# Absolute Configuration of Trixanolides from Trixis pallida 

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#### Abstract

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 Mutisieae ${ }^{1}$ of the family Compositae, produces sesquiterpenes based on the trixane skeleton as characteristic secondary metabol ites. ${ }^{2,3}$ 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 $\mathbf{1}$ showed bands at 3450, 3060, 1740, and $1670 \mathrm{~cm}^{-1}$, which were indicative of hydroxyl, a double bond with at least one attached hydrogen, and carbonyl groups. When the ${ }^{1} \mathrm{H}$ 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 $\delta 5.22$ (dq, J $=6.5,5.0 \mathrm{~Hz}$ ), 2.68 (dq, J $=7.0,5.0 \mathrm{~Hz}$ ), 2.03 (s), 1.27 (d, J $=6.5 \mathrm{~Hz}$ ), and $1.22(\mathrm{~d}, \mathrm{~J}=7.0 \mathrm{~Hz})$ indicated that a 3-acetyloxy-2methylbutanoate moiety was present. An additional acetate group signal appeared at $\delta 2.08$. The position of each ester residue was evident after comparison with the reported ${ }^{1} \mathrm{H}$ 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 $\mathrm{H}-14$ signal appeared as a doublet between $\delta 5.40$ and 5.50 . Furthermore, when this signal appeared in the narrow $\delta 5.40-5.43$ 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 $\mathrm{H}-14$ doublet appeared at $\delta 5.46-5.50$ (see Tables $1-3$ ). When an acetate group was attached to $\mathrm{C}-14$, the $\mathrm{H}-14$ doublet is shifted downfield to near $\delta 6.00 .{ }^{4}$ Taking these considerations into account, the acetate group in compound $\mathbf{1}$ was placed at C-3, while the 3-acetyloxy-2-methylbutanoate residue was placed at $\mathrm{C}-14$. This was further confirmed in the HMBC spectrum, which showed a correlation of $\mathrm{H}-14$ and the 2-methylbutanoate carbonyl. The assignments of the ${ }^{1} \mathrm{H}$ NMR signals corresponding to each acetyloxy group were made after comparison with the spectra of monoacetylated compounds 2-4.

[^0]The individual assignments of the protons attached to $\mathrm{C}-1$ and C-8 were deduced from the minimum energy conformation of $\mathbf{1}$, 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 $\mathrm{H}-1 \alpha$ and $\mathrm{H}-10$ of 1.7 Hz , and the other one between $\mathrm{H}-2$ and $\mathrm{H}-4$ of 2.0 Hz , as well as an unobservable coupling between $\mathrm{H}-1 \alpha$ and $\mathrm{H}-2$, which form a ca. $80^{\circ}$ dihedral angle.
The ${ }^{13} \mathrm{C}$ NMR spectrum of $\mathbf{1}$ showed 24 signals, four of them corresponding to carbonyl groups, two to $\mathrm{sp}^{2}$ carbons, and five to $\mathrm{sp}^{3}$ oxygen-bearing carbons. A DEPT experiment provided evidence of five $\mathrm{CH}_{3}$, two $\mathrm{CH}_{2}$, 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 $\mathbf{1}$ displayed a [ M ] ${ }^{+}$at $\mathrm{m} / \mathrm{z} 478.1851$, in agreement with the molecular formula $\mathrm{C}_{24} \mathrm{H}_{30} \mathrm{O}_{10}$.

The absolute configuration of the trixane skeleton was published as deduced using the H oreau method. ${ }^{6}$ H owever, 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 ${ }^{1} \mathrm{H}$ NMR chemical shift differences for selected signals in the spectra of the (R)- and (S)- $\alpha$-methoxy- $\alpha$ (trifluoromethyl) phenylacetate (MTPA) esters (25 and 26) were compared. ${ }^{8}$ The resulting absolute configuration is enantiomeric to that previously reported ${ }^{6}$ and in agreement with the proposed biosynthetic pathway for the trixane skeleton ${ }^{9}$ via guaiene or cyperene, on the basis of the wellknown fact that the isopropyl group attached to C-7 in guaienes isolated from higher plants is always $\beta$-oriented. ${ }^{10}$ Consequently, the absolute configuration of the trixane skeleton is $2 \mathrm{~S}, 6 \mathrm{R}, 7 \mathrm{R}, 10 \mathrm{R}$.
After a thorough study of the ${ }^{1} \mathrm{H}$ 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

