect between H-11 and the two H-1 protons is only consistent with the proposed structure. Furthermore, the base peak in the mass spectrum at *m*/*z* 248 could be explained in terms of a retro-Diels–Alder fragmentation process, as indicated by the loss of a C_5H_8 fragment from the molecular ion. The stereochemistry was elucidated from additional NOE experiments. On irradiation at H-20, increases in the intensities of the signals for axial H-2 (5%), H-6 (7%), 8-OH (8%), and H-19 (s) were observed. Therefore, the 8-hydroxy group must be oriented in an axial position. The vinyl group at C-13 also has to be

α-orientated, because of a dipolar interaction (5%) between OH-8 and H-15 observed after irradiation of OH-8.

Table 2. ^{1}H NMR spectroscopic data for pimarane derivatives **4** and **5**.

No.	**4** δ_H (J in Hz)	**5** δ_H (J in Hz)
1a	2.05–2.15 (m, 3 H)	2.10 (ddd, 1 H, 13.0, 6.5, 3.0)
1b	1.50–1.60 (m, 3 H)	1.85 (ddd, 1 H, 13.0, 12.5, 6.5)
2a	2.05–2.15 (m, 3 H)	2.73 (ddd, 1 H, 16.0, 12.5, 6.5)
2b	1.50–1.60 (m, 3 H)	2.49 (ddd, 1 H, 16.0, 6.5, 3.0)
3	3.25 (dd, 1 H, 12.0, 4.5)	–
5	1.12 (dd, 1 H, 11.0, 2.0)	1.53 (m, 1 H)
6a	2.05–2.15 (m, 3 H)	2.01 (dddd, 1 H, 12.5, 12.5, 12.5, 3.0)
6b	1.50–1.60 (m, 3 H)	1.53 (m, 1 H)
7a	1.90–1.65 (m, 2 H)	2.12 (m, 1 H)
7b	1.90–1.65 (m, 2 H)	1.40 (dddd, 1 H, 12.5, 12.5, 3.5, 3.0)
11a	2.28 (br. dd, 1 H, 15.5, 5.5)	5.91 (s, 1 H)
11b	1.90 (m, 1 H)	–
12	3.55 (dd, 1 H, 9.5, 5.5)	–
14a	2.00 (br. d, 1 H, 17.0)	2.13 (d, 1 H, 14.0)
14b	1.68 (br. d, 1 H, 17.0)	1.93 (d, 1 H, 14.0)
15	5.75 (dd, 1 H, 17.5, 11.0)	6.06 (dd, 1 H, 18.0, 10.5)
16_{cis}	5.18 (d, 1 H, 11.0)	5.16 (d, 1 H, 10.5)
16_{trans}	5.15 (d, 1 H, 17.5)	4.92 (d, 1 H, 18.0)
17	0.93 (s, 3 H)	1.20 (s, 3 H)
18	1.03 (s, 3 H)	1.13 (s, 3 H)
19	0.82 (s, 3 H)	1.11 (s, 3 H)
20	0.98 (s, 3 H)	1.44 (s, 3 H)
OH	not resolved	2.65 (d, 1 H, 3)

Compound **4** was obtained only in minute amounts, and its structure was deduced only from MS and ^{1}H NMR spectroscopic data. The HRM spectrum is indicative of a diol with five degrees of unsaturation. The presence of a tetrasubstituted double bond between C-8 and C-9 was supported by a downfield shift of the allylic protons H-7, H-11, and H-14. The stereochemistry at C-3 and C-12 was deduced from the H-3 and H-13 spin couplings and NOE data for neighboring centers.

