Full Papers

Biotransformation of the Diterpenes Epicandicandiol and Candicandiol by *Mucor plumbeus*

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The microbiological transformation of the diterpene epicandicandiol (**1**) with *Mucor plumbeus* gave foliol (**3**), sideritriol (**5**), and 7β,16α,17,18-tetrahydroxy-*ent*-kaurane (**7**), while the incubation of candicandiol (**2**) gave 7α,9β,18-trihydroxy-*ent*-kaur-16-ene (**10**), canditriol (**11**), and 7α,16α,17,18-tetrahydroxy-*ent*-kaurane (**12**). Thus, the main difference observed in both feedings, resulting from the spatial change in the orientation of the 7-hydroxyl, from *axial* in the substrate **1** to *equatorial* in **2**, was the formation of a 3α- and a 9β-hydroxylated derivative, respectively.

For several years, we have been interested in the study of the microbiological transformation of terpenoids with fungi. One of our aims was the biotransformation of *ent*-kaurene derivatives by *Gibberella fujikuroi*, the fungus that produces the gibberellin plant hormones. These studies have mainly been directed to preparing new gibberellin analogues and to obtaining information about the substrate specificity of the enzymes involved in the biosynthesis of gibberellins.[1−3] In this research we have used as substrates natural *ent*-kaurene diterpenes or synthetic derivatives prepared from them. To continue with these studies and to prepare new substrates, we have initiated the biotransformation of *ent*-kaurene derivatives with *Mucor plumbeus*, a fungus that has been used for the functionalization of unactivated carbons in sesqui-[4−9] and diterpenes.[10,11] On the other hand, these biotransformations should permit the development of models to rationalize diterpenoid microbiological hydroxylation by *M. plumbeus*. In this context, we have previously carried out incubations of labdane diterpenes with this fungus.[12−14]

In this work we describe the results of the biotransformation of the two *ent*-kaurene derivatives, epicandicandiol (7β,18-dihydroxy-*ent*-kaur-16-ene) (**1**) and candicandiol (7α,18-dihydroxy-*ent*-kaur-16-ene) (**2**), which are epimeric at C-7. Thus, we can also determine whether a spatial change in the orientation of the hydroxyl group at this carbon, from β-axial in the first to α-equatorial in the second, has any effect on the biotransformation.

Compounds **1** and **2** had been obtained from *Sideritis candicans*[15,16] and other species of this genus.[17] We have assigned the ^{1}H and ^{13}C NMR spectra of these substrates utilizing 2D NMR data (COSY, HSQC, and HMBC). The carbon spectra of **1** and **2** are included in Table 1. Previously we had reported the spectrum of **1** in deuterated pyridine.[18] Italian and Turkish authors have reported the isolation of candicandiol (**2**) from *Sideritis huber-morathii*,[19] but its ^{13}C NMR spectrum was different from that of **2**. Therefore, the structure had been erroneously assigned. We have now identified it with epicandicandiol (**1**). Its spectrum, conveniently reassigned, was identical with that of **1**.

Results and Discussion

The incubation of epicandicandiol (**1**) with *M. plumbeus* for 6 days afforded three hydroxylation products, **3**, **5**, and **7**. The metabolite **5** was obtained as its triacetate **6**, while **7** was isolated as the triacetate **8** and the tetraacetate **9**, by acetylation of the fractions containing them.

The least polar compound **3** was isolated as colorless needles in 7.3% yield. Its MS was in accordance with the formula $C_{20}H_{32}O_3$, indicating that a new oxygen was introduced in the molecule of **1**. This must form a part of a secondary hydroxyl group, because a new geminal proton was observed in the ^{1}H NMR spectrum resonating at δ 3.68 (dd, J = 8.9, 7.7 Hz). The form of resonance was typical of a β-axial hydrogen at C-1, C-3, or C-12, but the effect produced by the new alcohol in the resonance of the hydroxymethylene group (δ 3.35 and 3.59, each 1H, d) with respect to the substrate (δ 2.94 and 3.46) permitted it to be located at C-3(α). This location was confirmed by assignments of its ^{13}C NMR spectrum, and that of its acetate **4**, using 2D NMR data. Thus, the structure of this metabolite was determined as 3α,7β,18-trihydroxy-*ent*-kaur-16-ene. This compound has been isolated previously as a natural product from *Sideritis linearifolia* and named foliol (**3**).[20]