Table 1. ${ }^{1} \mathrm{H}$ NMR Data ( $\delta$ in ppm from TMS) of Compounds $\mathbf{1 - 6}{ }^{\text {a }}$

| proton | $1{ }^{\text {b }}$ | $2^{\text {¢ }}$ | $3^{\text {d }}$ | $4{ }^{\text {e }}$ | $5^{\text {d,f }}$ | $6^{\text {d,9 }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $1 \alpha$ | 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) |
| $1 \beta$ | 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 | $2.38 \mathrm{dr} \mathrm{dt}(7.0,2.0)$ | $2.39 \mathrm{br} \mathrm{dt}(7.0,2.0)$ | $2.39 \mathrm{br} \mathrm{dt}(7.0,2.0)$ | $2.39 \mathrm{br} \mathrm{dt}(7.0,2.0)$ | $2.38 \mathrm{br} \mathrm{dt}(7.0,2.0)$ | $2.38 \mathrm{br} \mathrm{dt}(7.0,2.0)$ |
| 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 ( $8.8,5.0$ ) |
| $8 \alpha$ | 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) |
| $8 \beta$ | 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) | $4.40 \mathrm{brt}(5.0)$ | $4.40 \mathrm{brt}(5.0)$ | $4.42 \mathrm{br} \mathrm{t} \mathrm{(5.0)}$ | $4.42 \mathrm{brt}(5.0)$ | $4.42 \mathrm{br} \mathrm{t} \mathrm{(5.0)}$ |
| 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 $300 \mathrm{MHz} ; \mathrm{CDCl}_{3}$ as solvent; J in parentheses in Hz. ${ }^{\text {b }}$ 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 ( $\mathrm{s}, \mathrm{OAc}$ ); 1.27 (d, 6.5, H-4'); 1.22 (d, 7.0, H-5'). cerythro-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'). $\mathrm{d}^{2}$ threo-3-OH-2-MeBu: 3.94 (quintet, $7.0, \mathrm{H}-3^{\prime}$ ); 2.55 (quintet, $7.0, \mathrm{H}-2^{\prime}$ ); 1.25 ( $\mathrm{d}, 7.0, \mathrm{H}-5^{\prime}$ ); 1.20 (d, $7.0, \mathrm{H}-4^{\prime}$ ). e 3-OHiVal: 2.59 (s, H-2'a,b); 1.34 (s, H-5'); 1.32 (s, H-4'). ${ }^{\text {fiVal: }} 2.21$ obscured; 2.08 (hept, 7.0, H-3'); 0.95 (d, 7.0, H-4'); 0.95 (d, 7.0, H-5'). ${ }^{\text {g }} 2-\mathrm{MeBu}: 2.41$ (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. ${ }^{1} \mathrm{H}$ NMR Data ( $\delta$ in ppm from TMS) of Compounds $\mathbf{7 - 1 2}{ }^{\text {a }}$

| proton | 7 7 ,f | $8^{\text {b,g }}$ | 9 ${ }^{\text {b,h }}$ | 10 ${ }^{\text {b,i }}$ | $11^{\text {ef }}$ | $12^{\text {d,g }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $1 \alpha$ | 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) |
| $1 \beta$ | 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) |
| $8 \alpha$ | 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) |
| $8 \beta$ | 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 $300 \mathrm{MHz} ; \mathrm{CDCl}_{3}$ as solvent; J in parentheses in Hz. ${ }^{\text {b }}$ 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
 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-OHiVal: 2.59 (s, H-2'a,b); 1.34 (s, H-5'); 1.32 (s, H-4'). ${ }^{\text {f } i V a l: ~} 2.21$ 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'). ${ }^{\text {h } 3-O A c-i V a l: ~}$ 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'). ${ }^{i} 3-\mathrm{OH}-\mathrm{iVal}: 2.56$ (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. ${ }^{1} \mathrm{H}$ NMR Data ( $\delta$ in ppm from TMS) of Compounds $\mathbf{1 3 - 1 7}{ }^{\text {a }}$

