

Absolute Configuration of Trixanolides from *Trixis pallida*

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The aerial parts of *Trixis pallida* afforded seven known trixanolides (**18–24**) together with 17 new trixanolides (**1–17**). Their structures were determined by 1D and 2D NMR techniques, while the absolute configuration of the trixane skeleton was determined using the Mosher method and turned out to be enantiomeric to that reported using the Horeau method. This result is further discussed from the biosynthetic point of view.

The genus *Trixis*, subtribe Nassauviinae, tribe Mutisieae¹ of the family Compositae, produces sesquiterpenes based on the trixane skeleton as characteristic secondary metabolites.^{2,3} In the present article, we report the isolation of 24 trixanolides from *Trixis pallida* Less., with 17 of these (**1–17**) being new. The absolute configuration of these compounds was revised, and the result is further discussed from the biosynthetic point of view.

Results and Discussion

The IR spectrum of **1** showed bands at 3450, 3060, 1740, and 1670 cm⁻¹, which were indicative of hydroxyl, a double bond with at least one attached hydrogen, and carbonyl groups. When the ¹H NMR spectrum was compared with those of trixanolide diesters isolated by one of us from *Trixis grisebachii*,³ a set of signals corresponding to the 3-14-diester-9-hydroxytrixanolide skeleton was readily evident. In addition, signals at δ 5.22 (dq, $J = 6.5, 5.0$ Hz), 2.68 (dq, $J = 7.0, 5.0$ Hz), 2.03 (s), 1.27 (d, $J = 6.5$ Hz), and 1.22 (d, $J = 7.0$ Hz) indicated that a 3-acetyloxy-2-methylbutanoate moiety was present. An additional acetate group signal appeared at δ 2.08. The position of each ester residue was evident after comparison with the reported ¹H NMR data of related compounds^{2–4} and with the spectra of other trixanolides isolated during the present investigation. We observed that when an ester other than acetate is placed at C-14, the H-14 signal appeared as a doublet between δ 5.40 and 5.50. Furthermore, when this signal appeared in the narrow δ 5.40–5.43 range, a 3-acetyloxy group was present as a part of the ester residue attached to C-14. Conversely, when a 3-hydroxy group was present as a part of the ester residue, the H-14 doublet appeared at δ 5.46–5.50 (see Tables 1–3). When an acetate group was attached to C-14, the H-14 doublet is shifted downfield to near δ 6.00.⁴ Taking these considerations into account, the acetate group in compound **1** was placed at C-3, while the 3-acetyloxy-2-methylbutanoate residue was placed at C-14. This was further confirmed in the HMBC spectrum, which showed a correlation of H-14 and the 2-methylbutanoate carbonyl. The assignments of the ¹H NMR signals corresponding to each acetyloxy group were made after comparison with the spectra of monoacetylated compounds **2–4**.

The individual assignments of the protons attached to C-1 and C-8 were deduced from the minimum energy conformation of **1**, obtained using the PCMODEL program.⁵ The dihedral angles and calculated coupling constant values given in Table 4 are in good agreement with the observed values given in Table 1. The molecular geometry is consistent with two long-range *W*-type couplings, with one between H-1 α and H-10 of 1.7 Hz, and the other one between H-2 and H-4 of 2.0 Hz, as well as an unobservable coupling between H-1 α and H-2, which form a ca. 80° dihedral angle.

The ¹³C NMR spectrum of **1** showed 24 signals, four of them corresponding to carbonyl groups, two to sp² carbons, and five to sp³ oxygen-bearing carbons. A DEPT experiment provided evidence of five CH₃, two CH₂, ten CH, and seven quaternary carbons. All protonated carbon atom assignments were determined from a HETCOR experiment, while the quaternary carbon signal assignments were made with the aid of an HMBC experiment, thus showing that the C-2 and C-7 chemical shift assignments should be interchanged from those given in a previous report.³ The chemical shift values are given in Table 5. The HREIMS of **1** displayed a [M]⁺ at m/z 478.1851, in agreement with the molecular formula C₂₄H₃₀O₁₀.

The absolute configuration of the trixane skeleton was published as deduced using the Horeau method.⁶ However, as errors in absolute configuration determination occur when this method is used,⁷ we carried out an independent study to determine the absolute configuration of the trixane skeleton. Thus, the absolute configuration at C-9 was determined using the Mosher method (see Table 6), in which the ¹H NMR chemical shift differences for selected signals in the spectra of the (*R*)- and (*S*)- α -methoxy- α -(trifluoromethyl)phenylacetate (MTPA) esters (**25** and **26**) were compared.⁸ The resulting absolute configuration is enantiomeric to that previously reported⁶ and in agreement with the proposed biosynthetic pathway for the trixane skeleton⁹ via guaiane or cyperene, on the basis of the well-known fact that the isopropyl group attached to C-7 in guaienes isolated from higher plants is always β -oriented.¹⁰ Consequently, the absolute configuration of the trixane skeleton is 2*S*,6*R*,7*R*,10*R*.

After a thorough study of the ¹H NMR spectra of the 24 trixanolides isolated in this work and the 12 trixanolides previously isolated from *T. grisebachii*³ some chemical shifts of ester residues seem to be indicative of their position on the trixanolide skeleton. Thus, when the

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Table 1. ¹H NMR Data (δ in ppm from TMS) of Compounds **1–6**^a