The second compound obtained in this biotransformation with a 0.93% yield was **5**, identified as its triacetate **6** by acetylation of the fraction containing it. Spectroscopic data of **6** were the same as those described in the literature for the triacetate of sideritriol.[21] We have now assigned the ^{1}H and ^{13}C NMR spectra of this compound utilizing two-dimensional NMR data. The corresponding alcohol sideritriol (**5**) had been isolated from the corollas of *Sideritis sicula*.[21,22]

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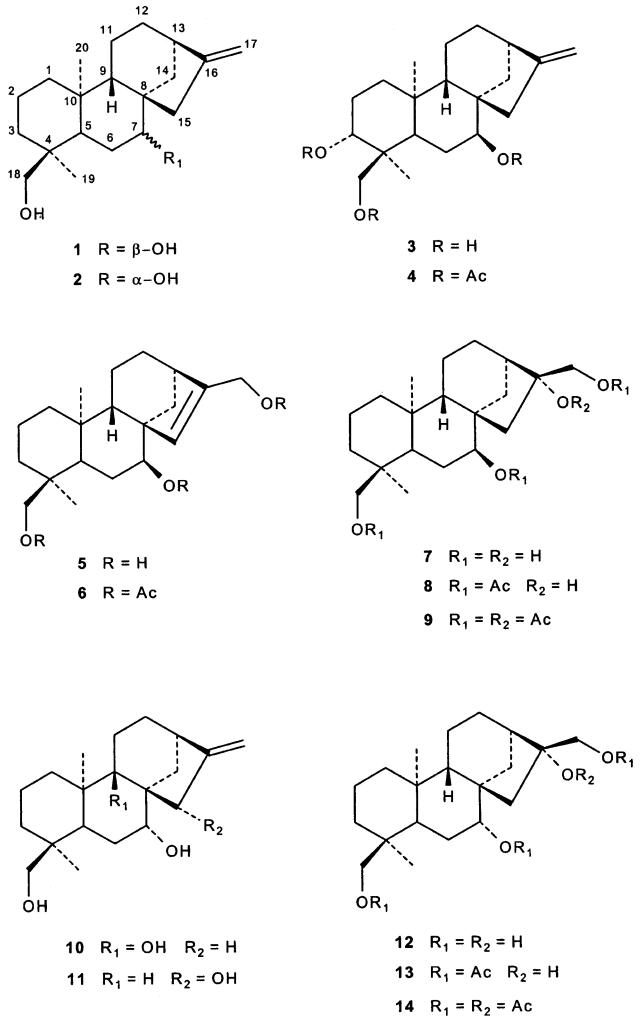
Table 1. ^{13}C NMR Data of Compounds **3**, **4**, **6**, **8**−**11**, **13**, and **14**

carbon	1	2	3	4	6	8	9	10	11	13	14
1	39.9	39.6	38.5	37.7	39.4	39.4	39.3	31.9	39.6	39.5	39.5
2	17.7	17.8[a]	26.9	23.0[a]	18.0	17.6	17.7[a]	17.8	17.8	17.6[a]	17.6[a]
3	35.1	34.9	74.1	73.8	35.4	35.4	35.3	34.7	34.8	35.6	35.6
4	37.0	37.3	41.6	40.3	36.0	36.1	36.0	37.5	37.3	36.2	36.2
5	38.5	45.8	38.6	39.6	41.3	41.5	41.4	38.8	44.9	46.4	46.3
6	26.9	29.0	27.0	24.2[a]	23.8	24.7	24.5	29.4	28.5	25.7	25.6
7	77.3	74.7	77.0	79.2	77.0	79.6	79.6	70.5	73.6	76.1	76.4
8	48.3	49.7	48.2	46.7	51.6	47.2	46.7	54.6	52.4	48.9	48.4
9	50.7	55.2	50.4	50.8	43.9	51.6	51.3	78.5	52.9	55.7	55.6
10	38.9	39.1	39.0	38.6	39.1	38.9	38.0	43.7	39.1	38.9	38.9
11	17.7	17.9[a]	17.8	18.0	17.0	17.6	17.5[a]	30.9	17.8	17.7[a]	17.8[a]
12	33.6	33.5	33.5	33.2	25.1	26.2	25.9	34.1	33.1	26.2	25.9
13	43.7	43.2	43.6	43.4	41.3	45.8	43.0	41.6	41.2	44.8	42.0
14	38.6	30.5	38.5	38.0	41.8	35.7	35.6	30.0	27.5	29.4	29.3
15	45.1	43.1	45.0	44.9	133.3	49.4	47.6	37.3	84.0	47.7	45.3
16	155.0	154.9	154.6	154.0	142.1	79.3	89.9	154.6	160.2	79.2	89.9
17	103.6	103.4	103.8	103.8	62.1	68.2	63.1	103.4	107.8	68.3	63.4
18	71.0	71.6	68.3	64.9	72.3	72.4	72.2	71.9	71.2	72.1	72.1
19	17.7	17.4	11.7	12.8	17.1	17.2	17.2	17.6	17.5	17.4	17.4
20	18.0	18.1	68.3	17.7	17.5	17.9	17.8	19.7	18.1	18.2	18.2

[a] These values can be interchanged.