The structures of kauranes **9** and **12** were deduced from spectroscopic data that were in part similar to those of co-occurring derivatives (see Table 3 and electronic supporting information). In the ^{1}H NMR spectra of **9** and **12** the signals for the *exo*-methylene group in **6** were replaced by an ABX system, which is typical for the hydroxymethyl group. The stereochemistry at C-16 and the substitution pattern in ring A for compound **12** followed from NOE experiments. Dipolar interactions between H-17, H-11β, and the β-oriented H-15 were observed and settled the configuration at C-16 (Figure 3). The β orientation of H-15 followed from the long-range coupling with H-14α (zig-zag in-plane), which itself showed a strong NOE after saturation of the resonance of H-20. The substitution in the A-ring was deduced from NOE effects between H-18 and the axial H-3, which itself interacts with axial H-1 (long-range coupling with H-20). The configuration at C-16 in **9** followed from ^{13}C NMR spectroscopic data for the C/D rings almost identical to those for **12**.

Table 3. ^{13}C NMR spectroscopic data for kaurane derivatives **9** and **12**.

No.	**9** δ_C	**12** δ_C
1	39.3 (CH_2)	53.5 (CH_2)
2	34.1 (CH_2)	211.0 (C)
3	218.3 (C)	82.9 (CH)
4	47.1 (C)	45.4 (C)
5	54.3 (CH)	54.4 (CH)
6	21.7 (CH_2)	20.4 (CH_2)
7	40.0 (CH_2)	41.3 (CH_2)
8	44.0 (C)	44.3 (C)
9	56.0 (CH)	56.6 (CH)
10	41.0 (C)	45.3 (C)
11	19.4 (CH_2)	19.1 (CH_2)
12	25.8 (CH_2)	25.7 (CH_2)
13	36.9 (CH)	36.9 (CH)
14	38.4 (CH_2)	39.8 (CH_2)
15	43.3 (CH_2)	43.4 (CH_2)
16	43.1 (CH)	43.0 (CH)
17	64.2 (CH_2)	64.1 (CH_2)
18	27.3 (CH_3)	29.6 (CH_3)
19	21.7 (CH_3)	16.4 (CH_3)
20	17.8 (CH_3)	18.7 (CH_3)

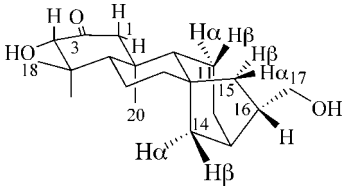


Figure 3. Conformational representation of **12**.

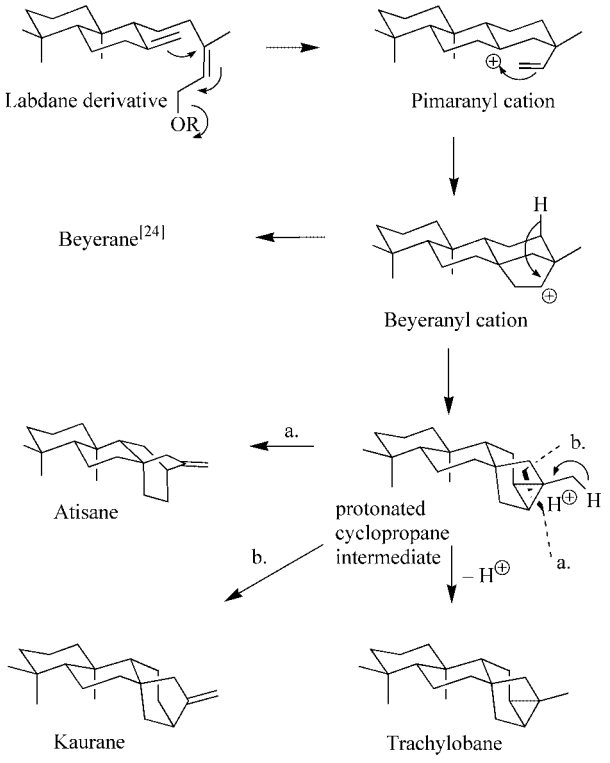
A literature survey for known 16,17-dioxy diterpenes with kaurane constitutions revealed that some of the structures were a matter of controversy. It would go beyond the scope of this paper to cover all derivatives described. To clear up the existing confusion in this structural class of diterpenes we have added a short discussion on that topic, which is included in the Supporting Information.

