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On the Biotransformation of *ent*-Trachylobane to *ent*-Kaur-11-ene Diterpenes

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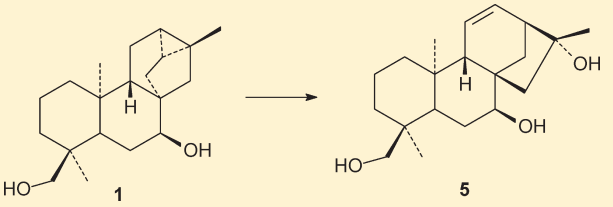
Supporting Information

ABSTRACT: The microbiological transformation of trachinodiol (**1**) by the fungus *Mucor plumbeus* afforded the corresponding 1α, 2α, 3α, and 17-hydroxy derivatives (**2**−**4** and **6**), respectively. 7β,16α,18-Trihydroxy-*ent*-kaur-11-ene (sicanatriol) (**5**) was also obtained in this feeding. The biotransformation of **1** to give **5** by this fungus may occur by enzymatic abstraction of a hydrogen atom, allylic to the cyclopropane ring, and subsequent cleavage of this ring. This route is similar to that postulated by us in plants of the genus *Sideritis*, where *ent*-trachylobane and *ent*-kaur-11-ene diterpenes coexist. This study confirms that hydroxylation of diterpenes by *M. plumbeus* occurs preferably at ring A carbons.



The isolation, chemistry, and microbiological transformation of diterpenes have been a matter of our interest for the past several years. During these studies, we have obtained *ent*-kaur-11-ene derivatives, together with other diterpenes, from species of the genus *Sideritis* endemic to the Canary Islands. Thus, sidendrodiol (16α,18-dihydroxy-*ent*-kaur-11-ene) was isolated from *S. dendrochahorra*,[1] its 18-monoacetate from *S. argosphacelus* var. *spicata*, *S. discolor*, *S. soluta*,[2] *S. ferrensis*,[3] and *S. massoniana* var. *pumila*.[4] Sicanadiol (7β,16α-dihydroxy-*ent*-kaur-11-ene) was found in *S. canariensis* var. *pannosa*,[5] and its 7-monoacetate was isolated from *S. ferrensis*.[3] Sicanatriol (**5**) has been isolated only as its 7,18-diacetate (**5b**) from *S. argosphacelus* var. *spicata*.[2] These *ent*-kaur-11-ene diterpenes are rare in nature, having been isolated only from plants containing *ent*-trachylobane diterpenes.[5]

We have proposed that diterpenes with an *ent*-trachylobane skeleton may be the biogenetic precursors of *ent*-kaur-11-ene derivatives. Thus, enzymatic abstraction of a hydrogen at C-11 in **1** assists the cleavage of the cyclopropane ring, giving a carbenium ion at C-16, which is then neutralized with an OH anion, probably of water origin, to form the alcohol **5** (Scheme 1).[1,6] The sesquiterpene (−)-maaliol **7** also has a cyclopropane ring. Recently, its microbiological transformation into the maalioxide derivatives **9** and **10** by the fungus *Mucor plumbeus* was reported.[7] We later indicated that the formation of these two metabolites may occur via an intermediate (**8**), which can be formed by enzymatic abstraction of an allylic proton to the cyclopropane, with concomitant cleavage of this ring (Scheme 2).[8] Consequently, we decided to attempt biotransformation of trachinodiol (**1**)[9] by this fungus, with the aim of obtaining the corresponding *ent*-kaur-11-ene derivative **5**. We have previously carried out microbiological transformations of diterpenes by *M. plumbeus*, with a view to developing models to explain the hydroxylation of these compounds by this fungus.[10−14]

RESULTS AND DISCUSSION

Incubation of trachinodiol (**1**) with *M. plumbeus* for 6 days led to the compounds **2**−**6** (Scheme 3). The chromatographic separation of these five trihydroxylated metabolites was difficult. Thus, for ease of separation we decided to reduce the polarity of these products by acetylation of mixtures containing them. The triacetates **2a**−**6a** were then obtained, using column chromatography (CC) and HPLC. Partial acetylation also occurred, which permitted the isolation of diacetates **3b**, **4b**, and **5b** and the monoacetates **4c** and **5c**.

The high-resolution mass spectrum of triacetate **2a** was in accordance with the molecular formula $C_{26}H_{38}O_6$. In comparison with the corresponding spectra of the diacetate **1a**, the ^{13}C NMR spectrum of **2a** showed the resonance of a new oxymethylene group at δ_C 82.4, and the ^1H NMR spectrum the presence of a new proton geminal to an oxygenated function at δ_H 4.62 (dd, J = 11.2 and 4.2 Hz). The resonance of this proton was typical of a β-axial hydrogen at C-1 or C-3. The HMBC correlations of this hydrogen with C-9 and C-20 indicated that it was located at C-1. The configuration of this center was confirmed by the NOE effect between H-1 and H-5 in the NOESY experiment. All of the data were in accordance with the structure **2a**. Thus, the original metabolite obtained in the fermentation was 1α,7β,18-trihydroxy-*ent*-trachylobane (**2**).