In addition to **3** and **5**, a more polar metabolite **7** was isolated from the *M. plumbeus* culture. The fraction containing it was acetylated and chromatographed to give the triacetate **8** and the tetraacetate **9** in 13.8% and 1.3% yield, respectively. The NMR spectrum of **8** was similar to that for **1** with the exception of carbon and proton signals at positions 16 and 17. ^1H and ^{13}C spectra of **8** showed the presence of a new acetoxymethylene group and a tertiary hydroxyl. This observation was consistent with the absence of vinylic hydrogen signals of H-17 and the appearance in its ^1H NMR spectrum of a 2H singlet at δ 4.18. The existence of the tertiary hydroxyl group was reflected in the ^{13}C NMR spectrum by the resonance of a quaternary carbinol carbon at δ 79.3. The MS spectrum of **8** was in full accordance with these assignments with $[M]^+$ at *m/z* 464.2786 ($C_{26}H_{40}O_7$). On the other hand, HSQC and HMBC spectral analysis showed that the tertiary carbinol carbon was three-bond correlated with the protons at C-14 and C-12, while the methylene carbon at C-17 correlated with H-13 and H-15, thus confirming the hydroxylation pattern. The stereochemistry C-16(R) for **8** followed from the observation of a NOE effect between H-13 and H-17. This interaction is only possible if the acetoxymethylene group has a β-orientation. Thus, the structure of this triacetate was determined as 7β,17,18-triacetoxy-16α-hydroxy-*ent*-kaurane. A study of 2D NMR data of the tetraacetate **9** confirmed this assignment. The two H-17 appear now as a pair of doublets at δ 4.43 and 4.86 (J = 12.5 Hz); C-16 resonates at 89.9 and C-17 at δ 63.1. These carbon chemical shifts are in accordance with a 16α-OH stereochemistry.[23,24] Therefore, the original metabolite obtained in the feeding was the corresponding alcohol 7β,16α,17,18-tetrahydroxy-*ent*-kaurane (**7**).

The incubation of candicandiol (**2**) with *M. plumbeus* for 6 days afforded the hydroxylation products **10**−**12**. This last compound was obtained as the triacetate **13** and the tetraacetate **14** by acetylation of the fractions containing it.



The least polar metabolite **10** was isolated as colorless crystals in 1.3% yield. The molecular formula was found to be $C_{20}H_{30}O_2$ on the basis of an ion peak at *m/z* 302.2194 $[M - H_2O]^+$ and other spectroscopic data. The ^1H and ^{13}C NMR spectra of **10** were similar to those of **2** except for resonances attributed to rings B and C. The ^{13}C NMR spectrum revealed the presence of an additional quaternary carbinol signal at δ 78.5. This hydroxyl group was determined from the HMBC spectrum to be located at C-9 due to the presence of correlations between this carbon (δ 78.5) and the methyl group protons at H-20 (δ 1.17, s), the methylene protons at H-11 (δ 1.73, m), H-14 (1.26, m and 1.98, dd), and H-15 (δ 2.43, dt, and 2.46, d), and the methine proton of H-5 (δ 1.89, dd). This was further supported by the expected downfield shifts for C-8 ($\Delta\delta$ = 4.9 ppm), C-10 ($\Delta\delta$ = 4.6 ppm), and C-11 ($\Delta\delta$ = 13.0 ppm)

and the upfield shift, due to γ-gauche effects, for C-1 ($\Delta\delta$ = 7.7 ppm), C-5 ($\Delta\delta$ = 7.0 ppm), C-7 ($\Delta\delta$ = 4.2 ppm), and C-15 ($\Delta\delta$ = 7.8 ppm) relative to those of **2**. Moreover, shift displacements in the carbon resonances of C-8, C-9, C-10, and C-11 in comparison with those of the diterpene 7α,9α,16*β*,17-tetrahydroxy-*ent*-kaurane[24] are in accordance with this assignment. