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TRIXANOLIDE AND GERMACRATRIENE DERIVATIVES FROM *TRIXIS GRISEBACHII*

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Key Word Index—*Trixis grisebachii*; Compositae; Mutisieae; trixanolides; trixanes; germacratrienes; sesquiterpenes; dammaranes; triterpenes.

Abstract—Chemical investigation of the aerial parts of *Trixis grisebachii* afforded a number of new sesquiterpene di- and triesters related to trixanolide and two new tetraesters derived from germacrene A, as well as some known compounds including dipterocarpol and 3-*O*-acetyldammarenediol-II.

INTRODUCTION

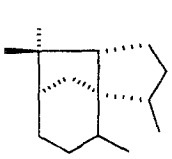
Sesquiterpenes based on the trixane skeleton **1** appear to be characteristic metabolites of the New World subtribe Nassauviinae of Mutisieae [1–11]. In the following we describe our work on *Trixis grisebachii* O. Kuntze (syn. *Trixis frutescens* var. *cacalioides* Griseb.), a member of the largest genus within the subtribe. Isolated from the aerial parts were the acid **2**, two triesters **3a**, **b**, 10 diesters **4a–d**, **4e** or **4e′**, **4f** or **4f′**, **4h**, **4i** or **4i′**, **4j** or **4j′**, all based on the trixane skeleton, two tetraesters **5a**,**b** derived from germacrene A, dipterocarpol and 3-*O*-acetyldammarenediol-II as well as common triterpenes and plant sterols. Compound **2** has been obtained previously as the methyl ester from *T. antimenorrhea* and *T. vautheri* [4] and as the acid from *T. paradoxa* [7].

RESULTS AND DISCUSSION

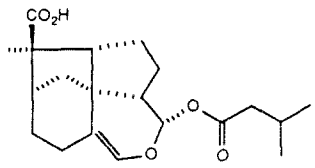
That the triesters **3a** and **3b** were lactones of the so-called trixikingolide or trixanolide type [2–4] carrying an ester function at C-9 as well as at C-3 and C-14 was evident from a comparison of their ^{1}H NMR spectra (Table 1) with spectra of diesters of type **4** from *T. grisebachii* (*vide infra*) and in the literature [4], the triplet of H-9 being displaced from near δ4.4 to 5.28. Decoupling established the sequence H-1a,b through H-4 and H-7 through H-10, with H-7 also being coupled to H-14 at δ5.50. The nature of the three ester functions of **3a** and **3b** could be deduced from the mass spectrum, the ^{1}H NMR and, in case of **3a**, the ^{13}C NMR spectra (Table 2). Allocation of the two β-hydroxyisovaleryl groups of **3a** to C-3 and C-9 was made possible by partial hydrolysis of **3a** (K_2CO_3–MeOH) to **3c** with loss of the valerate and a concomitant upfield shift of the H-14 resonance from δ5.50 to 4.54. By analogy and because the H-3, H-4, H-9 and H-14 resonances of both triesters are essentially identical, we assume that the α-methylbutyryl group of triester **3b** is on C-14 as well.

The 10 diesters **4a** or **a′**–**4j** or **j′**, almost all present in only very small amounts, were difficult to separate even by HPLC and five of them were obtained only in the form of two-component mixtures **4e** or **e′** with **4f** or **f′**, **4g** or **g′** with **4h** and **4i** or **i′** with **4j** or **j′**. The basic carbon skeleton and the stereochemistry was deduced by extensive decoupling of the ^{1}H NMR spectra and comparison with data in the literature [4]; the nature of the various ester functions was deduced from the mass and ^{1}H NMR spectra and, in the case of **4b**, **4c**, **4e** (or **e′**) and **4f** (or **f′**) also from the ^{13}C NMR spectra. Similar compounds (**4f** or **4f′**, **4j** and **4j′**, and **4k** or **4k′**) have been reported from *T. vautheri* and *T. antimenorrhea* [4]. While the differences in the ^{1}H NMR spectra of **4j** and **4j′**, one of which must be identical with one of our compounds from *T. grisebachii*, were insignificant [4], the ester distibution in **4j**, and by exclusion that in **4j′**, was established by partial saponification of **4j** to **4l**. Since partial saponification of the two remaining diesters was unsuccessful they were provisionally assigned formulas **4f′** and **4k′**, respectively [4]. In the case of our six diesters **4a** or **a′**–**4f** or **f′**, each containing a β-hydroxyisovaleryl residue, one might draw the inference that the constancy of the H-14 resonance (δ5.50–5.52), contrasted with the somewhat greater variability of the H-3 resonance (δ4.95–δ5.01—see Table 1), indicates the attachment in all six compounds of the β-hydroxyisovalerate residue to C-14. However, in the diesters **4g** or **g′**, **4j** or **j′** and the triesters **3a**, **b**, all of which differ in the nature of the ester residues attached to C-14, the frequency of H-14 covers nearly the same narrow range, i.e. δ5.48–5.49.