The triacetate of metabolite **3a** was an isomer of **2a**. Its ^1H NMR spectra showed the resonance of the new geminal proton of the new acetoxyl group at δ_H 4.74 (dd, J = 11.8 and 4.9 Hz), the coupling constants being similar to those of H-1 in **2a**. Consequently, this acetate group was assigned to the C-3(α)

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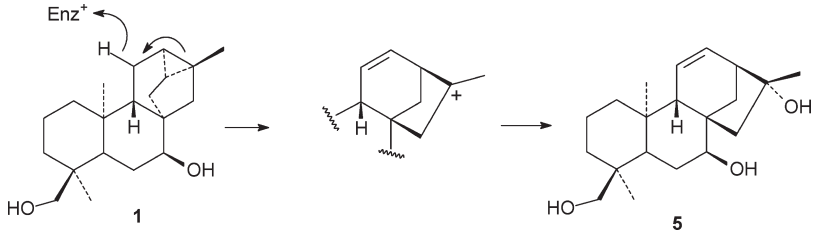
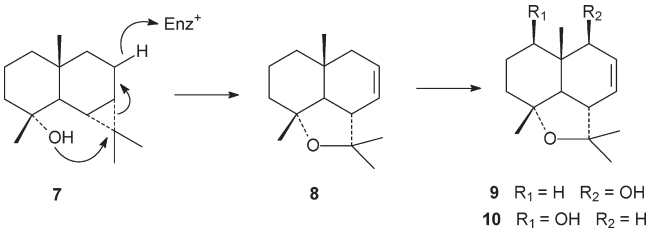
Scheme 1

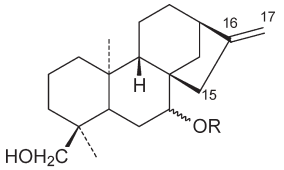


Scheme 2



position. The HMBC spectrum confirmed this with correlations of H-3 to C-4 and C-19 and of C-3 to H-18 and H-19. The NOESY experiment showed cross-peaks of H-3 with H-5 and H-1(β). Moreover, the C-3 oxymethine resonated at δ_C 74.1 in **3a**, a value similar to that observed in 3α,7β,18-triacetoxy-*ent*-kaur-16-ene (δ_C 73.8).[10] Therefore, the triol (**3**) formed in the incubation must have the structure 3α,7β,18-trihydroxy-*ent*-trachylobane.

Metabolite (**4**), obtained in this microbiological transformation, was assigned the structure 2α,7β,18-trihydroxy-*ent*-trachylobane. This compound, after the acetylation of fractions containing it, was isolated as its triacetate **4a**, its 7β,18-diacetate **4b**, and its 18-monoacetate **4c**. Their mass spectra indicated that metabolite **4** was an isomer of **2** and **3** with the molecular formula $C_{20}H_{32}O_3$. The new OH introduced in metabolite **4** was assigned to the 2α position with the following considerations: In the ^{1}H NMR spectrum of the 7β,18-diacetate (**4b**), the hydrogen geminal to the new alcohol resonated at δ_H 4.18, while in the triacetate **4a** it appeared at δ_H 5.16. The resonance was a quintuplet in both cases, with similar coupling constants (4.8 and 5.0 Hz, respectively). These data were typical of a 2β-equatorial hydrogen having symmetrical couplings with the 1- and 3-hydrogens in a chair A-ring. Moreover, the relatively low value of the C-2 resonance, at δ_C 67.1, is also characteristic of an oxymethylene carbon situated between two methylene groups.