The ^{13}C NMR spectrum of the trachylobane derivative **16** showed signals for a keto group and a carboxylic acid. The multiplicities of all signals required a pentacyclic core, which was in full agreement with the mass spectrum. The chemical shifts of several signals indicated a cyclopropane substructure, which led us to assume a trachylobane derivative. Spin decoupling confirmed this assumption. The final confirmation was achieved by comparison of the ^{13}C NMR spectroscopic data with those of known trachylobanes.[22] The exchange of the methyl group for a carboxylate caused the expected effects on the chemical shifts of the neighboring carbon atoms.

HR-MS analysis of **17** calculated $C_{20}H_{30}O_2$ for the molecular ion. The natures of the oxygen functions followed from the ^{13}C NMR spectrum, which showed signals for a tertiary alcohol and a keto group. Taking into account the six degrees of unsaturation, a pentacyclic compound had to be present. Surprisingly, the ^{1}H NMR spectrum (which was in part similar to those of co-occurring kauranes and atis-

ane derivatives, in particular the signals for the A/B rings) showed only three methyl signals. In $CDCl_3$ most of the signals appeared as overlapping multiplets, but in C_6D_6 partial separation of signals allowed tracing of sequences using 2D homo- and heterocorrelation experiments (Table 4). In particular, the information collected from the sequence starting with H-11β was crucial and led to elucidation of the C/D/E rings. The geminal coupling of the H-17 pair (J = 8 Hz) indicated the presence of a four-membered ring. The placement of the hydroxy group at C-16 and not at C-13 followed from the NOE interaction between H-20 and H-14α, which was itself coupled to H-13. The long-range correlations observed in HMBC experiments confirmed the structure and allowed the assignment of quaternary carbon signals. A series of long-range coupling constants observed in the ^{1}H NMR spectrum as a consequence of a zig-zag in-plane orientation of the corresponding protons in the bridged bicyclooctane moiety is worth noting. The formation of this unusual compound is interesting in the biogenetic context of several tetra- and pentacyclic diterpenes and the relationships between them. A possible plausible explanation is depicted in Scheme 1 and Scheme 2. Starting with kaurane derivatives, the formation of these polycyclic diterpenes can be explained by the generation of a protonated nonclassical cyclopropylium ion.

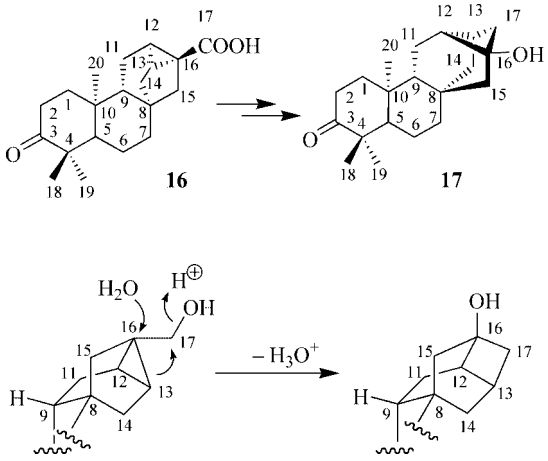
In conclusion, we report a detailed analysis of diterpenes found in *Stillingia sanguinolenta* (collected in Mexico). Besides known compounds, several new natural products could be identified. Two novel diterpenes named tonantzit-



Scheme 1. Suggested biosynthetic correlations of diterpenes from *S. sanguinolenta* based on the work of Whalley (a. and b. refer to rearrangements and breaking of cyclopropane bonds).[23]

Table 4. ^{1}H and ^{13}C NMR spectroscopic data for **16** and **17**.