Examination of Table 1 and Table 1 of ref. [4] reveals, however, that the diesters can be divided into two groups—those whose H-4 resonance are found in the range δ4.93–4.95 and those whose H-4 signal occurs in the range δ4.84–4.86, and that H-4 of both **4j** and **4j′** [4] resonates at the higher frequency. It is reasonable therefore to suppose that the presence of an angelate, tiglate or epoxyangelate on C-3 is responsible for the paramagnetic shift of H-4 and that formulas **4a–d** and **4h** can be assigned to five of our 10 diesters. That the effect of an unsaturated side chain at C-3 is exerted in the neighbourhood of C-4 may be deduced also by comparing the



1



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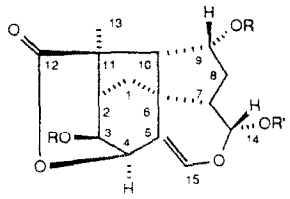
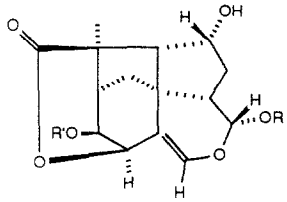
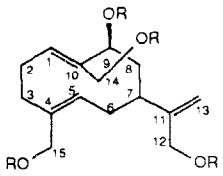


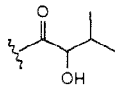
3 a R=A, R'=iVal
b R=A, R'=MeBu
c R=A, R'=H



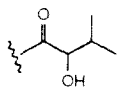
4 a R=A, R'=Tig
a' R=Tig, R'=A
b R=A, R'=MeBu
b' R=MeBu, R'=A
c R=A, R'=Ang
c' R=Ang, R'=A
d R=A, R'=Epoxyang
d' R=Epoxyang
e R=A, R'=AcB
e' R=AcB, R'=A
f R=A, R'=AcA
f' R=AcA, R'=A
g R=B, R'=MeBu
g' R=MeBu, R'=B
h R=B, R'=Ang
h' R=Ang, R'=B
i R=B, R'=AcB
i' R=AcB, R'=B
j R=B, R'=AcA
j' R=AcA, R'=B
k R=AcA, R'=MeBu
k' R=MeBu, R'=AcA
l R=H, R'=AcA

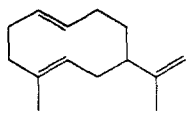


5 a R=Ac, Ac, iBu,



b R=Ac, Ac, MeBu,





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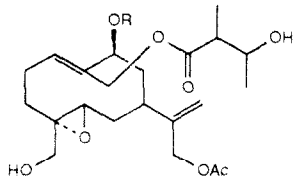
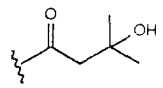
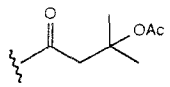


7

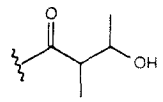
A =



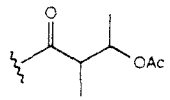
AcA =



B =



AcB =



chemical shift of C-4 in **4c** (Table 3) with the chemical shift of C-4 in **4b**, **4e** (or **4e'**) and **4f** (or **4f'**). In all other respects the ^{13}C NMR spectra of the sesquiterpene nuclei in the diesters are essentially identical. The generalization extends to triester **3a** where only the frequencies of C-8, C-9 and C-10 depart in a predictable way from the corresponding signals of the diesters. Attempts to provide more secure evidence for the location of the two different acyl groups on the carbon skeleton of **4b** by detecting long range ^{13}C–^{1}H couplings to the various carbonyl groups failed because of sample size and instrumental difficulties but did permit assignment of the δ176.97 signal to the lactone carbonyl (three bond couplings to H-13), thus correcting an earlier assignment [2].

Small amounts of two additional sesquiterpenoids **5a** and **5b** were obtained from *T. grisebachii*, **5a** being isolated only in admixture with **2**. That both were derivatives of germacrene A (**6**) with oxygenation at C-9, C-12, C-14 and C-15 was clear from the ^{1}H and ^{13}C NMR spectra (Tables 2 and 3), comparison with spectra of related compounds and decoupling experiments. The absence of NOE's between H-1 or H-5 and any of the two proton AB systems of H-12, H-14 and H-15 showed that the 1(10)-double bond was *E* and the 4,5-double bond was *Z*. The stereochemistry at C-9 follows from the coupling constants if it is assumed that the C-7 side chain is equatorial and that the 10-membered ring adopts the favoured crown conformation. The nature of the four acyl residues—two acetates, one isobutyrate and one α-hydroxyisovalerate in **5a** and one α-methylbutyrate in **5b** replacing the isobutyrate of **5a**—was also deduced from the NMR spectra, but their distribution over the carbon

Table 1. ^{1}H NMR spectra of compounds **3a–c**, **4a–j** (270 MHz, $CDCl_3$)