proton	1 ^b	2 ^c	3 ^d	4 ^e	5 ^{d,f}	6 ^{d,g}
1 α	1.42 dd (11.7, 1.7)	1.42 dd (11.9, 1.7)	1.42 dd (11.9, 1.7)	1.43 dd (11.9, 1.7)	1.42 dd (11.7, 1.7)	1.42 dd (11.7, 1.7)
1 β	3.16 dd (11.7, 7.0)	3.16 dd (11.9, 7.0)	3.16 dd (11.9, 7.0)	3.15 dd (11.9, 7.0)	3.16 dd (11.7, 7.0)	3.16 dd (11.7, 7.0)
2	2.38 br dt (7.0, 2.0)	2.39 br dt (7.0, 2.0)	2.39 br dt (7.0, 2.0)	2.39 br dt (7.0, 2.0)	2.38 br dt (7.0, 2.0)	2.38 br dt (7.0, 2.0)
3	4.82 dd (3.0, 2.0)	4.82 dd (3.0, 2.0)	4.82 dd (3.0, 2.0)	4.83 dd (3.0, 2.0)	4.85 dd (3.0, 2.0)	4.85 dd (3.0, 2.0)
4	4.95 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)	4.97 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)
7	2.59 td (9.0, 5.0)	2.61 td (8.8, 5.0)	2.61 td (8.8, 5.0)	2.60 td (9.0, 5.0)	2.60 td (8.8, 5.0)	2.60 td (8.8, 5.0)
8 α	2.21 dd (15.0, 9.0)	2.21 dd (15.0, 8.8)	2.21 dd (15.0, 8.8)	2.23 dd (15.0, 9.0)	2.20 dd (15.3, 8.8)	2.20 dd (15.3, 8.8)
8 β	1.87 dt (15.0, 5.0)	1.87 dt (15.0, 5.0)	1.87 dt (15.0, 5.0)	1.87 dt (15.0, 5.0)	1.88 dt (15.3, 5.0)	1.88 dt (15.3, 5.0)
9	4.40 br t (5.0)	4.40 br t (5.0)	4.40 br t (5.0)	4.42 br t (5.0)	4.42 br t (5.0)	4.42 br t (5.0)
10	2.17 dd (5.0, 1.7)	2.17 dd (5.0, 1.7)	2.17 dd (5.0, 1.7)	2.18 dd (5.0, 1.7)	2.16 dd (5.0, 1.7)	2.16 dd (5.0, 1.7)
13	1.59 s	1.60 s	1.60 s	1.60 s	1.60 s	1.60 s
14	5.41 d (9.0)	5.46 d (8.8)	5.46 d (8.8)	5.49 d (9.0)	5.46 d (8.8)	5.46 d (8.8)
15	6.51 s	6.52 s	6.52 s	6.52 s	6.52 s	6.52 s
OAc	2.08 s	2.08 s	2.08 s	2.08 s		

^a 300 MHz; CDCl₃ as solvent; *J* in parentheses in Hz. ^b *erythro*-3-OAc-2-MeBu: 5.22 (dq, 6.5, 5.0, H-3'); 2.68 (qd, 7.0, 5.0, H-2'); 2.03 (s, OAc); 1.27 (d, 6.5, H-4'); 1.22 (d, 7.0, H-5'). ^c *erythro*-3-OH-2-MeBu: 4.11 (dq, 6.5, 3.0, H-3'); 2.56 (qd, 7.0, 3.0, H-2'); 1.23 (d, 7.0, H-5'); 1.22 (d, 6.5, H-4'). ^d *threo*-3-OH-2-MeBu: 3.94 (quintet, 7.0, H-3'); 2.55 (quintet, 7.0, H-2'); 1.25 (d, 7.0, H-5'); 1.20 (d, 7.0, H-4'). ^e 3-OH-*N*Val: 2.59 (s, H-2'a,b); 1.34 (s, H-5'); 1.32 (s, H-4'). ^f *N*Val: 2.21 obscured; 2.08 (hept, 7.0, H-3'); 0.95 (d, 7.0, H-4'); 0.95 (d, 7.0, H-5'). ^g 2-MeBu: 2.41 (sext, 7.0, H-2'); 1.66 (ddq, 14.0, 7.0, 7.0, H-3'a); 1.48 (ddq, 14.0, 7.0, 7.0, H-3'b); 1.13 (d, 7.0, H-5'); 0.91 (t, 7.0, H-4').

Table 2. ¹H NMR Data (δ in ppm from TMS) of Compounds **7–12**^a

proton	7 ^{c,f}	8 ^{b,g}	9 ^{b,h}	10 ^{b,i}	11 ^{e,f}	12 ^{d,g}
1 α	1.44 dd (11.7, 1.7)	1.43 dd (11.7, 1.7)	1.43 dd (11.7, 1.7)	1.44 dd (11.7, 1.7)	1.42 dd (11.7, 1.7)	1.43 dd (11.9, 1.8)
1 β	3.15 dd (11.7, 7.0)	3.17 dd (11.7, 7.0)	3.17 dd (11.7, 7.0)	3.18 dd (11.7, 7.0)	3.16 dd (11.7, 7.0)	3.16 dd (11.9, 7.0)
2	2.38 br dt (7.0, 2.0)	2.38 br dt (7.0, 2.0)	2.38 br dt (7.0, 2.0)	2.41 br dt (7.0, 2.0)	2.38 br dt (7.0, 2.0)	2.39 br dt (7.0, 2.0)
3	4.85 dd (3.0, 2.0)	4.86 dd (3.0, 2.0)	4.86 dd (3.0, 2.0)	4.90 dd (3.0, 2.0)	4.83 dd (3.0, 2.0)	4.85 dd (3.0, 2.0)
4	4.95 dd (3.0, 2.0)	4.95 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)	4.97 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)	4.96 dd (3.0, 2.0)
7	2.60 td (9.0, 5.0)	2.60 td (9.0, 5.0)	2.60 td (9.0, 5.0)	2.60 td (9.0, 5.0)	2.60 td (8.8, 5.0)	2.60 td (8.9, 5.0)
8 α	2.23 dd (15.3, 9.0)	2.21 dd (15.3, 9.0)	2.21 dd (15.3, 9.0)	2.22 dd (15.3, 9.0)	2.20 dd (15.3, 8.8)	2.23 dd (15.3, 8.9)
8 β	1.87 dt (15.3, 5.0)	1.87 dt (15.3, 5.0)	1.87 dt (15.3, 5.0)	1.88 dt (15.3, 5.0)	1.86 dt (15.3, 5.0)	1.87 dt (15.3, 5.0)
9	4.42 br t (5.0)	4.41 br t (5.0)	4.41 br t (5.0)	4.41 br t (5.0)	4.41 br t (5.0)	4.43 br t (5.0)
10	2.19 dd (5.0, 1.7)	2.18 dd (5.0, 1.7)	2.18 dd (5.0, 1.7)	2.18 dd (5.0, 1.7)	2.16 dd (5.0, 1.7)	2.18 dd (5.0, 1.8)
13	1.60 s	1.59 s	1.59 s	1.60 s	1.60 s	1.60 s
14	5.47 d (9.0)	5.40 d (9.0)	5.40 d (9.0)	5.42 d (9.0)	5.48 d (8.8)	5.47 d (8.9)
15	6.52 s	6.51 s	6.52 s	6.52 s	6.52 s	6.53 s