	16		**17** (C_6D_6)	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1α	37.6 (CH_2)	1.45 (ddd, 1 H, 13.5, 7.0, 3.0)	38.4 (CH_2)	1.42 (ddd, 1 H, 13.5, 7.0, 3.5)
1β		0.95 (m, 1 H)		0.95 (m, 1 H)
2α	33.8 (CH_2)	2.29 (ddd, 1 H, 16.0, 12.0, 7.0)	33.9 (CH_2)	2.34 (ddd, 1 H, 16.0, 12.0, 7.0)
2β		2.12 (ddd, 1 H, 16.0, 6.0, 3.0)		2.23 (ddd, 1 H, 16.0, 6.5, 3.5)
3	216.7 (C)		217.4 (C)	
4	47.5 (C)		47.6 (C)	
5	55.0 (CH)	1.00 (m, 1 H)	55.6 (CH)	1.04 (m, 1 H)
6α,β	20.9 (CH_2)	1.35 (m, 2 H)	20.0 (CH_2)	1.42 (m, 2 H)
7α	37.5 (CH_2)	1.15 (m, 2 H)	40.0 (CH_2)	1.33 (ddd, 1 H, 13.5, 3.0, 3.0)
7β	37.5 (CH_2)	1.15 (m, 2 H)		1.05 (m, 1 H)
8	39.9 (C)		35.8 (C)	
9	51.2 (CH)	0.92 (m, 1 H)	52.9 (CH)	0.98 (m, 1 H)
10	37.6 (C)		37.7 (C)	
11α	19.2 (CH_2)	1.53 (ddd, 1 H, 15.0, 5.5, 2.0)	19.4 (CH_2)	1.18 (m, 1 H)
11β		1.77 (m, 1 H)		1.64 (ddd, 1 H, 15.0, 12.0, 3.0)
12	25.2 (CH)	1.72 (m, 1 H)	43.0 (CH)	1.74 (br. ddd, 1 H, 6.5, 3.0, 3.0)
13	31.8 (CH)	1.85 (dd, 1 H, 8.5, 3.5)	25.7 (CH)	1.93 (ddd, 1 H, 7.0, 7.0, 6.5)
14α	32.1 (CH_2)	1.06 (ddd, 1 H, 12.5, 3.5, 1.0)	33.5 (CH_2)	2.00 (dddd, 1 H, 13.0, 7.0, 3.0, 1.5)
14β		1.93 (d, 1 H, 12.5)		0.77 (dd, 1 H, 12.0, 2.0)
15α	42.5 (CH_2)	1.39 (d, 1 H, 12.0)	53.3 (CH_2)	1.64 (br. d, 1 H, 12.0)
15β		1.79 (d, 1 H, 12.0)		1.28 (ddd, 1 H, 12.0, 3.0, 3.0)
16	29.7 (C)		73.3 (C)	
17α	180.1 (C)		47.5 (CH_2)	1.83 (dddd, 1 H, 8.0, 7.0, 3.0, 1.5)
17β				1.42 (d, 1 H, 8.0)
18	26.0 (CH_3)	0.96 (s, 3 H)	26.1 (CH_3)	1.15 (s, 1 H)
19	21.4 (CH_3)	0.85 (s, 3 H)	21.4 (CH_3)	0.95 (s, 1 H)
20	14.1 (CH_3)	0.83 (s, 3 H)	14.5 (CH_3)	0.78 (s, 1 H)



Scheme 2. Hypothetical rearrangement of trachylobane **16** (after reduction of carboxylic group) to sanguinolane **17** (only CDE rings are depicted).

lolone (**2**) and tonantzitlolone B (**3**), with the rare flexibilane skeleton, two new pimaranes **4** and **5**, two new kauranes **9** and **12**, a new trachylobane **16**, and the new pentacyclic diterpene sanguinolane **17** with a novel skeleton were isolated.