H	3a	3b	3c	4a	4b	4c	4d	4e, 4j	4g	4h*	4i, 4j
1a	2.90 *dd* (11.5, 7)	2.92	2.93	3.15 *dd* (11.5, 7)	3.15	3.15	3.19	3.16	3.15	3.17	3.16
1b	1.51 *dd* (11.5, 2)	1.52	obsc.	1.47 *dd* (11.5, 2)	1.44	1.45	1.46	1.44	1.45	1.43	1.48
2	~2.40	~2.45	~2.40	2.42 *br dt* (7, 2.5)	2.38	2.44	2.41	2.38	~2.40	2.38	2.40
3	5.01 *dd* (2.5, 2.5)	4.99	4.96	4.97 *dd* (2.5, 2.5)	4.95	5.01	5.01	4.96, 4.95	4.95	5.01	4.96
4	4.90 *dd* (3, 2)	4.92	4.90	4.94 *dd* (3, 2)	4.84	4.95	4.94	4.86	4.85	4.93	4.86
7	~2.50	~2.50	obsc.	2.61 *ddd* (9, 8.5, 6.5)	2.60	2.60	2.61	2.60	2.62	2.59	2.62
8a	2.21 *dd* (16, 8)	2.20	obsc.	2.23 *dd* (15, 8)	2.23	2.22	2.24	2.22	2.23	2.22	2.24
8b	1.99 *ddd* (16, 6, 6)	2.01	obsc.	1.88 *ddd* (15, 7, 5)	1.87	1.89	1.91	1.87	1.88	1.87	1.88
9	5.28 *br t* (6)	5.28	5.27	4.44 *br t* (4.5)	4.42	4.43	4.44	4.43	4.43	4.42	4.42
10	~2.40	~2.40	obsc.	2.19 *dd* (5, 1.5)	2.19	2.20	2.22	2.19	2.20	2.19	2.19
13†	1.40 *s*	1.42	1.39	1.61 *s*	1.61	1.61	1.62	1.59	1.60	1.59	1.60
14	5.50 *d* (9)	5.50	4.54	5.50 *d* (9)	5.50	5.50	5.52	5.50	5.49	5.481	5.49
15	6.58 *s*	6.57	6.55	6.52 *s*	6.50	6.52	6.54	6.52, 6.51	6.52	6.51	6.53
R	A, A′	A, A′	A, A′	A	A	A	A	A, A‡	B‡	B	B, B‡
R′	*i*Val	MeBu		Tig	MeBu	Ang	Epoxy Ang	AcB, AcA‡	MeBu‡	Ang	AcB, AcA‡

*Chemical shifts from mixture of **4g** and **4h**.

†Intensity three protons.

‡or vice versa **4e**, **4f**, **4g**, **4i**, **4j**.

A = 2.57 *d* (1.4) (2H), 1.32 *s*, 1.31 *s*. A′ = 2.53 *d* (1.5) (2H), 1.30 *s*, 1.29 *s*. AcA = 2.59 *d* (14.5), 2.85 *d* (14.5), 1.52 *s*, 1.52 *s*, 2.01 *s*. B = 2.56 *dq* (7, 7), 4.11 *dq* (7, 7), 1.24 *d* (7), 1.13 *d* (7). AcB = 2.68 *dq* (7, 7), 5.11 *dq* (7, 7), 1.27 *d* (7), 1.18 *d* (7), 2.07 *s*. Ang = 6.16 *dq* (7, 1.5), 1.99 *dq* (7, 1.5), 1.87 *br t* (1.5). Epoxy-Ang = 3.07 *q* (5), 1.34 *d* (5), 1.57 *s*. *i*Val = 2.18 *d* (7), 2.11 *m*, 0.96 *d* (7), 0.97 *d* (7). MeBu = 2.34 *ddq* (7, 7, 7), 1.68 *ddq* (14, 7, 7), 1.48 *ddq* (14, 7, 7), 0.90 *t* (7), 1.14 *d* (7). Tig = 6.86 *qq* (7, 1.5), 1.79 *dq* (7, 1.5), 1.81 *q* (1.5).

Table 2. ^{13}C NMR spectra of compounds **3a**, **4b**, **4c**, **4e**, **f** and **5b** (67.89 MHz, $CDCl_3$)*