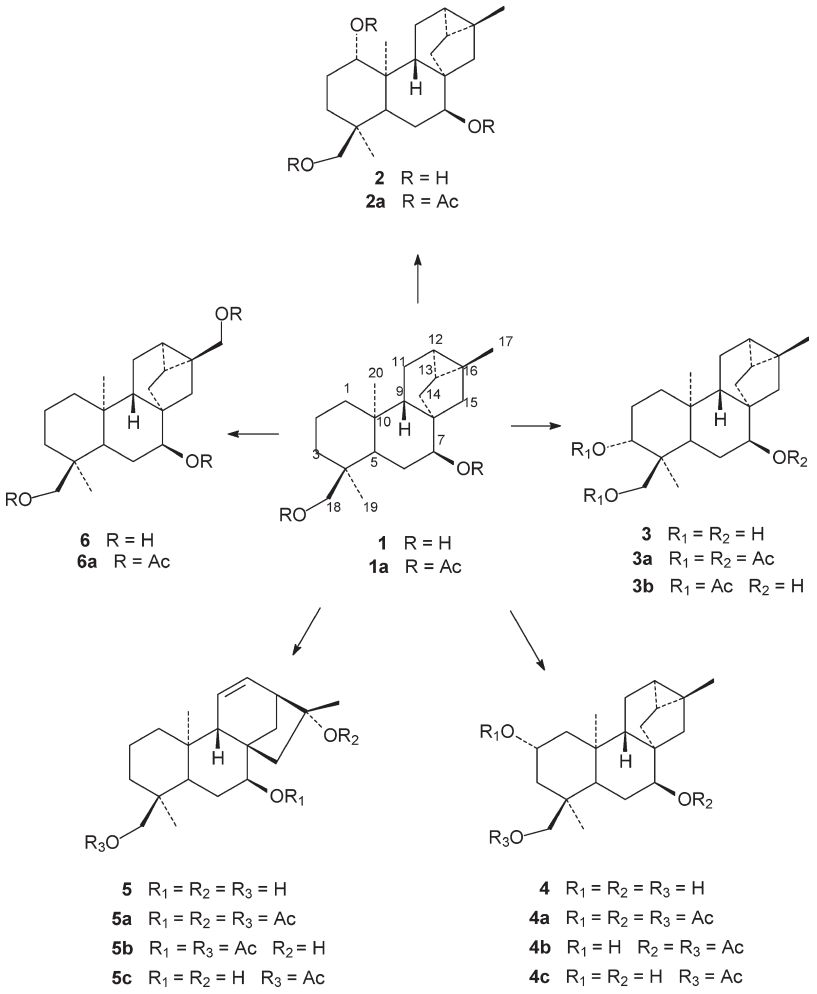
In the HREIMS of the triacetate **5a**, the molecular ion appeared at *m*/*z* 446.2668, in accordance with the molecular formula $C_{26}H_{38}O_6$. Its ^{1}H NMR spectrum lacked the cyclopropane hydrogens of the substrate and showed two new coupled vinylic signals at δ_H 5.59 (dd, *J* = 9.8 and 3.9 Hz) and 5.92 (ddt, *J* = 9.8, 6.6, and 1.2 Hz) and deshielding of the C-17 methyl at δ_H 1.55. The corresponding carbons bearing these substituents appeared at δ_C 127.4, 131.3, and 92.9, respectively. The values of the last proton and carbon resonances indicated that the C-17 methyl group was geminal to an oxygenated function. These facts suggested the opening of the cyclopropane ring of **1** and the possible formation of an *ent*-atis-13-ene or an *ent*-kaur-11-ene derivative. The latter skeleton was chosen considering the observed coupling constants of the vinylic protons, H-11 and H-12, and the COSY experiment. The α-orientation of the acetoxyl group at C-16 was assigned taking into consideration the NOE between H-17 and H-12. In the HMBC spectrum, in addition to the correlations observed for rings A and B, there appeared cross-peaks of H-11 with C-8, C-9, and C-13, of H-15 with C-7, C-8, C-9, C-16, and C-17, and of H-17 with C-15 and C-16. Consequently, the structure of compound **5** was 7β,16α,18-trihydroxy-*ent*-kaur-11-ene. This metabolite was also obtained in the acetylated fractions as its diacetate **5b** and its monoacetate **5c**. The former was identical with the 7β,18-diacetate of sicanatriol (**5b**), which had been isolated from *S. argosphacelus* var. *spicata*.[2]

The most polar of the compounds isolated in this biotransformation was the trachylobane derivative **6**, which was obtained as the triacetate **6a** after acetylation of the corresponding fractions. In comparison with the diacetate of trachinodiol (**1a**) the new acetate showed, in the NMR spectra, the absence of a methyl group and the presence of a new acetoxymethylene. The signals of the latter group, δ_C 68.9 and δ_H 4.00 and 4.12 (each 1H, d, *J* = 11.6 Hz), appeared correlated in the HSQC experiment. In the HMBC spectrum, cross-peaks of H-17 with C-12, C-13, C-15, and C-16 and of C-17 with H-13 and H-15 were observed, indicating that the new acetoxy group was at C-17. Therefore, metabolite **6** was determined to be 7β,17,18-trihydroxy-*ent*-trachylobane.

The following conclusions were made from the biotransformation of trachinodiol (**1**) by *M. plumbeus*:

1. The preference for equatorial hydroxylation at C-3 by *M. plumbeus* was observed as in the biotransformation of other diterpenes.[9−13] Furthermore, a C-2(α) hydroxylation also occurred in the incubations of some compounds of this type, such as 18-hydroxymanoyl oxide,[13] dehydroabietanol, and teideadiol.[14] These facts confirm that hydroxylation of similar diterpenes by this fungus occurs preferably at carbons of ring A.
2. The biotransformation of trachinodiol (**1**) to give sicanatriol (**5**) by *M. plumbeus* must occur by enzymatic abstraction of an H-11, in a way similar to that postulated by us in plants of the *Sideritis* genus, where *ent*-trachylobane and *ent*-kaur-11-ene diterpenes coexist.[1,6]

Scheme 3. Biotransformation of 1 by *M. plumbeus* Affording 2−6



3. Incubation of *ent*-trachylobane diterpenes with this fungus represents a new way to obtain *ent*-kaur-11-ene derivatives, which are difficult to prepare by chemical methods. The biotransformation of *ent*-trachyloban-18-oic acid by *Rhizopus arrhizus* also gave rearranged derivatives by cleavage of the cyclopropane ring.[15]
4. In this feeding with *M. plumbeus*, the functionalization of C-11 in substrate **1** to form **5** is likely favored because this carbon is allylic to the cyclopropane ring. This is analogous to the functionalization of C-15, allylic to the 16,17-double bond in *ent*-kaur-16-ene diterpenes, which was observed by us in the biotransformation of candicandiol (**11**) and epicandicandiol (**12**) by this fungus.[10] Therefore, the same enzyme may be responsible for both bioreactions.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500.13 and 125.03 MHz, respectively, with a Bruker AMX-500 spectrometer. Solvent was $CDCl_3$ unless otherwise stated. Mass spectra were taken at 70 eV (probe) in a Micromass Autospec spectrometer. HPLC was performed using a Beckman System Gold 125P column. Purification by HPLC was achieved using a silica gel column (Ultrasphere Si 5 μm, 10 × 250 mm). Dry column chromatography was performed on Merck 0.040−0.063 mm silica gel. The compounds were crystallized from petroleum ether−EtOAc except where otherwise indicated. The fungal strain was *Mucor plumbeus* F11 (CECT-20422).

*Trachinodiol (**1**):* ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.59 (1H, dt, J = 7.7 and 2.7 Hz, H-12), 0.68 (3H, s, H-19), 0.80 (1H, td, J = 12.8 and 4.0 Hz, H-1β), 0.88 (1H, dd, J = 7.7 and 3.2 Hz, H-13), 0.96 (3H, s, H-20), 1.15 (3H, s, H-17), 1.16 (1H, m overlapped with H-17, H-3), 1.20 (1H, ddd, J = 11.8, 3.3, and 1.7 Hz, H-14), 1.40 and 1.51 (each 1H, d, J = 11.0 Hz, H-15), 1.42 (1H, m overlapped with H-15, H-2), 1.48 (1H, br d overlapped with H-15, J = 12.8 Hz, H-1α), 1.50 (2H, m overlapped with H-15, H-6 and H-9), 1.57 (1H, br d, J = 13.8 Hz, H-3), 1.59 (1H, m overlapped with H-3, H-2), 1.67 (1H, ddd, J = 14.6, 6.5, and 2.7 Hz, H-11), 1.73 (1H, dd, J = 11.7 and 2.9 Hz, H-5), 1.91 (1H, ddd, J = 14.6, 11.5, and 2.7 Hz, H-11), 1.95 (1H, d, J = 11.8 Hz, H-14), 2.93 and 3.45 (each 1H, d, J = 11.4 Hz, H-18), 3.52 (1H, t, J = 2.8 Hz, H-7); EIMS m/z 304 $[M]^+$ (8), 286 (75), 255 (100), 241 (13), 213 (9), 199 (19), 185 (17), 173 (23), 159 (21), 145 (23); HREIMS $[M]^+$ m/z 304.2399 (calcd for $C_{20}H_{32}O_2$, 304.2402).

*Trachinodiol diacetate (**1a**):* ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.58 (1H, dt, J = 7.7 and 2.8 Hz, H-12), 0.79 (1H, td, J = 12.8 and 4.0 Hz, H-1β overlapped with H-19), 0.79 (3H, s, H-19), 0.88 (1H, dd, J = 7.7 and 3.1 Hz, H-13), 0.97 (3H, s, H-20), 1.12 (3H, s, H-17), 1.33 and 1.43 (each 1H, d, J = 11.4 Hz, H-15), 1.68 (1H, ddd, J = 14.6, 6.7, and 2.8 Hz, H-11α), 1.90 (1H, ddd, J = 14.6, 11.6, and 2.8 Hz, H-11β), 2.01 (1H, d, J = 12.1 Hz, H-14), 2.03 and 2.04 (each 3H, s, -OAc), 3.64 and 3.68 (each 1H, d, J = 11.4 Hz, H-18), 4.74 (1H, t, J = 2.6 Hz, H-7).

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Incubation of Trachinodiol (1). *M. plumbeus* was grown in shake culture at 25 °C for two days in 20 conical flasks (250 mL), each containing 75 mL of sterile medium comprising (per L) glucose (80 g), NH_4NO_3 (0.48 g), KH_2PO_4 (5 g), $MgSO_4$ (1 g), and trace elements solution (2 mL). The trace elements solution contained (per 100 mL) $Co(NO_3)_2$ (0.01 g), $CuSO_4$ (0.015 g), $ZnSO_4$ (0.16 g), $MnSO_4$ (0.01 g), and $(NH_4)_6Mo_7O_{24}$ (0.01 g). The substrate **1** (150 mg) dissolved in EtOH (4 mL) and Tween 80 (2 drops) was evenly distributed between the flasks, and the incubation was continued for six days. The broth was filtered and the culture filtrate extracted with EtOAc. The mycelium was treated with liquid nitrogen, crushed in a mortar, and extracted with EtOAc. Both extracts were combined and separated into "acid" and "neutral" fractions with aqueous $NaHCO_3$. The acid fraction was methylated with CH_2N_2.

The biotransformation gave, in the neutral fraction, starting material (115 mg), 1α,7β,18-trihydroxy-*ent*-trachylobane (**2**, 1 mg), 3α,7β,18-trihydroxy-*ent*-trachylobane (**3**, 2 mg), 2α,7β,18-trihydroxy-*ent*-trachylobane (**4**, 3 mg), 7β,16α,18-trihydroxy-*ent*-kaur-11-ene (**5**, 3 mg), and 7β,17,18-trihydroxy-*ent*-trachylobane (**6**, 5 mg). Compounds **2**−**6** were characterized as their acetates. No transformation products were observed in the acid fraction.

1α,7β,18-Triacetoxy-ent-trachylobane (***2a***): gum; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.56 (1H, br d, *J* = 7.8 Hz, H-12), 0.82 (3H, s, H-19), 0.89 (1H, dd, *J* = 7.8 and 3.2 Hz, H-13), 1.10 (3H, s, H-17), 1.17 (3H, s, H-20), 1.32 (2H, m overlapped with H-15, H-3, and H-14), 1.33 and 1.44 (each 1H, d, *J* = 11.6 Hz, H-15), 1.71 (1H, dd, *J* = 12.5 and 2.0 Hz, H-5), 1.81 (2H, m, H-9 and H-11), 1.94 (1H, d, *J* = 11.7 Hz, H-14), 2.02, 2.03, and 2.05 (each 3H, s, OAc), 2.08 (1H, m, H-11), 3.63 and 3.69 (each 1H, d, *J* = 11.3 Hz, H-18), 4.62 (1H, dd, *J* = 11.2 and 4.2 Hz, H-1), 4.69 (1H, t, *J* = 2.8 Hz, H-7); EIMS *m/z* 446 $[M]^+$ (0.3), 386 (11), 326 (33), 266 (50), 251 (33), 232 (26), 215 (22), 211 (21), 203 (19), 197 (18); HREIMS $[M]^+$ *m/z* 446.2669 (calcd for $C_{26}H_{38}O_6$, 446.2668).

3α,7β,18-Triacetoxy-ent-trachylobane (***3a***): colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.60 (1H, dt, *J* = 7.7 and 2.6 Hz, H-12), 0.80 (3H, s, H-19), 0.90 (1H, dd, *J* = 7.7 and 3.2 Hz, H-13), 1.00 (3H, s, H-20), 1.04 (1H, td, *J* = 13.3 and 3.8 Hz, H-1β), 1.13 (3H, s, H-17), 1.32 (1H, br d, *J* = 11.8 Hz, H-14), 1.35 and 1.45 (each 1H, d, *J* = 11.6 Hz, H-15), 1.74 (1H, dd, *J* = 12.7 and 2.1 Hz, H-5), 1.94 (1H, ddd, *J* = 14.6, 11.6, and 2.8 Hz, H-11), 1.99 (1H, d, *J* = 11.8 Hz, H-14), 2.01, 2.02, and 2.03 (each 3H, s, OAc), 3.55 and 3.87 (each 1H, d, *J* = 11.8 Hz, H-18), 4.74 (1H, dd, *J* = 11.8 and 4.9 Hz, H-3), 4.75 (1H, br s overlapped with H-3, H-7); EIMS *m/z* 446 $[M]^+$ (3), 386 (26), 326 (64), 266 (100), 251 (54), 225 (18), 197 (23), 185 (40), 157 (24), 133 (31); HREIMS $[M]^+$ *m/z* 446.2654 (calcd for $C_{26}H_{38}O_6$, 446.2668).

3α,18-Diacetoxy-7β-hydroxy-ent-trachylobane (***3b***): colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.59 (1H, br d, *J* = 7.7 Hz, H-12), 0.80 (3H, s, H-19), 0.88 (1H, dd, *J* = 7.7 and 3.0 Hz, H-13), 0.98 (3H, s, H-20), 1.02 (1H, td, *J* = 13.9 and 3.8 Hz, H-1β), 1.16 (3H, s, H-17), 1.21 (1H, br d, *J* = 11.6 Hz, H-14), 1.42 (1H, d, *J* = 11.3 Hz, H-15), 1.61 (1H, m, H-2), 1.66 (1H, ddd, *J* = 14.6, 6.5, and 2.6 Hz, H-11), 1.74 (1H, m, H-2), 1.79 (1H, dd, *J* = 9.7 and 4.9 Hz, H-6), 1.92 (1H, d, *J* = 11.6 Hz, H-14), 1.93 (1H, ddd, *J* = 14.6, 11.6, and 3.0 Hz, H-11), 2.03 and 2.06 (each 3H, s, OAc), 3.52 (1H, t, *J* = 2.8 Hz, H-7), 3.55 and 4.03 (each 1H, d, *J* = 11.8 Hz, H-18), 4.82 (1H, dd, *J* = 11.7 and 4.7 Hz, H-3); EIMS *m/z* 404 $[M]^+$ (2), 386 (10), 326 (64), 266 (100), 251 (50), 237 (11), 225 (18), 210 (14), 197 (24), 185 (29); HREIMS $[M]^+$ *m/z* 404.2552 (calcd for $C_{24}H_{36}O_5$, 404.2563).

2α,7β,18-Triacetoxy-ent-trachylobane (***4a***): colorless crystal, mp 146−148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.60 (1H, dt, *J* = 7.8 and 2.6 Hz, H-12), 0.90 (1H, dd, *J* = 7.8 and 3.1 Hz, H-13), 0.97 (3H, s, H-19), 1.13 (3H, s, H-17), 1.23 (3H, s, H-20), 1.26 (1H, m, H-1), 1.32 (1H, br d, *J* = 11.7 Hz, H-14), 1.34 and 1.42 (each 1H, d, *J* = 11.5 Hz, H-15), 1.74 (1H, ddd, *J* = 14.6, 6.7, and 2.6 Hz, H-11), 1.79 (1H, dd, *J* = 14.6 and 3.9 Hz, H-6), 1.94 (1H, ddd, *J* = 14.6, 11.4, and 2.6 Hz, H-11), 2.02 (1H, overlapped with OAc, H-14), 2.03 (6H, s, OAc), 2.05 (3H, s, OAc), 3.66 and 3.72 (each 1H, d, *J* = 11.1 Hz, H-18), 4.77 (1H, t, *J* = 2.6 Hz, H-7), 5.16 (1H, quint, *J* = 4.8 Hz, H-2); EIMS *m/z* 446 $[M]^+$ (5), 386 (25), 326 (100), 266 (85), 251 (38), 237 (13), 225 (18), 211 (17), 197 (18), 185 (33); HREIMS $[M]^+$ *m/z* 446.2671 (calcd for $C_{26}H_{38}O_6$, 446.2668).

7β,18-Diacetoxy-2α-hydroxy-ent-trachylobane (***4b***): colorless crystal, mp 132−134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.61 (1H, dt, *J* = 7.8 and 2.6 Hz, H-12), 0.90 (1H, dd, *J* = 7.8 and 3.1 Hz, H-13), 1.01 (3H, s, H-19), 1.13 (3H, s, H-17), 1.30 (3H, s, H-20), 1.32 (2H, m overlapped with H-15, H-1 and H-14), 1.34 and 1.43 (each 1H, d, *J* = 11.5 Hz, H-15), 1.79 (1H, ddd, *J* = 14.6, 6.7, and 2.5 Hz, H-11), 1.97 (1H, ddd, *J* = 14.6, 11.5, and 3.0 Hz, H-11), 2.03 and 2.05 (each 3H, s, OAc), 2.04 (1H, m overlapped with OAc, H-14), 3.67 and 3.75 (each 1H, d, *J* = 11.0 Hz, H-18), 4.18 (1H, quint, *J* = 5.0 Hz, H-2), 4.76 (1H, t, *J* = 2.7 Hz, H-7); EIMS *m/z* 404 $[M]^+$ (7), 344 (100), 326 (22), 284 (46), 269 (46), 266 (45), 251 (37), 229 (36), 197 (27), 185 (39); HREIMS $[M]^+$ *m/z* 404.2562 (calcd for $C_{24}H_{36}O_5$, 404.2563).

18-Acetoxy-2α,7β-dihydroxy-ent-trachylobane (***4c***): colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.60 (1H, dt, *J* = 7.7 and 2.6 Hz, H-12), 0.89 (1H, dd, *J* = 7.7 and 3.1 Hz, H-13), 1.02 (3H, s, H-19), 1.16 (3H, s, H-17), 1.20 (1H, ddd, *J* = 11.6, 3.1, and 1.6 Hz, H-14), 1.28 (1H, m overlapped with H-20, H-1), 1.29 (3H, s, H-20), 1.42 and 1.52 (each 1H, d, *J* = 11.2 Hz, H-15), 1.77 (1H, dd, *J* = 14.3 and 3.7 Hz, H-6), 1.78 (1H, m overlapped with H-6, H-11), 1.96 (1H, m overlapped with H-14, H-11), 1.97 (1H, d, *J* = 11.6 Hz, H-14), 2.06 (3H, s, OAc), 3.52 (1H, t, *J* = 2.7 Hz, H-7), 3.54 and 4.03 (each 1H, d, *J* = 11.0 Hz, H-18), 4.19 (1H, quint, *J* = 4.9 Hz, H-2); EIMS *m/z* 362 $[M]^+$ (1), 344 (12), 326 (10), 284 (26), 269 (40), 266 (34), 251 (22), 229 (27), 211 (16), 197 (27); HREIMS $[M]^+$ *m/z* 362.2465 (calcd for $C_{22}H_{34}O_4$, 362.2457).

7β,16α,18-Triacetoxy-ent-kaur-11-ene (***5a***): colorless crystal, mp 104−106 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (3H, s, H-19), 0.98 (3H, s, H-20), 1.06 (1H, td, *J* = 13.0 and 3.9 Hz, H-1β), 1.34 (2H, m, H-3), 1.50 (1H, m, H-2), 1.53 (2H, m, H-14 and H-15), 1.55 (3H, s, H-17), 1.58 (1H, m overlapped with H-17, H-6), 1.65 (1H, m, H-2), 1.72 (1H, dd, *J* = 12.8 and 1.7 Hz, H-5), 1.95 (3H, s, OAc), 2.05 and 2.06 (each 3H, s, OAc), 2.06 (1H, m overlapped with OAc, H-15), 2.92 (1H, dd, *J* = 6.8 and 3.4 Hz, H-13), 3.66 and 3.74 (each 1H, d, *J* = 11.1 Hz, H-18), 4.83 (1H, t, *J* = 2.8 Hz, H-7), 5.59 (1H, dd, *J* = 9.8 and 3.9 Hz, H-11), 5.92 (1H, ddt, *J* = 9.8, 6.8, and 1.2 Hz, H-12); ^{1}H NMR (500 MHz, C_6D_6) δ 0.61 (3H, s, H-19), 0.80 (3H, s, H-20), 0.83 (1H, br t, *J* = 12.9 Hz, H-1β), 1.22 (2H, m, H-3 and H-6), 1.31 (3H, m, H-2, H-3, and H-6), 1.43 (1H, m, H-2), 1.51 (1H, m overlapped with H-14, H-1α), 1.55 (1H, dd, *J* = 11.4 and 3.7 Hz, H-14), 1.59 (1H, dd, *J* = 15.3 and 1.7 Hz, H-15), 1.69 (3H, s, H-17), 1.68, 1.73, and 1.78 (each 3H, s, OAc), 2.39 (1H, dd, *J* = 15.3 and 1.1 Hz, H-15), 3.05 (1H, dd, *J* = 6.7 and 3.7 Hz, H-13), 3.70 and 3.77 (each 1H, d, *J* = 11.1 Hz, H-18), 5.05 (1H, t, *J* = 2.8 Hz, H-7), 5.36 (1H, dd, *J* = 9.7 and 3.9 Hz, H-11), 5.76 (1H, ddt, *J* = 9.7, 6.7, and 1.3 Hz, H-12); EIMS *m/z* 446 $[M]^+$ (1), 404 (1), 386 (9), 344 (100), 326 (63), 266 (28), 251 (21), 226 (64), 213 (77), 197 (16); HREIMS $[M]^+$ *m/z* 446.2671 (calcd for $C_{26}H_{38}O_6$, 446.2668).

7β,18-Diacetoxy-16α-hydroxy-ent-kaur-11-ene (***5b***): gum; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (3H, s, H-19), 0.98 (3H, s, H-20), 1.05 (1H, td, *J* = 13.0 and 3.8 Hz, H-1β), 1.33 (3H, s, H-17), 1.34 (2H, m, H-3), 1.73 (1H, dd, *J* = 12.6 and 1.7 Hz, H-5), 1.76 (2H, m, H-1α and H-6), 1.81 (1H, br d, *J* = 2.7 Hz, H-9), 1.85 (1H, d, *J* = 10.9 Hz, H-14), 2.04 and 2.05 (each 3H, s, OAc), 2.24 (1H, dd, *J* = 6.6 and 3.5 Hz, H-13), 3.66 and 3.74 (each 1H, d, *J* = 11.1 Hz, H-18), 4.90 (1H, t, *J* = 2.7 Hz, H-7), 5.52 (1H, dd, *J* = 9.8 and 4.0 Hz, H-11), 5.92 (1H, ddt, *J* = 9.8, 6.6, and 1.2 Hz, H-12); EIMS *m/z* 344 $[M - C_2H_4O_2]^+$ (28), 326 (84), 266 (39), 251 (68), 226 (45), 213 (61), 197 (37), 183 (48), 169 (32), 157 (40); HREIMS $[M - C_2H_4O_2]^+$ *m/z* 344.2343 (calcd for $C_{22}H_{32}O_3$, 344.2351).

18-Acetoxy-7β,16α-dihydroxy-ent-kaur-11-ene (***5c***): colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (3H, s, H-19), 0.97 (3H, s, H-20),

Table 1. ^{13}C NMR Data (δ) for Compounds 1, 1a, 2a, 3a, 4b, 5a, and 6a

position	1	1a	2a	3a	4b	5a	5a[b]	6a
1	38.7	38.4	82.4	36.6	45.1	38.6	38.8	38.3
2	17.5	17.2	24.0[a]	22.6	67.1	17.4	17.7	17.2
3	35.2	35.7	33.3	74.1	41.3	35.4	35.8	35.6
4	36.9	35.9	35.7	40.3	35.6	36.1	36.2	35.9
5	39.1	42.3	40.2	40.2	40.2	40.6	41.1	42.1
6	27.0	25.2	24.3[a]	24.7	25.6	24.7	25.1	25.2
7	76.0	77.9	77.6	77.6	77.6	79.4	79.1	77.5
8	45.4	43.9	44.3	43.8	43.9	45.5	45.9	43.6
9	47.7	48.3	48.0	48.0	48.4	57.9	58.4	48.1
10	38.1	38.1	42.1	37.8	38.4	38.1	38.2	38.2
11	19.3	19.3	20.8	19.3	19.2	127.4	128.6	19.0
12	20.6	20.3	20.4	20.2	20.2	131.3	131.6	19.0
13	24.1	23.8	23.8	23.8	23.8	45.5	46.0	22.4
14	32.7	32.5	32.8	32.4	32.2	32.4	32.8	31.9
15	45.3	45.1	45.1	44.9	45.1	52.3	52.7	40.9
16	23.1	22.8	23.2	22.8	22.7	92.9	92.6	26.8
17	20.4	20.3	20.1	20.3	20.2	20.7	20.6	68.9
18	71.0	72.5	71.4	64.9	72.2	72.5	72.5	72.4
19	17.7	17.3	17.4	12.8	19.7	17.3	17.3	17.3
20	14.8	14.8	12.2	15.2	17.9	17.6	17.6	14.9

[a] Interchangeable values. [b] Solvent C_6D_6.

1.04 (1H, td, J = 12.9 and 3.8 Hz, H-1β), 1.35 (3H, s, H-17), 1.75 (1H, br d, J = 12.9 Hz, H-1α), 1.78 (1H, br d, J = 10.8 Hz, H-14), 1.82 (1H, dd, J = 4.0 and 1.3 Hz, H-9), 1.86 (1H, dd, J = 10.7 and 3.8 Hz, H-6), 1.93 (1H, dd, J = 14.6 and 1.1 Hz, H-15), 2.08 (3H, s, OAc), 2.21 (1H, br s, H-13), 3.47 and 4.13 (each 1H, d, J = 11.1 Hz, H-18), 3.74 (1H, t, J = 2.8 Hz, H-7), 5.52 (1H, dd, J = 9.8 and 4.0 Hz, H-11), 5.90 (1H, ddt, J = 9.8, 6.6, and 1.3 Hz, H-12); EIMS m/z 344 $[M - H_2O]^+$ (48), 326 (20), 266 (31), 251 (87), 238 (21), 226 (22), 213 (33), 197 (18), 183 (19), 162 (41); HREIMS $[M - H_2O]^+$ m/z 344.2347 (calcd for $C_{22}H_{32}O_3$, 344.2351).

*7β,17,18-Triacetoxy-ent-trachylobane (**6a**):* colorless crystal, mp 110−112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 (3H, s, H-19), 0.81 (1H, td, J = 13.0 and 3.6 Hz, H-1β), 0.87 (1H, dt, J = 8.0 and 2.6 Hz, H-12), 0.98 (3H, s, H-20), 1.21 (1H, dd, J = 8.0 and 3.2 Hz, H-13), 1.32 (3H, m, H-3 and H-14), 1.43 (1H, m, H-2), 1.48 (1H, d, J = 11.7 Hz, H-15), 1.51 (1H, m overlapped with H-6 and H-15, H-1α), 1.53 (1H, dd, J = 13.4 and 2.1 Hz, H-6), 1.66 (1H, dd, J = 13.4 and 3.2 Hz, H-6), 1.72 (1H, ddd, J = 14.8, 6.4, and 2.6 Hz, H-11), 1.94 (1H, ddd, J = 14.8, 11.6, and 2.8 Hz, H-11), 2.03, 2.04, and 2.05 (each 3H, s, OAc), 2.05 (1H, m overlapped with OAc, H-14), 3.66 and 3.68 (each 1H, d, J = 11.3 Hz, H-18), 4.00 and 4.12 (each 1H, d, J = 11.6 Hz, H-17), 4.75 (1H, t, J = 2.7 Hz, H-7); EIMS m/z 446 $[M]^+$ (5), 386 (16), 344 (8), 326 (100), 311 (8), 266 (50), 251 (29), 237 (12), 197 (17), 183 (18); HREIMS $[M]^+$ m/z 446.2663 (calcd for $C_{26}H_{38}O_6$, 446.2668).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^{1}H and ^{13}C NMR spectra of compounds **2a**, **3a**, **4b**, **5a**, and **6a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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