Supporting Information (see also the footnote on the first page of this article): Descriptions of experimental procedures as well as analytical data for Mosher ester analysis, ^{1}H NMR spectroscopic data for kaurane derivatives **9** and **12**, and a discussion on controversial structure assignments of 16,17-dioxykauranes in the literature.

Experimental Section

General Remarks: Optical rotations were measured with a Perkin–Elmer 341 polarimeter. IR spectra were recorded with a Nicolet Magna 750 spectrophotometer. NMR spectra were recorded with a Bruker AM 400 and a Bruker ARX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. ^{1}H-^{1}H-COSY, NOESY, HMBC, and HMQC spectra were measured using standard Bruker pulse sequences. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^{1}H, 7.26 ppm) and $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards. Mass spectra were taken with a Varian MAT 711 (EI, 70 eV; direct inlet) spectrometer.

Plant Material: The plant material was collected in Valle Alto, Monterrey N. L., Mexico in May 1990 and March 1992. A herbarium specimen is deposited at the ITESM Herbarium (ID 8482).

Extraction and Isolation: The air-dried roots (800 g) were extracted overnight with a mixture of petroleum ether, methyl *tert*-butyl ether, and methanol. The extract, after evaporation of solvent (35 g), was dissolved in methanol and kept at –20 °C overnight. The soluble part (24 g) was separated into 10 fractions by CC using mixtures of petroleum ether, ethyl acetate, and methanol with increasing polarity. Fractions 1, 2, 4, 8, 9, and 10 were discarded. Fraction 3 was separated by HPLC (always RP 8; 8 × 250 mm) to give **2** (1 mg, methanol/H_2O, 9:1, R_t = 8.3 min). Fractions 5, 6, and 7 were combined and separated by MPLC (medium pressure liquid chromatography) with solvent mixtures comprising petroleum ether, methyl *tert*-butyl ether (MTB), and methanol in increasing polarities. 150 fractions were collected. After TLC checking the fractions were combined and in each case further separated by HPLC. The conditions of the final purification step are given in parentheses. Fractions 6–15 gave **13** (1 mg, methanol/H_2O, 13:7, R_t = 13.3 min). Fractions 37–47 gave **17** (3 mg, methanol/H_2O, 13:7, R_t = 13.9 min) and **4** (1 mg, R_t = 18.0 min). Fractions 48–67 gave **11** (4 mg, methanol/H_2O, 3:2, R_t = 11.9 min) and **8** (6 mg, R_t = 22.0 min). Fractions 68 –89 gave **14** (5 mg, methanol/H_2O, 3:2, R_t = 9.2 min), **10** (1 mg, R_t = 10.7 min), **11** (5 mg, R_t = 11.9 min), and **16** (4 mg, R_t = 19.6 min).

The aerial parts were extracted in the same manner as described above, yielding 31 g of extract. After defatting, the extract was separated by CC with mixtures comprising petroleum ether, MTB, and methanol to furnish eight fractions. Fractions 1, 7, and 8 were discarded. Fraction 2 gave, as judged by TLC (petroleum ether/MTB, 9:1), **1** (5 mg, R_f = 0.84), **6** (2 mg, R_f = 0.5), and **2** (15 mg, R_f = 0.35). Fraction 3 was separated by MPLC into several fractions, which after TLC checking were combined into four portions. Portions 2 and 3 were combined and separated by TLC (petroleum ether/MTB, 4:1) to give **3** (2 mg, R_f = 0.45), **7** (5 mg, R_f = 0.38), and **13** (2 mg, R_f = 0.3). Fractions 4, 5, and 6 were combined and separated by MPLC. The combined mixtures were further separated by TLC and/or HPLC. HPLC (methanol/H_2O, 13:7) afforded **5** (1 mg, R_t = 12.2 min) and **15** (2 mg, R_t = 20.7 min). TLC (CH_2Cl_2/toluene/MTB, 9:9:2) gave **9** (4 mg, R_f = 0.32). HPLC (methanol/H_2O, 13:7) afforded **12** (2 mg, R_t = 19.8 min), **8** (6 mg, R_t = 9.3 min), **14** (10 mg, R_t = 14.8 min), **10** (8 mg, R_t = 18.0 min), and **11** (40 mg, R_t = 12.2 min). Known compounds were identified by comparison of their spectroscopic data with those for authentic material or with literature data.