^a 300 MHz; CDCl₃ as solvent; *J* in parentheses in Hz. ^b *erythro*-3-OAc-2-MeBu: 5.22 (dq, 6.5, 5.0, H-3'); 2.68 (qd, 7.0, 5.0, H-2'); 2.03 (s, OAc); 1.27 (d, 6.5, H-4'); 1.22 (d, 7.0, H-5'). ^c *erythro*-3-OH-2-MeBu: 4.11 (dq, 6.5, 3.0, H-3'); 2.56 (qd, 7.0, 3.0, H-2'); 1.23 (d, 7.0, H-5'); 1.22 (d, 6.5, H-4'). ^d *threo*-3-OH-2-MeBu: 3.94 (quintet, 7.0, H-3'); 2.55 (quintet, 7.0, H-2'); 1.25 (d, 7.0, H-5'); 1.20 (d, 7.0, H-4'). ^e 3-OH-*N*Val: 2.59 (s, H-2'a,b); 1.34 (s, H-5'); 1.32 (s, H-4'). ^f *N*Val: 2.21 obscured; 2.08 (hept, 7.0, H-3'); 0.95 (d, 7.0, H-4'); 0.95 (d, 7.0, H-5'). ^g *threo*-3-OAc-2-MeBu: 5.11 (quintet, 6.5, H-3'); 2.68 (qd, 7.0, 6.5, H-2'); 2.06 (s, OAc); 1.26 (d, 6.5, H-4'); 1.17 (d, 7.0, H-5'). ^h 3-OAc-*N*Val: 2.95 (d, 14.5, H-2'a); 2.85 (d, 14.5, H-2'b); 2.00 (s, OAc); 1.53 (s, H-4'); 1.53 (s, H-5'). ⁱ 3-OH-*N*Val: 2.56 (d, 15.0, H-2'a); 2.52 (d, 15.0, H-2'b); 1.31 (s, H-5'); 1.29 (s, H-4').

Table 3. ¹H NMR Data (δ in ppm from TMS) of Compounds **13–17**^a

proton	13 ^{b,g}	14 ^{b,f}	15 ^{c,h,i}	16 ^{d,h,i}	17 ^{e,h,i}
1 α	1.43 dd (11.8, 2.0)	1.43 dd (11.8, 2.0)	1.52 dd (12.0, 1.5)	1.52 dd (12.0, 1.5)	1.52 dd (12.0, 1.5)
1 β	3.16 dd (11.8, 7.0)	3.16 dd (11.8, 7.0)	2.93 dd (12.0, 6.8)	2.93 dd (12.0, 6.8)	2.93 dd (12.0, 6.8)
2	2.38 br dt (7.0, 2.0)	2.38 br dt (7.0, 2.0)	2.46 br dt (6.8, 2.0)	2.46 br dt (6.8, 2.0)	2.46 br dt (6.8, 2.0)
3	4.83 dd (3.0, 2.0)	4.85 dd (3.0, 2.0)	4.92 dd (3.0, 2.0)	4.92 dd (3.0, 2.0)	4.92 dd (3.0, 2.0)
4	4.94 dd (3.0, 2.0)	4.94 dd (3.0, 2.0)	5.00 dd (3.0, 2.0)	5.00 dd (3.0, 2.0)	5.00 dd (3.0, 2.0)
7	2.60 td (8.8, 6.0)	2.60 td (8.8, 6.0)	2.54 td (9.0, 6.0)	2.54 td (9.0, 6.0)	2.54 td (9.0, 6.0)
8 α	2.21 dd (15.3, 8.8)	2.21 dd (15.3, 8.8)	2.21 dd (16.0, 9.0)	2.20 dd (16.0, 9.0)	2.21 dd (16.0, 9.0)
8 β	1.87 dt (15.3, 6.0)	1.87 dt (15.3, 6.0)	2.01 dt (16.0, 6.0)	2.01 dt (16.0, 6.0)	2.01 dt (16.0, 6.0)
9	4.41 br t (6.0)	4.41 br t (6.0)	5.27 br t (6.0)	5.27 br t (6.0)	5.27 br t (6.0)
10	2.18 dd (6.0, 2.0)	2.18 dd (6.0, 2.0)	2.42 dd (6.0, 1.5)	2.42 dd (6.0, 1.5)	2.42 dd (6.0, 1.5)
13	1.59 s	1.59 s	1.43 s	1.42 s	1.44 s
14	5.41 d (8.8)	5.41 d (8.8)	5.48 d (9.0)	5.47 d (9.0)	5.50 d (9.0)
15	6.51 s	6.51 s	6.58 s	6.59 s	6.58 s

^a 300 MHz; CDCl₃ as solvent; *J* in parentheses in Hz. ^b *erythro*-3-OAc-2-MeBu: 5.22 (dq, 6.5, 5.0, H-3'); 2.68 (qd, 7.0, 5.0, H-2'); 2.03 (s, OAc); 1.27 (d, 6.5, H-4'); 1.22 (d, 7.0, H-5'). ^c *erythro*-3-OH-2-MeBu: 4.11 (dq, 6.5, 3.0, H-3'); 2.56 (qd, 7.0, 3.0, H-2'); 1.23 (d, 7.0, H-5'); 1.22 (d, 6.5, H-4'). ^d *threo*-3-OH-2-MeBu: 3.94 (quintet, 7.0, H-3'); 2.55 (quintet, 7.0, H-2'); 1.25 (d, 7.0, H-5'); 1.20 (d, 7.0, H-4'). ^e 3-OH-*N*Val: 2.59 (s, H-2'a,b); 1.34 (s, H-5'); 1.32 (s, H-4'). ^f *N*Val: 2.21 obscured; 2.08 (hept, 7.0, H-3'); 0.95 (d, 7.0, H-4'); 0.95 (d, 7.0, H-5'). ^g 2-MeBu: 2.41 (sext, 7.0, H-2'); 1.66 (ddq, 14.0, 7.0, 7.0, H-3'a); 1.48 (ddq, 14.0, 7.0, 7.0, H-3'b); 1.13 (d, 7.0, H-5'); 0.91 (t, 7.0, H-4'). ^h 3-OH-*N*Val: 2.56 (d, 15.0, H-2'a); 2.52 (d, 15.0, H-2'b); 1.31 (s, H-5'); 1.29 (s, H-4'). ⁱ 2-MeBu: 2.37 (ddq, 7.0, 7.0, 7.0, H-2'); 1.68 (dq, 14.0, 7.0, H-3'a); 1.50 (dq, 14.0, 7.0, H-3'b); 1.16 (d, 7.0, H-5'); 0.94 (t, 7.0, H-4').