| proton | 13 ${ }^{\text {b,g }}$ | 14, ${ }^{\text {b,f }}$ | 15ch,i | $\mathbf{1 6}^{\text {d, h, }{ }^{\text {i }} \text {, }}$ | 17 ${ }^{\text {eh, }}$ i |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $1 \alpha$ | 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) |
| $1 \beta$ | 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 ( $6.8,2.0$ ) | $2.46 \mathrm{br} \mathrm{dt} \mathrm{(6.8}, \mathrm{2.0)}$ |
| 3 | 4.83 dd (3.0, 2.0) | 4.85 dd (3.0, 2.0) | $4.92 \mathrm{dd}(3.0,2.0)$ | $4.92 \mathrm{dd}(3.0,2.0)$ | 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) |
| $8 \alpha$ | 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) |
| $8 \beta$ | 1.87 dt (15.3, 6.0) | 1.87 dt (15.3, 6.0) | $2.01 \mathrm{dt}(16.0,6.0)$ | $2.01 \mathrm{dt}(16.0,6.0)$ | $2.01 \mathrm{dt}(16.0,6.0)$ |
| 9 | 4.41 br t (6.0) | 4.41 brt (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) | $2.42 \mathrm{dd}(6.0,1.5)$ | 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 $300 \mathrm{MHz} ; \mathrm{CDCl}_{3}$ as solvent; J in parentheses in Hz. ${ }^{\text {b }}$ 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 ( $\mathrm{s}, \mathrm{OAc}$ ); 1.27 (d, 6.5; H-4');1.22 (d, 7.0, H-5'). c erythro-3-OH-2-M eBu: 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'). ${ }^{\text {d }}$ 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-OHiVal: 2.59 (s, H-2'a, b); 1.34 (s, H-5'); 1.32 (s, H-4'). ${ }^{\text {f }}$ VVal: 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'). ${ }^{\text {h }} 3-\mathrm{OH}-\mathrm{iVal}: 2.56$ (d, 15.0, H-2'a); 2.52 (d, 15.0, H-2'b); 1.31 (s, H-5'); 1.29 (s, H-4'). ${ }^{\text {i } 2-M e B u: 2.37(d d q, ~ 7.0, ~ 7.0, ~ 7.0, ~ H-2 ') ; ~ 1.68 ~(d q, ~ 14.0, ~}$ 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 $\delta 2.59$ and the methyl group signals appeared at ca. $\delta 1.34$ 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 $\delta 2.53$ and the methyl groups gave two
singlets at ca. $\delta 1.31$ 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 ${ }^{2,3,11} \mathrm{H}-3$ and H-4 NMR chemical shifts have to be

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

| position | $\phi(\mathrm{deg})$ | J calcd $(\mathrm{Hz})$ | J obs $(\mathrm{Hz})$ |
| :---: | ---: | :---: | :---: |
| $1 \alpha, 2$ | -79 | 1.1 | a |
| $1 \beta, 2$ | 40 | 6.2 | 7.0 |
| 2,3 | 67 | 2.4 | 2.0 |
| 3,4 | -53 | 3.1 | 3.0 |
| $7,8 \alpha$ | -22 | 9.1 | 9.0 |
| $7,8 \beta$ | -142 | 8.3 | 5.0 |
| 7,14 | 164 | 8.6 | 9.0 |
| $8 \alpha, 9$ | -83 | 1.4 | a |
| $8 \beta, 9$ | 34 | 5.6 | 5.0 |
| 9,10 | -46 | 3.6 | 5.0 |

${ }^{\text {a }}$ Not observed.
reversed due to the observation of correlations of the lactone carbonyl ( $\mathrm{C}-12$ ) with $\mathrm{H}-4, \mathrm{H}-10$, and $\mathrm{Me}-13$

Compounds 2 and $\mathbf{3}$ were obtained as an inseparable 7:3 mixture. The ${ }^{1} \mathrm{H}$ NMR spectrum of this mixture showed signals similar to $\mathbf{1}$ (see Table 1), except those for the ester moiety at C-14. Signals for 3-hydroxy-2-methylbutanoate esters at $\delta 4.11,2.56,1.23$, and 1.22 for the major component and at $\delta 3.94,2.55,1.25$, 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 ${ }^{13} \mathrm{C}$ NMR spectrum of the mixture of 2 and $\mathbf{3}$ 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 ${ }^{1} \mathrm{H}$ NMR spectrum (see Tables 1 and 7). The relative stereochemistry of the 3-hydroxy-2methylbutanoate esters has already been studied, ${ }^{12}$ whereby it was found that the $\mathrm{H}-2$ and $\mathrm{H}-3$ 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 $\mathrm{H}-2$ and $\mathrm{H}-3$ was $65^{\circ}$ with a calculated J $=1.2 \mathrm{~Hz}$ for the erythro configuration, while for the threo configuration the dihedral angle was $175^{\circ}$ with a J $=10.8 \mathrm{~Hz}$. These data are in agreement with the fact that the H-3 signal appeared as a double 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 ${ }^{1} \mathrm{H}$ NMR spectra of compounds $\mathbf{4 - 1 4}$ showed the characteristic signals of a 3,14-diester-9-hydroxytrixanol ide skeleton, as described in detail for the above compounds. The nature of the different ester functions was deduced from the ${ }^{1} \mathrm{H}$ NMR spectra and, in the case of $\mathbf{4}$ and 7 , also from ${ }^{13} \mathrm{C}$ 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 trixanolides isolated from T. grisebachii, ${ }^{3}$ and comparison with literature data. ${ }^{2}$ They were further corroborated by detecting long-range ${ }^{13} \mathrm{C}-{ }^{1} \mathrm{H}$ interactions in HMBC measurements. Additionally, the HREIMS of 4 showed a [M] ${ }^{+}$at $\mathrm{m} / \mathrm{z} 436.1725$, in agreement with the molecular formula $\mathrm{C}_{22} \mathrm{H}_{28} \mathrm{O}_{9}$.

When the NMR spectra of compounds published as either $\mathbf{4 a} \mathbf{- j}$ or $\mathbf{4 a} \mathbf{a}^{\prime}-\mathbf{j}$ ' in the T . grisebachii report ${ }^{3}$ were evaluated under the above considerations, the correct structures were revised as $\mathbf{4 a} \mathbf{- j}$. Thus, the spectrum of compound $\mathbf{1 0}$, isolated in this work, mainly differed from that of compound $\mathbf{4 e}$, isol ated from $T$. grisebachii, in the chemical shift of the $\mathrm{H}-14$ signal. In $\mathbf{1 0}$ this signal appeared at $\delta 5.42$, indicating that the 3-acetyloxy-2-methyl butanoate residue was at $\mathrm{C}-14$, while in $\mathbf{4 e}$ the $\mathrm{H}-14$ signal appeared at $\delta$ 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 $\mathbf{1 0}$ the erythro-3-acetyloxy-2-methyl butanoate and 3-hydroxy-iso-


| carbon | $1{ }^{\text {b }}$ | $\mathbf{2}^{\text {c }}$ | $3^{\text {d }}$ | $4{ }^{\text {e }}$ | 7c,f | $\mathbf{1 6}^{\text {d,g,h }}$ | 18e, | 20 ${ }^{\text {c,j }}$ | 21 ${ }^{\text {e, }}$ | 23c, | 24c,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 |  |  |  |  |  |  |  |

[^1]Table 6. Selected ${ }^{1} \mathrm{H}$ NMR Data for (R)- and (S)-MTPA Esters 25 and 26

| H | $\mathbf{2 5}(\mathrm{R})$ | $\mathbf{2 6}(\mathrm{S})$ | $\Delta(\mathrm{S}-\mathrm{R})$ |
| :---: | :---: | :---: | :---: |
| 7 | 2.23 | 2.46 | +0.23 |
| $8 \alpha$ | 2.30 | 2.32 | +0.02 |
| $8 \beta$ | 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 | $\delta$ | multiplicity | compounds |
| :---: | :---: | :---: | :---: | :---: |
| acetyloxy acetyloxy of erythro-3-OAc-2-MeBu | C-3 | 2.08 | s (3H) | 1,2,3,4,25,26 |
|  |  | 2.03 |  |  |
|  | C-14 |  | s (3H) | $\begin{array}{r} \mathbf{1 , 8 , 9 , 1 0 , 1 3} \\ 14,25,26 \end{array}$ |
| threo-3-OAc-2-MeBu 3-OAc-iVal | C-3 | 2.06 | S (3H) | 8,12,21,24 |
|  | C-3 | 2.00 | S (3H) | 9,18,22,23 |
| $3-\mathrm{OAc}-\mathrm{iVal}$ | C-3 | 1.53 | $s$ (6H) | 9,18,22,23 |
| erythro-3-OAc-2-MeBu | C-14 | 1.27 | d (3H) | $\begin{array}{r} 1,8,9,10,13 \\ 14,25,26 \end{array}$ |
|  | C-3 | 1.22 | d (3H) |  |
| threo-3-OAc-2-MeBu |  | 1.26 | d (3H) | 8,12,21,24 |
|  |  | 1.17 | d (3H) |  |
| $3-\mathrm{OH}-\mathrm{iVal}$ | C-3 | 1.31 | $s$ (3H) | 10,15,16,17 |
|  |  | 1.29 | $s$ (3H) |  |
|  | C-14 | 1.34 | $s(3 \mathrm{H})$ | $\begin{gathered} \text { 4,11,17,18 } \\ 19,21 \end{gathered}$ |
|  |  | 1.32 | $s(3 \mathrm{H})$ |  |
| erythro-3-OH-2-MeBu | C-14 | 1.23 | d (3H) | $\begin{gathered} 2,7,15,20,23 \\ 24 \end{gathered}$ |
|  |  | 1.22 | d (3H) |  |
| threo-3-OH-2-MeBu | C-14 | 1.25 | d (3H) | $\begin{gathered} 3,5,6,12,16 \\ 22 \end{gathered}$ |
|  |  | 1.20 | d (3H) |  |
| iVal <br> 2-MeBu | $\begin{aligned} & C-3 \\ & C-3 \end{aligned}$ | 0.95 | d (6H) | $\begin{aligned} & 5,7,11,14 \\ & 6,13,19,20 \end{aligned}$ |
|  |  | 1.13 | d (3H) |  |
|  |  | 0.91 | $t$ (3H) |  |
|  | C-9 | 1.16 | d (3H) | 15,16,17 |