C	3a	4b, 4c	4e	4f‡	5b
1	43.93 *t*	41.99 *t*	42.07 *t*	42.04 *t*	129.95 *d*[a]
2	40.40 *d*	40.62 *d*†	40.66 *d*	40.62 *d*	28.44 *t*
3	76.34 *d*†	76.59 *d*†	76.68 *d*†	76.48 *d*	34.32 *t*
4	74.33 *d*†	74.04 *d*†	73.12 *d*†	74.62 *d*†	134.78 *s*[b]
5	117.20 *s*	118.02 *s*	118.04 *s*	117.88 *s*	130.19 *d*[a]
6	54.22 *s*	53.87 *s*	53.93 *s*	53.84 *s*	30.91 *t*
7	49.34 *d*	49.90 *d*†	49.49 *d*	49.32 *d*	41.22 *d*[c]
8	38.16 *t*	41.01 *t*†	41.00 *t*	41.01 *t*	40.10 *t*
9	74.24 *d*†	73.10 *d*†	73.12 *d*†	73.08 *d*†	75.29 *d*[d]
10	60.49 *d*	62.02 *d*†	62.04 *d*	62.06 *d*	135.21 *s*[b]
11	49.70 *s*	49.43 *s*	49.96 *s*	49.84 *s*	147.74 *s*
12	176.13 *s*	176.97 *s*	177.00 *s*	176.77 *s*	112.94 *t*
13	19.87 *q*†	20.06 *q*†	20.04 *q*	20.06 *q*	63.64 *t*[e]
14	95.18 *d*†	95.63 *d*†	95.64 *d*	95.59 *d*	63.90 *t*[e]
15	140.62 *d*†	139.87 *d*†	139.90 *d*	139.99 *d*	66.92 *t*[e]
1	171.46 *s*	170.92 *s*	170.91 *s*	170.90 *s*	174.54 *s*
2	46.89/46.74 *t*†	46.80 *t*	46.82 *t*	46.79 *t*	73.14 *d*[d]
3	69.20/69.12 *s*	69.17 *s*	69.17 *s*	69.18 *s*	32.20 *d*
4	29.30 *q*†	29.32 *q*†	29.32 *q*	29.32 *q*	18.85 *q*
5	29.23 *q*†	29.23 *q*	29.23 *q*	29.23 *q*†	16.57 *q*
1′	170.68 *s*	175.64 *s*	166.57 *s*	172.85 *s*	170.90 *s*
2′	41.47 *t*	41.06 *d*	127.14 *s*	44.50 *d*	41.22 *d*[c]
3′	25.60 *d*†	26.42 *t*	140.33 *d*	70.82 *d*	26.72 *t*
4′	22.37 *q*	16.24 *q*†	20.34 *q*	17.59 *q*†	11.54 *q*
5′	22.37 *q*†	11.41 *q*†	15.87 *q*	12.25 *q*†	16.10 *q*
OAc	—	—	170.34 *s*	—	170.59 *s*
	—	—	21.07 *q*†	—	170.59 *s*
	—	—	—	—	21.12 *q*
	—	—	—	—	20.89 *q*
1′	—	—	169.26 *s*	—	—
2′	—	—	obsc	—	—
3′	—	—	79.19 *s*	—	—
4′, 5′	—	—	26.58 *q*	—	—
OAc	—	—	170.47 *s*	—	—
	—	—	22.27 *q*	—	—

*Multiplicities established by DEPT pulse sequence.
†Assignments by single frequency heteronuclear decoupling.
‡Chemical shifts of a mixture 3:1 of **4e** and **4f**.
[a–e]Assignments may be interchanged.

skeleton remained uncertain although presumably the two compounds differ from each other only in substitution of the α-methylbutyrate for the isobutyrate at the same locus. A series of related diesters of type **7** has been isolated from *T. vautheri* [4].

The triterpene and plant sterol fraction of *T. grisebachii* contained in addition to commonly encountered substances the somewhat rarer dammarane derivatives dipterocarpol and 3-(*O*)acetyldammarenediol-II ([3*S*,20*S*]-3-acetoxy-20-hydroxydammar-24-ene) [12]. To the best of our knowledge the latter has been encountered only once previously as a natural product [13].

EXPERIMENTAL

General. For separation of mixtures HPLC using a differential refractometer was used. The column employed was a Sil C-18 column (10 μm, 10 mm i.d. × 50 cm). R_f's were measured from the solvent peak.

Plant material. Aerial parts of *Trixis grisebachii* O. Kuntze were collected on 22 August 1986 at the flowering stage in Timbo Viejo, Departamento Burruyacu, Tucumán Province, Argentina. A voucher specimen (P. R. Lgname 9276) is deposited in the herbarium of the Instituto Miguel Lillo.

Extraction. Flowers and leaves (507 g) were extracted with $CHCl_3$ (2 × 6 l) at room temp. for 7 days to give 33 g (6.5%) of crude ext which was suspended in 570 ml of EtOH at 50–55°, dil with 375 ml of H_2O and extracted successively with *n*-hexane (3 × 500 ml) and $CHCl_3$ (3 × 500 ml). Evapn of the hexane fr gave 23.6 g of residue which was dissolved in heptane–EtOAc (2:1), decolorized with charcoal, filtered and evapd. The residue was chromatograped over silica gel (600 g) using hexane and increasing amounts of Et_2O (from 20 to 65%), all frs being monitored by TLC. Fractions with higher R_f than β-amyrin (hexane–EtOAc,

Table 3. ^{1}H NMR spectra of compounds **5a** and **5b** (270 MHz)