Tonantzitlolone (2): $[\alpha]_D^{20}$ = +134 ($CHCl_3$; *c* = 0.25). IR (KBr): $\tilde{\nu}_{max}$ = 3381, 1741, 1684 cm^{-1}. EIMS (probe, 70 eV) *m*/*z* (rel. int.): 464.2774 (2) $[M]^+$ (calcd. for $C_{26}H_{40}O_7$: 464.2774), 446 $[M - H_2O]^+$ (6), 350, $[M - RCOOH]^+$ (2), 332 $[350 - H_2O]^+$ (1), 304, $[332 - CO]^+$ (1), 245 (5), 180 (6), 97 $[RCO]^+$ (100).

4′-Acetoxytonantzitlolone (3): EIMS (probe, 70 eV) *m*/*z* (rel. int.): 522.2829 (8) $[M]^+$ (calcd. for $C_{28}H_{42}O_9$: 522.2829), 504 $[M - H_2O]^+$ (4), 464 (3), 422 (2), 345 (9), 316 (3), 113 (100).

***ent*-3β,11α-Dihydroxypimara-8,15-diene (4):** EIMS (probe, 70 eV) *m*/*z* (rel. int.): 304.2402 (3) $[M]^+$ (calcd. for $C_{20}H_{32}O_2$: 304.2402), 286 $[M - H_2O]^+$ (3), 271 $[286 - Me]^+$ (7), 253 (8), 218 (3), 73 (100).

***ent*-8β-Hydroxy-3,12-dioxopimara-9(11),15-diene (5):** EIMS (probe, 70 eV) *m*/*z* (rel. int.): 316.2038 (17) $[M]^+$ (calcd. for $C_{20}H_{28}O_3$: 316.2038), 248 $[M - RDA]^+$ (100), 233 $[248 - Me]^+$ (55), 205 (13), 191(10), 121 (14), 69 (30), 55 (32).

***ent*-16β-H-3-Oxokauran-17-ol (9):** EIMS (probe, 70 eV) *m*/*z* (rel. int.): 304.2402 (45) $[M]^+$ (calcd. for $C_{20}H_{32}O_2$: 304.2402), 218 (80), 205 (21), 152 (76), 73 (56), 57 (100).

***ent*-16β-H-2-Oxokauran-3β,17-diol (12):** EIMS (probe, 70 eV) *m*/*z* (rel. int.): 320.2351 (43) $[M]^+$ (calcd. for $C_{20}H_{32}O_3$: 320.2352), 247 (100), 229 (23), 205 (14), 105 (32), 47 (50).

***ent*-3-Oxotrachylobane-17-carboxylic Acid (16):** IR (CCl_4): $\tilde{\nu}_{max}$ = 1707, 1684 cm^{-1}. EIMS (probe, 70 eV) *m*/*z* (rel. int.): 316.2039 (15) $[M]^+$ (calcd. for $C_{20}H_{28}O_3$: 316.2039), 298 (6), 230 (13), 135 (10), 95 (100), 55 (62).

***ent*-16-Hydroxy-3-oxosanguinane (17):** IR (CCl_4): $\tilde{\nu}_{max}$ = 3612, 1706 cm^{-1}. EIMS (probe, 70 eV) *m*/*z* (rel. int.): 302.2246 (63) $[M]^+$ (calcd. for $C_{20}H_{30}O_2$: 302.2246), 287 $[M - Me]^+$ (90), 244 (100), 159 (55), 91 (77), 55 (72).

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