valerate residues were at C-14 and C-3, respectively, while in $4 \mathbf{e}$ they occurred at the C-3 and C-14 positions, respectively.

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

The ${ }^{1} \mathrm{H}$ NMR spectra of the triesters $\mathbf{1 5 - 1 7}$ showed in all cases the signals corresponding to $\mathrm{H}-3$ and $\mathrm{H}-9$ at $\delta 4.92$ and 5.27, respectively, whileH-14 appeared at $\delta 5.48,5.47$, and 5.50 in 15, 16, and 17, respectively. Compound 15 showed the $\mathrm{CH}_{2}-2^{\prime} \mathrm{AB}$ system of the 3 -hydroxy-iso-valerate ester residue centered at $\delta 2.54$, indicating that it was located at C-3. The erythro-3-hydroxy-2-methylbutanoate signals indi cated 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 $\mathbf{1 6}$ and $\mathbf{1 7}$ with those of $\mathbf{1 5}$ (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, whilea 3-hydroxy-iso-val erate ester was found at C-14 in 17. Finally, HREIMS of the three isomeric substances (15-17) displayed [M] at $\mathrm{m} / \mathrm{z} 578.2729$, 578.2713, and 578.2703, respectively, in agreement with the molecular formula $\mathrm{C}_{30} \mathrm{H}_{42} \mathrm{O}_{11}$.

Table 8


| compd | R | $\mathrm{R}^{\prime}$ | R" |
| :---: | :---: | :---: | :---: |
| 1 | erythro-3-OAc-2-MeBu | Ac | H |
| 2 | erythro-3-OH-2-MeBu | Ac | H |
| 3 | threo-3-OH-2-MeBu | Ac | H |
| 4 | $3-\mathrm{OH}-\mathrm{iVal}$ | Ac | H |
| 5 | threo-3-OH-2-MeBu | iVal | H |
| 6 | threo-3-OH-2-MeBu | 2-MeBu | H |
| 7 | erythro-3-OH-2-MeBu | iVal | H |
| 8 | erythro-3-OAc-2-MeBu | threo-3-OAc-2-MeBu | H |
| 9 | erythro-3-OAc-2-MeBu | 3-OAc-iVal | H |
| 10 | erythro-3-OAc-2-MeBu | 3-OH-iVal | H |
| 11 | $3-\mathrm{OH}-\mathrm{iVal}$ | iVal | H |
| 12 | threo-3-OH-2-MeBu | threo-3-OAc-2-MeBu | H |
| 13 | erythro-3-OAc-2-MeBu | 2-MeBu | H |
| 14 | erythro-3-OAc-2-MeBu | iVal | H |
| 15 | erythro-3-OH-2-MeBu | 3-OH-iVal | 2-MeBu |
| 16 | threo-3-OH-2-MeBu | $3-\mathrm{OH}-\mathrm{iVal}$ | 2-MeBu |
| 17 | $3-\mathrm{OH}-\mathrm{iVal}$ | $3-\mathrm{OH}-\mathrm{iVal}$ | 2-MeBu |
| 18 | $3-\mathrm{OH}-\mathrm{iVal}$ | 3-OAc-iVal | H |
| 19 | $3-\mathrm{OH}-\mathrm{iVal}$ | 2-MeBu | H |
| 20 | erythro-3-OH-2-MeBu | 2-MeBu | H |
| 21 | $3-\mathrm{OH}-\mathrm{iVal}$ | threo-3-OAc-2-MeBu | H |
| 22 | threo-3-OH-2-MeBu | 3-OAc-iVal | H |
| 23 | erythro-3-OH-2-MeBu | 3-OAc-iVal | H |
| 24 | erythro-3-OH-2-MeBu | threo-3-OAc-2-MeBu | H |
| 25 | erythro-3-OAc-2-MeBu | Ac | R-MTPA |
| 26 | erythro-3-OAc-2-MeBu | Ac | S-MTPA |