H	**5a** $CDCl_3$	$CDCl_3$	**5b*** C_6D_6†
1	5.66 *dd* (10, 7)	5.66 *dd* (10, 7)	5.50 *br dd* (10, 7)
2a	obsc.	2.58 *m*	2.56 *m*
2b	obsc.	obsc.	~1.95
3a	obsc.	obsc.	2.21 *m*
3b	obsc.	obsc.	1.83 *m*
5	~5.60	~5.60	5.34 *br t* (8)
6a	obsc.	obsc.	~2.20–2.25 *m*
6b	obsc.	obsc.	—
7	obsc.	obsc.	2.45 *m*
8a	1.89 *dd* (15, 5)	1.88 *dd* (15, 5)	1.94 *dd* (15, 5)
8b	obsc.	obsc.	~1.70
9	5.51 *dd* (5, 1.5)	5.52 *br d* (5)	5.67 *dd* (5, 1.5)
12a	5.10 *br s*	5.10 *br s*	4.91 *br s*
12b	5.00 *br s*	5.00 *br s*	4.85 *br s*
13a	4.71 *d* (14)	4.69 *d* (14)	4.83 *d* (12)
13b	4.64 *d* (14)	4.63 *d* (14)	4.29 *d* (12)
14a,b	4.73 *d* (12)	4.72 *d* (12)	4.76 *d* (12.5)
	4.20 *d* (12)	4.22 *d* (12)	4.61 *d* (12)
15a,b	4.61 *d* (13.5)	4.62 *d* (14)	4.61 *d* (13.5)
	4.53 *d* (13.5)	4.50 *d* (14)	4.51 *d* (12.5)
OAc	2.03 *s*	2.04 *s*	1.71 *s*
2.00s	2.00 *s*	2.00 *s*	1.66 *s*
2-OH *i* Val			
2	4.07 *d* (4)	3.96 *d* (4)	4.11 *d* (4)
3	2.13 *dq* (7, 4)	2.10 *dq* (7, 4)	2.13 *dq* (7, 4)
4	1.03 *d* (7)	1.04 *d* (7)	1.04 *d* (7)
5	0.89 *d* (7)	0.89 *d* (7)	0.96 *d* (7)
MeBu			
2	—	2.36 *ddq* (7, 7, 7)	2.26 *ddq* (7, 7, 7)
3a	—	1.71 *ddq* (14, 7, 7)	1.75 *ddq* (14, 7, 7)
3b	—	1.52 *ddq* (14, 7, 7)	1.43 *ddq* (14, 7, 7)
4	—	0.92 *t* (7)	0.88 *t* (7)
5	—	1.17 *d* (7)	1.14 *d* (7)
*i*Bu			
2	2.59 *hept* (7)	—	—
3	1.19 *d* (7)	—	—
4	1.19 *d* (7)	—	—

*Chemical shifts from mixture of **2** and **5a**.

†No change on addition of TAl except for **2**-OH *i*Val H-2, 5.00 *d* (4); H-4, 0.97 *d* (7), H-5, 0.92 *d* (7), and the AB system from 4.61–4.51 to 4.72 *d* (12)–4.41 *d* (12).

5:2) were combined to yield 16.3 g of waxy material which was not examined further. Fractions 9–12 which showed two major spots on TLC, the lower with the same R_f as β-amyrin, were combined (1.3 g); a small portion of this material was processed by RP-HPLC (MeOH, 4 ml/min) to give crystalline palmitic acid (3.6 mg, R_t 9.8 min, identified by M and NMR), 3-*O*-acetyldammarenediol-II (**8**, [3S, 20S]-3-acetoxy-20-hydroxydammar-24-ene, 7.4 mg, R_t 14.5 min), lupeol (11 mg, R_t 34 min), β-amyrin (4.6 mg, R_t 39.5 min). Fractions with R_t 6, 7.5, 29 and 45.5 min were discarded. Fractions 13–19 which showed one major spot were combined (1.9 g). A small portion of this material was subjected to HPLC (MeOH, 3 ml/min) to give 5.2 mg of germanicol (R_t 6 min), dipterocarpol (**9**, [20*S*]-20-hydroxydammar-24-en-one, 25 mg, R_t 11.5 min), β-amyrin (6.0 mg, R_t 45 min) and α-amyrin (4 mg, R_t 55.5 min). Frs R_t 8.5, 19 and 21 min were discarded as were frs 20 and 21 of the CC (58 mg). Frs 22–24 of the CC contained primarily stigmasterol and sitosterol. Compounds **8** and **9** were identified by MS which exhibited fragmentation patterns typical of a 3-acetoxy- and a 3-keto-20-hydroxydammar-24-ene and by NMR, for **8**, 5.11 *tt* ($J = 7$, 1.5 Hz, H-24), 4.48 *dd* ($J = 10$, 6 Hz, α-orientated H-3), 2.02 (Ac), 1.68 *br s* and 1.62 *br s* (H-26 and H-27), 1.14 *s* (H-21), 0.98 *s* (8-Me), 0.87 *s*, 0.84 *s*, 0.84 *s*, 0.84 *s* (4α, β, 10, 14-Me) [12], for **9**, 5.13 *br t* ($J = 7$ Hz, H-24), 2.4 *m* and 2.35 *t* (H-2a, b), 1.70 *br s* and 1.63 *br s* (H-26 and H-27), 1.18 (H-21), 1.09 (4β-Me), 1.05 (4α-Me), 1.01 (8-Me), 0.97 (10-Me) and 0.90 (14-Me) [14] which showed that the C-20 configuration was *S* rather than *R*. This was also evident from the ^{13}C NMR spectrum of **9** which exhibited the C-21 and C-22 frequencies for a 20*S* rather than a 20*R* epimer at δ25.51 and 40.54, respectively [15].