3-hydroxyl-*iso*-valerate ester residue was placed at C-14, the protons attached to C-2' gave a singlet around δ 2.59 and the methyl group signals appeared at ca. δ 1.34 and 1.32 as singlets. Conversely, when this ester residue was at C-3, the protons at C-2' appeared as an AB system centered around δ 2.53 and the methyl groups gave two

singlets at ca. δ 1.31 and 1.29. The chemical shifts of the methyl groups owing to the ester residues and their location on the trixanolide skeleton are given in Table 7.

In a similar fashion, evaluation of HMBC contour plots of many trixanolides revealed systematically that published^{2,3,11} H-3 and H-4 NMR chemical shifts have to be

Table 4. Dihedral Angles and Calculated and Observed Coupling Constant Values for **1**

position	ϕ (deg)	J_{calcd} (Hz)	J_{obs} (Hz)
1 α ,2	-79	1.1	<i>a</i>
1 β ,2	40	6.2	7.0
2,3	67	2.4	2.0
3,4	-53	3.1	3.0
7,8 α	-22	9.1	9.0
7,8 β	-142	8.3	5.0
7,14	164	8.6	9.0
8 α ,9	-83	1.4	<i>a</i>
8 β ,9	34	5.6	5.0
9,10	-46	3.6	5.0

^a Not observed.

reversed due to the observation of correlations of the lactone carbonyl (C-12) with H-4, H-10, and Me-13.

Compounds **2** and **3** were obtained as an inseparable 7:3 mixture. The ¹H NMR spectrum of this mixture showed signals similar to **1** (see Table 1), except those for the ester moiety at C-14. Signals for 3-hydroxy-2-methylbutanoate esters at δ 4.11, 2.56, 1.23, and 1.22 for the major component and at δ 3.94, 2.55, 1.25, and 1.20 for the minor component were present. In a previous report, Bohlmann et al.² also observed different chemical shifts for H-3' of 3-hydroxy-2-methylbutanoate residues, but in the discussion, the authors concluded that the chemical shift difference should be attributed to a different position of the ester residue on the trixane ring. However, the ¹³C NMR spectrum of the mixture of **2** and **3** showed duplicated signals for C-5, C-8, C-14, and C-15 and for the 3-hydroxy-2-methylbutanoate carbonyl group, while no duplication was observed for C-2, C-4, C-12, and the acetate carbonyl group signals, which are in the neighborhood of the C-3 atom. These observations allowed us to conclude that both compounds have isomeric 3-hydroxy-2-methylbutanoate ester residues at C-14, which produced the signal doubling described above. Furthermore, the acetate ester moiety should be placed at C-3, in consonance with the methyl group chemical shift in the ¹H NMR spectrum (see Tables 1 and 7). The relative stereochemistry of the 3-hydroxy-2-methylbutanoate esters has already been studied,¹² whereby it was found that the H-2 and H-3 signals for the *erythro* isomer appeared at lower fields than those for the *threo*

isomer, while the doublet corresponding to Me-5 appeared at a higher field for the *erythro* isomer as compared to the *threo* isomer. The minimum energy conformation of the *erythro*- and *threo*-3-hydroxy-2-methylbutanoate esters showed that the dihedral angle between H-2 and H-3 was 65° with a calculated $J = 1.2$ Hz for the *erythro* configuration, while for the *threo* configuration the dihedral angle was 175° with a $J = 10.8$ Hz. These data are in agreement with the fact that the H-3 signal appeared as a doublet quartet in the *erythro* isomer and as a quintet-like signal in the *threo* isomer.¹² Therefore, the major constituent of the mixture has an *erythro*-3-hydroxy-2-methylbutanoate residue and was assigned to **2**, while the *threo* configuration corresponded to the minor constituent **3**.

The ¹H NMR spectra of compounds **4**–**14** showed the characteristic signals of a 3,14-diester-9-hydroxytrioxanolid skeleton, as described in detail for the above compounds. The nature of the different ester functions was deduced from the ¹H NMR spectra and, in the case of **4** and **7**, also from ¹³C NMR spectra. The ester residue positions on the trixane skeleton were assigned on the basis of the above observations, as shown in Table 7, by direct comparison with the spectra of trioxanolides isolated from *T. grisebachii*,³ and comparison with literature data.² They were further corroborated by detecting long-range ¹³C–¹H interactions in HMBC measurements. Additionally, the HREIMS of **4** showed a [M]⁺ at m/z 436.1725, in agreement with the molecular formula C₂₂H₂₈O₉.

When the NMR spectra of compounds published as either **4a**–**j** or **4a'**–**j'** in the *T. grisebachii* report³ were evaluated under the above considerations, the correct structures were revised as **4a**–**j**. Thus, the spectrum of compound **10**, isolated in this work, mainly differed from that of compound **4e**, isolated from *T. grisebachii*, in the chemical shift of the H-14 signal. In **10** this signal appeared at δ 5.42, indicating that the 3-acetyloxy-2-methylbutanoate residue was at C-14, while in **4e** the H-14 signal appeared at δ 5.50, which demonstrated that the ester attached to C-14 had a 3-hydroxy group instead of a 3-acetyloxy group. Further support was given by the ester residue signals (see Tables 1 and 7), which clearly indicated that in **10** the *erythro*-3-acetyloxy-2-methylbutanoate and 3-hydroxy-*iso*-

Table 5. ¹³C NMR Data (δ in ppm from TMS) of Compounds **1**–**4**, **7**, **16**, **18**, **20**, **21**, **23**, and **24**^a