## Experimental Section

General Experimental Procedures. Melting points were obtained on a Fisher-J ohns melting point apparatus and are uncorrected. Optical rotations were performed on a PerkinElmer 241 polarimeter. IR spectra were recorded on a PerkinElmer 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 HewlettPackard 5989A spectrometers. HRMS were measured on a VG 7070 high-resolution mass spectrometer at the UCR Mass Spectrometry F acility, 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 $\mathrm{C}_{18}(5 \mu \mathrm{~m}, 10 \times 250 \mathrm{~mm})$. 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 $60^{\circ} \mathrm{C}$, diluted with 40 mL of $\mathrm{H}_{2} \mathrm{O}$, and extracted successively with hexane $(2 \times 50 \mathrm{~mL})$ and $\mathrm{CH}_{2} \mathrm{Cl}_{2}(2 \times 50 \mathrm{~mL})$. Evaporation under vacuum of the hexane extract gave known compounds as outlined in the Supporting Information. Evaporation under reduced pressure of the $\mathrm{CH}_{2^{-}}$ $\mathrm{Cl}_{2}$ extract gave 1.95 g of residue, which was column chromatographed over Si gel using $\mathrm{CHCl}_{3}$ with increasing amounts of EtOAc ( $0.20 \%$ ) to give 164 fractions. Fractions 33-46 (116 mg ) were processed by HPLC ( $\mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}, 2: 1,2 \mathrm{~mL} \mathrm{~min}^{-1}$ ) to give $\mathbf{1}\left(25.1 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 7 \mathrm{~min}\right.$ ) and a 1:1 mixture of $\mathbf{8}$ and 9 ( 14.0 $\left.\mathrm{mg}, \mathrm{t}_{\mathrm{R}} 20 \mathrm{~min}\right)$. F ractions $81-118$ were combined, and a portion $(280 \mathrm{mg})$ was processed by HPLC ( $\mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}, 64: 36,2 \mathrm{~mL}$ $\mathrm{min}^{-1}$ ) to give 4 ( $11.6 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 3 \mathrm{~min}$ ); a 3:1 mixture of 10 and

18 (12.8 mg, $\mathrm{t}_{\mathrm{R}} 6 \mathrm{~min}$ ); a 18:13:11:8 mixture of $11,5,19$, and 6 ( $52.8 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 10 \mathrm{~min}$ ); a 3:1 mixture of 7 and $20\left(2.0 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 12\right.$ $\mathrm{min}) ; 17\left(4.1 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 16 \mathrm{~min}\right) ; 15\left(2: 1 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 17 \mathrm{~min}\right)$, and 16 (28.4 mg, $t_{R} 18 \mathrm{~min}$ ). Fractions 119-141 (230 mg) were processed by HPLC ( $\mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}, 31: 19,2 \mathrm{~mL} \mathrm{~min}^{-1}$ ) to give a $2: 1$ mixture of 3 and 4 ( 8.1 mg , $\mathrm{t}_{\mathrm{R}} 8 \mathrm{~min}$ ); a $3: 2$ mixture of 18 and 21 ( $15.1 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 9 \mathrm{~min}$ ); a 7:3 mixture of 6 and 5 ( 2.1 mg , $\left.t_{R} 15 \mathrm{~min}\right)$; a $3: 1$ mixture of 7 and $20\left(37.2 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 16 \mathrm{~min}\right)$, and 16 ( $0.5 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 25 \mathrm{~min}$ ). Fractions $142-164$ (193 mg) were processed by HPLC as before to give a 7:3 mixture of 2 and 3 ( $16.9 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 5 \mathrm{~min}$ ); a 7:5:5:3 mixture of $12,22,21$, and 18 ( $61.2 \mathrm{mg}, \mathrm{t}_{\mathrm{R}} 11 \mathrm{~min}$ ), and a $3: 2 \mathrm{mixture}$ of $\mathbf{2 3}$ and $\mathbf{2 4}$ ( 51.7 mg , $\mathrm{t}_{\mathrm{R}} 13 \mathrm{~min}$ ).
(2S,3S,4S,6R,7R,9R,10R,11R,14S)-3-Acetyloxy-9-hydroxy-14-(erythro-2-methyl-3-acetyloxybutanoyloxy)-14,15-ep-oxytrix-5(15)-en-4,12-olide (1): gum; $[\alpha]_{589}-36.5^{\circ},[\alpha]_{578}$ $-38.5^{\circ},[\alpha]_{546}-44.2^{\circ},[\alpha]_{436}-67.3^{\circ},[\alpha]_{365}-96.1^{\circ}$ (с 0.52, $\mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3060,1740,1670 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ 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 $\mathrm{C}_{24} \mathrm{H}_{30} \mathrm{O}_{10}, 478.1839$ ).

3-Acetyloxy-9-hydroxy-14-(erythro-2-methyl-3-hydroxy-butanoyloxy)-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, $\mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3020,1735,1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ 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-hydroxy-butanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide(3): gum (in 3:7 mixture with 2); $[\alpha]_{589}-30.8^{\circ},[\alpha]_{578}-32.5^{\circ},[\alpha]_{546}$ $-35.0^{\circ},[\alpha]_{336}-55.5^{\circ},[\alpha]_{365}-78.6^{\circ}$ (c $1.17, \mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3020,1735,1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ 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-methylbutanoyl-oxy)-14,15-epoxytrix-5(15)-en-4,12-olide (4): gum; $[\alpha]_{589}$ $-29.7^{\circ},[\alpha]_{578}-31.1^{\circ},[\alpha]_{546}-35.1^{\circ},[\alpha]_{436}-51.3^{\circ},[\alpha]_{365}-72.9^{\circ}$ ( $\mathrm{c} 0.74, \mathrm{CHCl}_{3}$ ); IR (KBr) $\nu_{\max } 3450,3030,1735,1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ 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 $\mathrm{C}_{22} \mathrm{H}_{28} \mathrm{O}_{9}, 436.1733$ ).

3-(3-Methylbutanoyloxy)-9-hydroxy-14-(threo-2-meth-yl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12olide (5): mp 198-202 ${ }^{\circ} \mathrm{C}$ (in 3:7 mixture with 6); IR (KBr) $v_{\max } 3450,3030,1735,1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ NMR, see Table 1; EIMS $(20 \mathrm{eV}) \mathrm{m} / \mathrm{z}[\mathrm{M}]^{+} 478$ (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-meth-yl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12 olide (6): mp 198-202 ${ }^{\circ} \mathrm{C}$ (in 7:3 mixture with 5); IR (KBr) $\nu_{\max } 3450,3030,1735,1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ NMR, see Table 1; EIMS $(20 \mathrm{eV}) \mathrm{m} / \mathrm{z}[\mathrm{M}]^{+} 478$ (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-meth-yl-3-hydroxybutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12olide (7): $\mathrm{mp} 198-202{ }^{\circ} \mathrm{C}$ (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 $3.69, \mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3030,1735 ; 1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR, see Tables 2 and 5, respectively; EIMS $(20 \mathrm{eV}) \mathrm{m} / \mathrm{z}[\mathrm{M}]^{+} 478$ (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-ep-oxytrix-5(15)-en-4,12-olide (8): $\mathrm{mp} 124-127^{\circ} \mathrm{C}$ (in mixture $1: 1$ with 9 ); $[\alpha]_{589}-33.1^{\circ},[\alpha]_{578}-35.2^{\circ},[\alpha]_{546}-38.6^{\circ},[\alpha]_{436}$ $-63.4^{\circ},[\alpha]_{365}-97.9^{\circ}\left(\mathrm{c} 1.45, \mathrm{CHCl}_{3}\right)$; IR (KBr) $v_{\max } 3450,3060$,

1740, $1670 \mathrm{~cm}^{-1}$; 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-epoxy-trix-5(15)-en-4,12-olide (9): mp 124-127 ${ }^{\circ} \mathrm{C}$ (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, $\mathrm{CHCl}_{3}$ ); IR (KBr) $\nu_{\text {max }} 3450,3060,1740$, $1670 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ 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-epoxy-trix-5(15)-en-4,12-olide (10): $\mathrm{mp} 130.5-133{ }^{\circ} \mathrm{C}$ (in 3:1 mixture with 18); $[\alpha]_{589}-34.5^{\circ},[\alpha]_{578}-36.2^{\circ},[\alpha]_{546}-39.6^{\circ}$, $[\alpha]_{336}-60.3^{\circ},[\alpha]_{365}-84.5^{\circ}\left(\mathrm{c} 0.56, \mathrm{CHCl}_{3}\right) ;$ IR (KBr) $v_{\max } 3450$, 3030, 1735, $1665 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ 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-meth-yl-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 , $\mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3050,1735,1710,1665 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ 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-meth-yl-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 , $\left.\mathrm{CHCl}_{3}\right) ; \mathrm{IR}(\mathrm{KBr}) v_{\max } 3450,3050,1735,1710,1665 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ NMR, seeTable 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-methylbu-tanoyloxy)-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, $\mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3030,1735,1665 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ 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 (cal cd for $\mathrm{C}_{30} \mathrm{H}_{42} \mathrm{O}_{11}$, 578.2727).