Evapn of the $CHCl_3$ extract gave a residue (5.5 g) which was chromatographed over silica gel (260 g) using $CHCl_3$ and increasing amounts of Et_2O (0–40%), 124 frs being collected. Frs 1–4 were discarded. HPLC of fraction 5 (MeOH–H_2O, 4:1) gave frs whose properties were reminiscent of the trixanes from *T. praestans* [11] but which decomposed before they could be examined in detail. Frs 6–12 were combined; a small portion on HPLC (MeOH–H_2O 4:1, 3 ml/min) yielded 10 mg of **2** (R_t 18 min) whose MS and ^1H NMR spectrum corresponded to the lit [7], 12 mg of a mixt of **2** and **5a** (R_t 19.5 min) and 10 mg of **5b** (R_t 25 min). Frs 13–28 were discarded. Frs 29–38 were combined; a portion processed by HPLC (MeOH–H_2O 2:1, 3 ml/min) gave 4 mg of **3b** (gum, R_t 37.5 min) and 35 mg of **3a** (gum, R_t 39.5 min). Frs 39–49 were mainly mixts of **4a** and **4b** with a small amount of **3a**. Frs 50–61 were combined; a portion processed by HPLC gave 1.2 mg of **4a** slightly contaminated by **4b** (R_t 39.1 min), 15 mg of **4b** (solid, R_t 42.6 min) and 16 mg of **4c** (solid, R_t 43.7 min.). Frs 62–71 were mixts. Frs 72–79 were combined; a portion subjected to HPLC (MeOH–H_2O 2:1, 2.5 ml/min) gave 2.5 mg of **4d** (gum, R_t 8 min), 8 mg of a 3:1 mixt of **4e** (or **4e'**) and **4f'** (or **4f'**) (gum, R_t 15.5 min), 2.5 mg of **4g** or **4g'** (gum, R_t 26 min) and 4 mg of a 1:1 mixt of **4g** (or **4g'**) and **4h** (gum, R_t 27 min). Frs 80–95 were a mixt of **4d**, **4e** (or **e'**), **4f** (or **f'**) and small amounts of **4i** and **4j** (or **4i'** and **4j'**). Frs 96–108 on HPLC (MeOH–H_2O 2:1, 2.5 ml/min) gave 1.6 mg of a 2:1 mixt of **4i** and **4j** (gum, R_t 14 min).

(2R*,3S*,4R*,6R*,7R*,8R*,10R*,11R*,14S*)-3,9-*Di*-(3-*hydroxy*-3-*methylbutanoyloxy*)-14-(3-*methylbutanoyloxy*)-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide* (**3a**). Gum; IR ν_{max}^{KBr} cm^{-1}: 3500, 1760, 1730, 1665; EIMS *m/z* (rel. int. %) 578 (0.7, $[M]^+$), 478 (5.5, $[M-C_5H_8O_2]^+$), 460 (2.2, $[M-C_5H_{10}O_3]^+$), 378 (100, $[M-2\,C_5H_8O_2]^+$), 361 (24.1), 360 (21.7), 359 (39.5), 343 (21.2), 334 (43.9), 332 (51.1), 316 (12.1), 276 (6.8), 259 (5.8); PCIMS *m/z* (rel. int. %) 579 (25.0, $[M+1]^+$), 561 (4.2), 479 (9.9), 461 (100), 443 (40.9), 379 (5.1), 361 (33.7), 345 (13.2), 343 (6.9), 317 (7.4), 299 (4.5), 277 (11.1), 259 (6.0), 243 (2.2), 119 (99.1), 103 (9.1), 101 (80.8), 85 (3.7), 83 (4.9); ^1H and ^{13}C NMR spectra in Tables 1 and 3.

Hydrolysis of 3 mg of **3a** in 1 ml of MeOH with 10 mg of K_2CO_3 in 0.1 ml of H_2O for 30 min at room temp followed by the usual work-up afforded 2 mg of **3c** whose ^1H NMR spectrum is listed in Table 1.

(2R*,3S*,4R*,6R*,7R*,8R*,10R*,11R*,14S*)-3,9-(*Di*-(3-*hydroxy*-3-*methylbutanoyloxy*)-14-(2-*methylbutanoyloxy*)-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide*(**3b**). Gum; IR ν_{max}^{KBr} cm^{-1}: 3478, 1750, 1730, 1665; EIMS *m/z* (rel. int.%) 578 (0.8, $[M]^+$), 478 (3.9, $[M-C_5H_8O_2)^+$], 460 (1.2, $[M-C_5H_{10}O_3]^+$), 378 (100, $[M-2\,C_5H_8O_2]^+$), 361 (22.1), 360 (23.7), 359 (38.2), 343 (22.3), 334 (74.2), 332 (90.1), 276 (55.5), 259 (71.3), 241 (16.3), 101 (14.2), 85 (38.1), 83 (43.4); PCIMS *m/z* (rel. int. %) 579 (12.1, $[M+1]^+$), 561 (3.3) 479 (5.5), 461 (100), 443 (48.4), 379 (4.6), 361 (43.8), 345 (13.5), 343 (9.7), 317 (6.2), 299 (3.6), 277 (3.2), 277 (3.2), 259 (10.5), 243 (4.0), 119 (76.9), 103 (18.5), 101 (93.0), 85 (7.0), 83 (8.1); ^1H NMR spectrum in Table 1.