carbon	1 ^b	2 ^c	3 ^d	4 ^e	7 ^{c,f}	16 ^{d,g,h}	18 ^{e,i}	20 ^{c,j}	21 ^{e,k}	23 ^{c,i}	24 ^{c,k}
1	41.9	42.0	42.0	42.0	41.9	41.4	42.0	41.8	41.9	42.0	41.9
2	49.1	49.2	49.2	49.2	49.3	49.2	49.1	49.3	49.2	49.1	49.2
3	74.4	74.4	74.4	74.4	74.1	74.3	74.4	74.0	74.5	74.4	74.6
4	76.5	76.6	76.6	76.6	76.6	76.4	76.5	76.6	76.4	76.5	76.5
5	117.7	117.8	117.7	117.8	117.8	117.0	117.8	117.9	117.8	117.7	117.7
6	53.9	53.9	53.9	53.8	53.8	54.2	53.8	53.8	53.8	53.8	53.8
7	40.5	40.6	40.6	40.5	40.5	40.3	40.4	40.5	40.4	40.5	40.5
8	40.8	40.9	40.8	41.0	40.9	38.1	40.9	40.9	40.9	40.9	40.9
9	73.0	73.0	73.0	73.0	72.9	74.5	72.8	72.9	72.8	72.9	72.9
10	61.8	61.9	61.9	61.9	61.8	60.4	61.9	61.8	61.8	61.9	61.9
11	49.8	49.8	49.8	49.8	49.8	49.7	49.7	49.8	49.7	49.8	49.8
12	177.2	177.1	177.1	177.1	177.2	176.2	176.9	177.2	176.9	176.9	176.9
13	20.0	20.0	20.0	20.0	20.0	19.9	19.9	20.0	20.0	19.9	20.0
14	95.8	95.5	95.6	95.6	95.6	95.3	95.5	95.6	95.5	95.5	95.6
15	140.0	140.0	140.1	140.0	139.9	140.8	139.9	139.9	139.9	140.0	140.0
OAc	170.1	170.1	170.1	170.1							
	21.1	20.9	20.9	20.9							

^a 75.4 MHz; CDCl₃ as solvent. ^b *erythro*-3-OAc-2-MeBu: 172.0 (C-1); 44.3 (C-2); 70.4 (C-3); 17.7 (C-4); 11.3 (C-5); 170.2, 20.9 (OAc). ^c *erythro*-3-OH-2-MeBu: 173.9 (C-1); 45.8 (C-2); 68.1 (C-3); 20.1 (C-4); 10.8 (C-5). ^d *threo*-3-OH-2-MeBu: 174.0 (C-1); 47.2 (C-2); 69.4 (C-3); 20.7 (C-4); 13.7 (C-5). ^e 3-OH-*i*Val: 171.0 (C-1); 46.7 (C-2); 69.2 (C-3); 29.3 (C-4); 29.2 (C-5). ^f *i*Val: 172.0 (C-1); 43.2 (C-2); 25.6 (C-3); 22.3 (C-4); 22.3 (C-5). ^g 3-OH-*l*Val: 171.6 (C-1); 46.8 (C-2); 69.2 (C-3); 29.3 (C-4); 29.2 (C-5). ^h 2-MeBu: 175.2 (C-1); 41.2 (C-2); 26.7 (C-3); 16.0 (C-4); 11.5 (C-5). ⁱ 3-OAc-*l*Val: 170.5 (C-1); 44.4 (C-2); 79.2 (C-3); 26.5 (C-4); 26.5 (C-5); 169.2, 22.3 (OAc). ^j 2-MeBu: 175.6 (C-1); 41.0 (C-2); 26.4 (C-3); 16.2 (C-4); 11.4 (C-5). ^k *threo*-3-OAc-MeBu: 172.8 (C-1); 44.3 (C-2); 70.8 (C-3); 17.6 (C-4); 12.2 (C-5); 170.4, 21.1 (OAc).

Table 6. Selected ¹H NMR Data for (*R*)- and (*S*)-MTPA Esters **25** and **26**

H	25 (<i>R</i>)	26 (<i>S</i>)	Δ(<i>S</i> – <i>R</i>)
7	2.23	2.46	+0.23
8α	2.30	2.32	+0.02
8β	2.09	2.11	+0.02
13	1.16	0.89	–0.27
14	5.40	5.43	+0.03

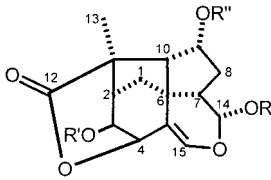
Table 7. Methyl Chemical Shifts of the Ester Residues and Their Location on the Trixanolide Skeleton

ester residue	position	δ	multi- plicity	compounds
acetyloxy	C-3	2.08	s (3H)	1,2,3,4,25,26
acetyloxy of <i>erythro</i> -3-OAc-2-MeBu	C-14	2.03	s (3H)	1,8,9,10,13,14,25,26
<i>threo</i> -3-OAc-2-MeBu	C-3	2.06	s (3H)	8,12,21,24
3-OAc- <i>N</i> Val	C-3	2.00	s (3H)	9,18,22,23
3-OAc- <i>N</i> Val	C-3	1.53	s (6H)	9,18,22,23
<i>erythro</i> -3-OAc-2-MeBu	C-14	1.27	d (3H)	1,8,9,10,13,14,25,26
		1.22	d (3H)	
<i>threo</i> -3-OAc-2-MeBu	C-3	1.26	d (3H)	8,12,21,24
		1.17	d (3H)	
3-OH- <i>N</i> Val	C-3	1.31	s (3H)	10,15,16,17
		1.29	s (3H)	
	C-14	1.34	s (3H)	4,11,17,18,19,21
		1.32	s (3H)	
<i>erythro</i> -3-OH-2-MeBu	C-14	1.23	d (3H)	2,7,15,20,23,24
		1.22	d (3H)	
<i>threo</i> -3-OH-2-MeBu	C-14	1.25	d (3H)	3,5,6,12,16,22
		1.20	d (3H)	
<i>N</i> Val	C-3	0.95	d (6H)	5,7,11,14
2-MeBu	C-3	1.13	d (3H)	6,13,19,20
		0.91	t (3H)	
	C-9	1.16	d (3H)	15,16,17
		0.94	t (3H)	

valerate residues were at C-14 and C-3, respectively, while in **4e** they occurred at the C-3 and C-14 positions, respectively.

Compounds **12** and **22** differed from **4i**³ and **4j**³ of the *T. grisebachii* report,³ respectively, in the nature of the 3-hydroxy-2-methylbutanoate ester residue, being *erythro* in **4i** and **4j** and *threo* in **12** and **22**. On the other hand, the ¹H NMR spectra of compounds **18** and **20** were essentially identical to those of compounds **4f**³ and **4g**³ respectively.