3-(3-Hydroxy-3-methylbutanoyloxy)-9-(2-methylbu-tanoyloxy)-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, $\mathrm{CHCl}_{3}$ ); IR (KBr) $v_{\max } 3450,3030,1735,1665 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ 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 $\mathrm{m} / \mathrm{z} 578.2713$ (calcd for $\mathrm{C}_{30} \mathrm{H}_{42} \mathrm{O}_{11}, 578.2727$ ).
3,14-Bis(3-hydroxy-3-methylbutanoyloxy)-9-(2-meth-ylbutanoyloxy)-14,15-epoxytrix-5(15)-en-4,12-olide (17): gum; $[\alpha]_{589}-47.9^{\circ},[\alpha]_{578}-50.0^{\circ},[\alpha]_{546}-55.2^{\circ},[\alpha]_{436}-87.5^{\circ}$, $[\alpha]_{365}-123.9^{\circ}\left(\mathrm{c} 0.96, \mathrm{CHCl}_{3}\right)$; IR ( $\mathrm{CHCl}_{3}$ ) $\nu_{\text {max }} 3544,3028,1736$, $1668 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ 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 $\mathrm{m} / \mathrm{z} 578.2703$ (calcd for $\mathrm{C}_{30} \mathrm{H}_{42} \mathrm{O}_{11}, 578.2727$ ).
Preparation of (R)- and (S)- $\alpha$-Methoxy- $\alpha$-(trifluoromethyl)phenylacetates 25 and 26. A solution of $\mathbf{1}$ ( 5 mg in 0.5 mL of $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ ) was treated with $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ solutions of dicyclohexylcarbodiimide ( 20 mg in 0.5 mL ), 4-(dimethylamino)pyridine ( 3 mg in 0.5 mL ), and either (R)- or (S)- $\alpha-$ methoxy- $\alpha$-(trifluoromethyl)phenylacetic acid ( 10 mg in 0.5 mL ), at room temperature for 24 h . In each case, the M osher 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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[^1]:    a $75.4 \mathrm{MHz} ; \mathrm{CDCl}_{3}$ as solvent. ${ }^{\text {b }}$ 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-M eBu: 173.9 (C-1'); 45.8 (C-2'); 68.1 (C-3'); 20.1 (C-4'); 10.8 (C-5'). ${ }^{\text {d }}$ 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-iVal: 171.0 (C-1'); 46.7 (C-2'); 69.2 (C-3'); 29.3 (C-4'); $29.2\left(\mathrm{C}-5^{\prime}\right) .{ }^{\mathrm{f}} \mathrm{i} V \mathrm{Val}: 172.0\left(\mathrm{C}-1^{\prime}\right) ; 43.2\left(\mathrm{C}-2^{\prime}\right) ; 25.6$
     26.7 (C-3'); 16.0 (C-4'); 11.5 (C-5'). ${ }^{\text {i }} 3-\mathrm{OAc-iVal:} 170.5$ (C-1'); 44.4 (C-2'); 79.2 (C-3'); 26.5 (C-4'); 26.5 (C-5'); 169.2, 22.3 (OAc). ${ }^{\text {j } 2-M e B u: ~}$ 175.6 (C-1'); 41.0 (C-2'); 26.4 (C-3'); $16.2\left(\mathrm{C}-4^{\prime}\right) ; 11.4\left(\mathrm{C}-5^{\prime}\right) .{ }^{\mathrm{k}}$ threo-3-OAc-MeBu: 172.8 (C-1'); $44.3\left(\mathrm{C}-2^{\prime}\right) ; 70.8\left(\mathrm{C}-3^{\prime}\right) ; 17.6\left(\mathrm{C}-4^{\prime}\right) ; 12.2$ (C-5'); 170.4, 21.1 (OAc).