(2R*,3S*,4R*,6R*,7R*,8R*,10R*,11R*,14S*)-9-*Hydroxy*-14-(3-*hydroxy*-3-*methylbutanoyloxy*)-3-*tiglyloxy*-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide* (**4a**). Gum; EIMS *m/z* (rel. int. %) 476 (0.2, $[M]^+$), 359 (3.3), 332 (1.5), 294 (5.6), 276 (4.5), 259 (1.3), 258 (0.7), 248 (15.5), 230 (1.7), 221 (12.2), (1.3), 100 (0.9), 73 (19.7), 55 (48.9); PCIMS *m/z* (rel. int. %) 477 (2.7, $[M+1]^+$), 459 (0.5), 378 (5.5), 377 (12.9), 376 (3.8), 362 (11.8), 361 (53.4), 360 (23.9), 359 (97.5), 358 (7.2), 341 (13.3), 333 (2.9), 277 (58.5), 261 (8.1), 259 (70.6), 249 (3.9), 231 (14.4), 215 (11.4), 119 (27.3), 101 (100), 83 (26.7); ^1H NMR spectrum in Table 1.

(2R*,3S*,4R*,6R*,7R*,8R*,10R*,11R*,14S*)-9-*Hydroxy*-14-(3-*hydroxy*-3-*methylbutanoyloxy*)-3-(2-*methylbutanoyloxy*)-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide* (**4b**). Mp 228–231°; IR ν_{max}^{KBr} cm^{-1}: 3415, 1758, 1730, 1665; EIMS *m/z* (rel. int. %) 478 (0.2, $[M]^+$), 378 (1.9), 361 (4.9), 334 (2.8), 294 (11.4), 276 (3.8), 259 (1.7), 258 (0.7), 248 (16.6), 230 (2.3), 117 (3.2), 85 (26.9), 57 (100); PCIMS *m/z* (rel. int. %) 479 (2.7, $[M+1]^+$), 461 (0.2), 378 (2.6), 377 (2.8), 362 (22.4), 361 (100), 277 (10.0), 259 (34.7), 231 (5.8), 215 (2.7), 119 (26.1), 103 (11.3), 101 (42.6), 85 (12.6); ^1H and ^{13}C NMR spectra in Tables 1 and 2.

(2R*,3S*,4R*,6R*,7R*,8R*,10R*,11R*,14S*)-3-*Angelyloxy*-9-*hydroxy*-14-(3-*hydroxy*-3-*methylbutanoyloxy*)-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide* (**4c**). Mp 188–190°; IR ν_{max}^{KBr} cm^{-1}: 3430, 1756, 1728, 1662; EIMS *m/z* (rel. int. %) 476 (0.1, $[M]^+$), 359 (4.7), 294 (18.4), 276 (7.7), 259 (1.7), 258 (1.1), 248 (17.0), 230 (1.8), 221 (1.3), 118 (1.2), 100 (0.7), 83 (100), 55 (52.5); PCIMS *m/z* (rel. int. %) 477 (4.1, $[M+1]^+$), 459 (0.5), 378 (3.1), 377 (7.3), 362 (12.2), 361 (57.8), 360 (22.9), 359 (100), 358 (3.4), 341 (6.5), 333 (1.1), 294 (2.2), 277 (20.1), 261 (6.7), 259 (54.1), 249 (1.6), 231 (10.9), 215 (3.6), 119 (65.7), 101 (87.9), 83 (54.0); ^1H and ^{13}C NMR spectra in Tables 1 and 2.

(2R*,3S*,4R*,6R*,7R*,8R*,10R*,11R*,14S*)-3-(2,3-*Epoxy*-2-*methylbutanoyloxy*)-9-*hydroxy*-14-(3-*hydroxy*-3-*methylbutanoyloxy*)-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide* (**4d**). Gum; EIMS *m/z* (rel. int. %) 492 (4.7, $[M]^+$), 448 (3.2), 393 (5.7), 392 (26.0), 377 (2.7), 375 (50.4), 374 (8.3), 348 (70.5), 294 (13.9), 276 (19.1), 259 (18.2), 258 (8.4), 248 (45.2), 230 (22.6), 118 (13.5), 116 (21.0), 110 (18.2), 99 (10.6), 73 (19.9), 71 (25.8); PCIMS *m/z* (rel. int. %) 493 (41.7, $[M+1]^+$), 475 (2.7), 394 (6.7), 393 (30.6), 392 (4.1), 378 (3.8), 377 (15.9), 376 (22.6) 375 (100), 359 (5.1), 357 (8.1), 349 (2.2), 293 (1.4), 277 (43.1), 261 (14.2), 259 (26.7), 249 (3.5), 231 (6.4), 215 (15.9), 119 (57.5), 117 (20.2), 101 (56.8), 99 (8.8); ^1H NMR spectrum in Table 1.