The ¹H NMR spectra of the triesters **15**–**17** showed in all cases the signals corresponding to H-3 and H-9 at δ 4.92 and 5.27, respectively, while H-14 appeared at δ 5.48, 5.47, and 5.50 in **15**, **16**, and **17**, respectively. Compound **15** showed the CH₂-2' AB system of the 3-hydroxy-*iso*-valerate ester residue centered at δ 2.54, indicating that it was located at C-3. The *erythro*-3-hydroxy-2-methylbutanoate signals indicated that this ester residue is attached to C-14 (see Tables 3 and 7), and consequently the 2-methylbutanoate residue should be placed at C-9. By comparing the ester moiety signals of **16** and **17** with those of **15** (Table 3), one may conclude that the 3-hydroxy-*iso*-valerate and 2-methylbutanoate ester residues should be placed at C-3 and C-9, respectively, in both compounds. On the other hand, a *threo*-3-hydroxy-2-methylbutanoate residue was attached to C-14 in **16**, while a 3-hydroxy-*iso*-valerate ester was found at C-14 in **17**. Finally, HREIMS of the three isomeric substances (**15**–**17**) displayed [M]⁺ at *m/z* 578.2729, 578.2713, and 578.2703, respectively, in agreement with the molecular formula C₃₀H₄₂O₁₁.

Table 8


compd	R	R'	R''
1	<i>erythro</i> -3-OAc-2-MeBu	Ac	H
2	<i>erythro</i> -3-OH-2-MeBu	Ac	H
3	<i>threo</i> -3-OH-2-MeBu	Ac	H
4	3-OH- <i>N</i> Val	Ac	H
5	<i>threo</i> -3-OH-2-MeBu	<i>N</i> Val	H
6	<i>threo</i> -3-OH-2-MeBu	2-MeBu	H
7	<i>erythro</i> -3-OH-2-MeBu	<i>N</i> Val	H
8	<i>erythro</i> -3-OAc-2-MeBu	<i>threo</i> -3-OAc-2-MeBu	H
9	<i>erythro</i> -3-OAc-2-MeBu	3-OAc- <i>N</i> Val	H
10	<i>erythro</i> -3-OAc-2-MeBu	3-OH- <i>N</i> Val	H
11	3-OH- <i>N</i> Val	<i>N</i> Val	H
12	<i>threo</i> -3-OH-2-MeBu	<i>threo</i> -3-OAc-2-MeBu	H
13	<i>erythro</i> -3-OAc-2-MeBu	2-MeBu	H
14	<i>erythro</i> -3-OAc-2-MeBu	<i>N</i> Val	H
15	<i>erythro</i> -3-OH-2-MeBu	3-OH- <i>N</i> Val	2-MeBu
16	<i>threo</i> -3-OH-2-MeBu	3-OH- <i>N</i> Val	2-MeBu
17	3-OH- <i>N</i> Val	3-OH- <i>N</i> Val	2-MeBu
18	3-OH- <i>N</i> Val	3-OAc- <i>N</i> Val	H
19	3-OH- <i>N</i> Val	2-MeBu	H
20	<i>erythro</i> -3-OH-2-MeBu	2-MeBu	H
21	3-OH- <i>N</i> Val	<i>threo</i> -3-OAc-2-MeBu	H
22	<i>threo</i> -3-OH-2-MeBu	3-OAc- <i>N</i> Val	H
23	<i>erythro</i> -3-OH-2-MeBu	3-OAc- <i>N</i> Val	H
24	<i>erythro</i> -3-OH-2-MeBu	<i>threo</i> -3-OAc-2-MeBu	H
25	<i>erythro</i> -3-OAc-2-MeBu	Ac	<i>R</i> -MTPA
26	<i>erythro</i> -3-OAc-2-MeBu	Ac	<i>S</i> -MTPA

Experimental Section

General Experimental Procedures. Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were performed on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrophotometer. NMR spectra were recorded on Varian XL-300GS and Mercury 300 spectrometers. LREIMS were obtained on JEOL JMS SX 102 or Hewlett-Packard 5989A spectrometers. HRMS were measured on a VG 7070 high-resolution mass spectrometer at the UCR Mass Spectrometry Facility, University of California, Riverside. For column chromatography, Si gel Merck 70–230 or 230–400 mesh ASTM was used. For separation of mixtures, HPLC with a differential refractive index detector was used. The column employed was a Beckman C₁₈ (5 μm, 10 × 250 mm). Retention times (*t_R*) were measured from the solvent peak.

Plant Material. Aerial parts of *T. pallida* were collected in April 1997, at Yatay-Poñy, Corrientes Province, Argentina. A voucher specimen (Leg. A Schinini and O. Ahumada, No. 12411) is on deposit at the Instituto de Botánica del Noreste, Corrientes, Argentina.

Extraction and Isolation. Aerial parts (283.5 g) of *T. pallida* were extracted with EtOAc at room temperature for 2 days to give 6.0 g of a residue (yield 2.1%), which was suspended in 50 mL of EtOH at 60 °C, diluted with 40 mL of H₂O, and extracted successively with hexane (2 × 50 mL) and CH₂Cl₂ (2 × 50 mL). Evaporation under vacuum of the hexane extract gave known compounds as outlined in the Supporting Information. Evaporation under reduced pressure of the CH₂-Cl₂ extract gave 1.95 g of residue, which was column chromatographed over Si gel using CHCl₃ with increasing amounts of EtOAc (0.20%) to give 164 fractions. Fractions 33–46 (116 mg) were processed by HPLC (MeOH–H₂O, 2:1, 2 mL min⁻¹) to give **1** (25.1 mg, *t_R* 7 min) and a 1:1 mixture of **8** and **9** (14.0 mg, *t_R* 20 min). Fractions 81–118 were combined, and a portion (280 mg) was processed by HPLC (MeOH–H₂O, 64:36, 2 mL min⁻¹) to give **4** (11.6 mg, *t_R* 3 min); a 3:1 mixture of **10** and

18 (12.8 mg, t_R 6 min); a 18:13:11:8 mixture of **11**, **5**, **19**, and **6** (52.8 mg, t_R 10 min); a 3:1 mixture of **7** and **20** (2.0 mg, t_R 12 min); **17** (4.1 mg, t_R 16 min); **15** (2:1 mg, t_R 17 min), and **16** (28.4 mg, t_R 18 min). Fractions 119–141 (230 mg) were processed by HPLC (MeOH–H₂O, 31:19, 2 mL min⁻¹) to give a 2:1 mixture of **3** and **4** (8.1 mg, t_R 8 min); a 3:2 mixture of **18** and **21** (15.1 mg, t_R 9 min); a 7:3 mixture of **6** and **5** (2.1 mg, t_R 15 min); a 3:1 mixture of **7** and **20** (37.2 mg, t_R 16 min), and **16** (0.5 mg, t_R 25 min). Fractions 142–164 (193 mg) were processed by HPLC as before to give a 7:3 mixture of **2** and **3** (16.9 mg, t_R 5 min); a 7:5:5:3 mixture of **12**, **22**, **21**, and **18** (61.2 mg, t_R 11 min), and a 3:2 mixture of **23** and **24** (51.7 mg, t_R 13 min).

(2S,3S,4S,6R,7R,9R,10R,11R,14S)-3-Acetyloxy-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (1): gum; $[\alpha]_{589} -36.5^\circ$, $[\alpha]_{578} -38.5^\circ$, $[\alpha]_{546} -44.2^\circ$, $[\alpha]_{436} -67.3^\circ$, $[\alpha]_{365} -96.1^\circ$ (*c* 0.52, CHCl₃); IR (KBr) ν_{\max} 3450, 3060, 1740, 1670 cm⁻¹; ¹H NMR and ¹³C NMR, see Tables 1 and 5, respectively; EIMS (70 eV) m/z [M]⁺ 478 (45), 434 (40), 378 (95), 334 (51), 332 (45), 285 (58), 232 (60), 231 (85), 230 (61), 143 (86), 117 (66), 83 (100); HRDEIMS m/z 478.1851 (calcd for C₂₄H₃₀O₁₀, 478.1839).

3-Acetyloxy-9-hydroxy-14-(erythro-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (2): gum (in 7:3 mixture with **3**); $[\alpha]_{589} -30.8^\circ$, $[\alpha]_{578} -32.5^\circ$, $[\alpha]_{546} -35.0^\circ$, $[\alpha]_{436} -55.5^\circ$, $[\alpha]_{365} -78.6^\circ$ (*c* 1.17, CHCl₃); IR (KBr) ν_{\max} 3450, 3020, 1735, 1665 cm⁻¹; ¹H NMR and ¹³C NMR, see Tables 1 and 5, respectively; EIMS (20 eV) m/z [M]⁺ 436 (2), 368 (2), 348 (2), 336 (37), 319 (48), 294 (68), 292 (80), 276 (21), 248 (100), 230 (26), 213 (38).

3-Acetyloxy-9-hydroxy-14-(threo-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (3): gum (in 3:7 mixture with **2**); $[\alpha]_{589} -30.8^\circ$, $[\alpha]_{578} -32.5^\circ$, $[\alpha]_{546} -35.0^\circ$, $[\alpha]_{436} -55.5^\circ$, $[\alpha]_{365} -78.6^\circ$ (*c* 1.17, CHCl₃); IR (KBr) ν_{\max} 3450, 3020, 1735, 1665 cm⁻¹; ¹H NMR and ¹³C NMR, see Tables 1 and 5, respectively; EIMS (20 eV) m/z [M]⁺ 436 (2), 368 (2), 348 (2), 336 (37), 319 (48), 294 (68), 292 (80), 276 (21), 248 (100), 230 (26), 213 (38).

3-Acetyloxy-9-hydroxy-14-(3-hydroxy-3-methylbutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (4): gum; $[\alpha]_{589} -29.7^\circ$, $[\alpha]_{578} -31.1^\circ$, $[\alpha]_{546} -35.1^\circ$, $[\alpha]_{436} -51.3^\circ$, $[\alpha]_{365} -72.9^\circ$ (*c* 0.74, CHCl₃); IR (KBr) ν_{\max} 3450, 3030, 1735, 1665 cm⁻¹; ¹H NMR and ¹³C NMR, see Tables 1 and 5, respectively; EIMS (70 eV) m/z [M]⁺ 436 (20), 392 (6), 378 (8), 336 (70), 319 (100), 294 (94), 292 (91), 276 (18), 248 (63), 230 (16), 213 (18), 164 (10); HRDEIMS m/z 436.1725 (calcd for C₂₂H₂₈O₉, 436.1733).

3-(3-Methylbutanoyloxy)-9-hydroxy-14-(threo-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (5): mp 198–202 °C (in 3:7 mixture with **6**); IR (KBr) ν_{\max} 3450, 3030, 1735, 1665 cm⁻¹; ¹H NMR, see Table 1; EIMS (20 eV) m/z [M]⁺ 478 (0.4), 378 (15), 361 (25), 334 (24), 332 (11), 294 (56), 276 (21), 250 (50), 249 (20), 248 (100), 232 (24), 214 (24), 213 (25).

3-(2-Methylbutanoyloxy)-9-hydroxy-14-(threo-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (6): mp 198–202 °C (in 7:3 mixture with **5**); IR (KBr) ν_{\max} 3450, 3030, 1735, 1665 cm⁻¹; ¹H NMR, see Table 1; EIMS (20 eV) m/z [M]⁺ 478 (0.4), 378 (15), 361 (25), 334 (24), 332 (11), 294 (56), 276 (21), 250 (50), 249 (20), 248 (100), 232 (24), 214 (24), 213 (25).

3-(3-Methylbutanoyloxy)-9-hydroxy-14-(erythro-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (7): mp 198–202 °C (in 3:1 mixture with **20**); $[\alpha]_{589} -27.1^\circ$, $[\alpha]_{578} -27.9^\circ$, $[\alpha]_{546} -31.7^\circ$, $[\alpha]_{436} -48.8^\circ$, $[\alpha]_{365} -66.4^\circ$ (*c* 3.69, CHCl₃); IR (KBr) ν_{\max} 3450, 3030, 1735; 1665 cm⁻¹; ¹H NMR and ¹³C NMR, see Tables 2 and 5, respectively; EIMS (20 eV) m/z [M]⁺ 478 (0.5), 378 (16), 361 (25), 334 (25), 294 (60), 276 (20), 250 (51), 248 (100), 232 (23), 214 (22), 213 (24).

3-(threo-2-Methyl-3-acetyloxybutanoyloxy)-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (8): mp 124–127 °C (in mixture 1:1 with **9**); $[\alpha]_{589} -33.1^\circ$, $[\alpha]_{578} -35.2^\circ$, $[\alpha]_{546} -38.6^\circ$, $[\alpha]_{436} -63.4^\circ$, $[\alpha]_{365} -97.9^\circ$ (*c* 1.45, CHCl₃); IR (KBr) ν_{\max} 3450, 3060,

1740, 1670 cm⁻¹; ¹H NMR, see Table 2; EIMS (20 eV) m/z [M]⁺ 578 (0.2), 436 (0.8), 419 (3), 294 (2), 259 (3), 231 (5), 143 (69), 83 (100).

3-(3-Methyl-3-acetyloxybutanoyloxy)-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (9): mp 124–127 °C (in mixture 1:1 with **8**); $[\alpha]_{589} -33.1^\circ$, $[\alpha]_{578} -35.2^\circ$, $[\alpha]_{546} -38.6^\circ$, $[\alpha]_{436} -63.4^\circ$, $[\alpha]_{365} -97.9^\circ$ (*c* 1.45, CHCl₃); IR (KBr) ν_{\max} 3450, 3060, 1740, 1670 cm⁻¹; ¹H NMR, see Table 2; EIMS (20 eV) m/z [M]⁺ 578 (0.2), 436 (0.8), 419 (3), 294 (2), 259 (3), 231 (5), 143 (69), 83 (100).

3-(3-Hydroxy-3-methylbutanoyloxy)-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (10): mp 130.5–133 °C (in 3:1 mixture with **18**); $[\alpha]_{589} -34.5^\circ$, $[\alpha]_{578} -36.2^\circ$, $[\alpha]_{546} -39.6^\circ$, $[\alpha]_{436} -60.3^\circ$, $[\alpha]_{365} -84.5^\circ$ (*c* 0.56, CHCl₃); IR (KBr) ν_{\max} 3450, 3030, 1735, 1665 cm⁻¹; ¹H NMR, see Table 2; EIMS (20 eV) m/z [M]⁺ 536 (0.1), 436 (1), 419 (1), 392 (1), 377 (3), 376 (3), 294 (14), 277 (12), 276 (12), 248 (12), 143 (33), 83 (100).

3-(2-Methylbutanoyloxy)-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (13): gum (in 3:2 mixture with **14**); $[\alpha]_{589} -28.6^\circ$, $[\alpha]_{578} -28.6^\circ$, $[\alpha]_{546} -38.1^\circ$, $[\alpha]_{436} -47.6^\circ$, $[\alpha]_{365} -61.9^\circ$ (*c* 0.21, CHCl₃); IR (KBr) ν_{\max} 3450, 3050, 1735, 1710, 1665 cm⁻¹; ¹H NMR, see Table 3; EIMS (20 eV) m/z [M]⁺ 520 (0.3), 476 (0.6), 392 (1), 361 (4), 259 (2), 232 (3), 231 (3), 213 (2), 143 (55), 83 (100).

3-(3-Methylbutanoyloxy)-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (14): gum (in 2:3 mixture with **13**); $[\alpha]_{589} -28.6^\circ$, $[\alpha]_{578} -28.6^\circ$, $[\alpha]_{546} -38.1^\circ$, $[\alpha]_{436} -47.6^\circ$, $[\alpha]_{365} -61.9^\circ$ (*c* 0.21, CHCl₃); IR (KBr) ν_{\max} 3450, 3050, 1735, 1710, 1665 cm⁻¹; ¹H NMR, see Table 3; EIMS (20 eV) m/z [M]⁺ 520 (0.3), 476 (0.6), 392 (1), 361 (4), 259 (2), 232 (3), 231 (3), 213 (2), 143 (55), 83 (100).

3-(3-Hydroxy-3-methylbutanoyloxy)-9-(2-methylbutanoyloxy)-14-(erythro-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (15): gum; $[\alpha]_{589} -48.7^\circ$, $[\alpha]_{578} -51.3^\circ$, $[\alpha]_{546} -56.4^\circ$, $[\alpha]_{436} -89.7^\circ$, $[\alpha]_{365} -128.2^\circ$ (*c* 0.39, CHCl₃); IR (KBr) ν_{\max} 3450, 3030, 1735, 1665 cm⁻¹; ¹H NMR, see Table 3; EIMS (70 eV) m/z [M]⁺ 578 (3), 478 (7), 378 (15), 311 (50), 310 (100), 264 (27), 190 (15), 91 (37), 57 (40); HRDEIMS m/z 578.2729 (calcd for C₃₀H₄₂O₁₁, 578.2727).

3-(3-Hydroxy-3-methylbutanoyloxy)-9-(2-methylbutanoyloxy)-14-(threo-2-methyl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (16): gum; $[\alpha]_{589} -34.9^\circ$, $[\alpha]_{578} -36.3^\circ$, $[\alpha]_{546} -41.0^\circ$, $[\alpha]_{436} -64.4^\circ$, $[\alpha]_{365} -91.5^\circ$ (*c* 2.95, CHCl₃); IR (KBr) ν_{\max} 3450, 3030, 1735, 1665 cm⁻¹; ¹H NMR and ¹³C NMR, see Tables 3 and 5, respectively; EIMS (70 eV) m/z [M]⁺ 578 (4), 478 (25), 443 (12), 434 (18), 378 (100), 359 (27), 334 (55), 332 (43), 276 (12), 259 (14), 232 (16); HRDEIMS m/z 578.2713 (calcd for C₃₀H₄₂O₁₁, 578.2727).

3,14-Bis(3-hydroxy-3-methylbutanoyloxy)-9-(2-methylbutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (17): gum; $[\alpha]_{589} -47.9^\circ$, $[\alpha]_{578} -50.0^\circ$, $[\alpha]_{546} -55.2^\circ$, $[\alpha]_{436} -87.5^\circ$, $[\alpha]_{365} -123.9^\circ$ (*c* 0.96, CHCl₃); IR (CHCl₃) ν_{\max} 3544, 3028, 1736, 1668 cm⁻¹; ¹H NMR, see Table 3; EIMS (70 eV) m/z [M]⁺ 578 (12), 563 (5), 534 (7), 520 (5), 478 (32), 461 (12), 443 (20), 434 (22), 378 (100), 359 (22), 334 (41), 332 (36), 232 (16); HRDEIMS m/z 578.2703 (calcd for C₃₀H₄₂O₁₁, 578.2727).

Preparation of (R)- and (S)- α -Methoxy- α -(trifluoromethyl)phenylacetates **25 and **26**.** A solution of **1** (5 mg in 0.5 mL of CH₂Cl₂) was treated with CH₂Cl₂ solutions of dicyclohexylcarbodiimide (20 mg in 0.5 mL), 4-(dimethylamino)pyridine (3 mg in 0.5 mL), and either (R)- or (S)- α -methoxy- α -(trifluoromethyl)phenylacetic acid (10 mg in 0.5 mL), at room temperature for 24 h. In each case, the Mosher esters were purified by flash column chromatography on Si gel using hexanes–EtOAc (7:3) as eluent.

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Supporting Information Available: Isolation of known compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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