(2R*, 3S*, 4R*, 6R*,7R*,8R*,10R*,11R*,14S*)-9-*Hydroxy*-14 *or* 3-(3-*hydroxy*-2-*methylbutanoyloxy*)-3 *or* 14-(2-*methylbutanoyloxy*)-14,15-*epoxytrix*-5(15)-*en*-4,12-*olide* (**4g** or **4g'**). Gum; IR ν_{max}^{KBr} cm^{-1}: 3460, 1755, 1720, 1670 cm^{-1}; EIMS *m/z* (rel. int. %) 478 (2.2, $[M]^+$), 378 (25.0), 361 (40.0), 334 (22.8), 294 (81.5), 276 (25.5), 259 (7.9), 258 (9.9), 250 (49.5), 248 (100), 230 (13.1), 221 (3.0), 117 (8.9), 101 (23.9), 85 (40.7), 73 (15.0), 57 (69.2); PCIMS *m/z* (rel. int. %) 479, (8.5, $[M+1]^+$), 461 (0.2), 379 (8.3), 377 (4.7), 362 (21.4), 361 (100), 359 (8.7), 343 (5.2) 294 (0.7), 277 (14.0), 261 (8.8), 259 (29.2), 249 (1.2), 231 (5.5), 215 (4.7), 119 (86.2), 103 (28.2), 101 (93.6), 85 (7.2); ^1H- and ^{13}C NMR spectra in Tables 1 and 2.

Mixture of **4e** (*or* **4e'**) and **4f** (or **4f'**). Gum; EIMS *m/z* (rel. int. %) 536 (1.0, $[M]^+$), 436 (4.0), 419 (11.0), 408 (3.2), 392 (4.6), 376 (2.2), 294 (12.5), 276 (5.2), 259 (3.3), 258 (1.8), 248 (15.9), 230 (2.7), 221 (1.5), 161 (2.2), 143 (22.0), 117 (3.9), 101 (7.0), 83 (100), 55 (11.8); PCIMS *m/z* (rel. int. %) 537 (9.3, $[M+1]^+$), 519 (0.3), 479 (4.3), 477 (1.2), 437 (7.7), 419 (53.7), 377 (4.5), 359 (20.6), 277 (14.7), 261 (14.2), 259 (8.9), 249 (1.5), 231 (2.9), 215 (7.3), 161 (68.5), 193 (47.8), 119 (83.3), 101 (100); ^1H and ^{13}C NMR spectra in Tables 1 and 2.

Mixture of **4g** (*or* **4g'**) *and* **4h**. Gum; IR and MS essentially identical with that of pure **4g** (or **4g'**); ^1H NMR spectrum of **4h** taken from spectrum of mixt in Table 1.

Mixture of **4i** (*or* **4i'**) *and* **4j** (*or* **4j'**). Gum; IR ν_{max}^{KBr} cm^{-1}: 3460, 1753, 1735, 1665; EIMS *m/z* (rel. int. %) 536 (0.5, $[M]^+$), 436 (5.0), 419 (12.1), 408 (3.1), 259 (6.3), 258 (2.5), 248 (38.7), 230 (6.5), 221 (2.5), 161 (7.2), 143 (53.0), 117 (5.4), 101 (17.2), 83 (100), 55 (12.0); PCIMS *m/z* (rel. int. %) 537 (8.9, $[M+1]^+$, 477 (1.2), 437 (5.4), 419 (24.6), 359 (17.9), 294 (0.2), 277 (18.2), 261 (17.9), 259 (9.1), 231 (3.3), 215 (6.8), 161 (95.5), 143 (57.8), 119 (88.3), 101 (100), 85 (21.3).

(7S*,9S*)-9,12,14,15-*Diacetoxy*)-2-*Hydroxy*-3-*methylbutanoyloxy*, 2-*methylbutanoyloxygermacra*-1(10),4,11(13)-*triene* (**5b**). Gum; IR ν_{max}^{KBr} cm^{-1}: 3480, 1735; EIMS *m/z* (rel. int. %) 536 (0.2, $[M]^+$), 477 (28.9), 435 (2.3), 419 (9.4), 418 (26.3), 404 (9.9), 403

(25.3), 316 (11.7), 315 (47.6), 314 (35.2), 257 (12.0), 216 (11.6), 215 (48.4), 214 (50.89), 213 (12.9), 199 (28.6), 198 (52.1), 197 (100), 196 (81.2), 195 (16.8), 183 (33.0), 157 (37.1), 143 (31.9), 85 (32.7), 73 (13.0), 57 (28.2); PCIMS *m/z* (rel. int. %) 537 (0.6, $[M+1]^+$), 478 (28), 477 (100), 435 (3), 419 (30.0), 417 (8.5), 405 (5.9), 375 (21.3), 315 (69.2), 299 (3.0), 277 (9.9), 257 (11.9), 215 (25.8), 197 (27.4), 119 (6.2), 103 (9.1); ^1H and ^{13}C NMR spectra in Tables 2 and 3.

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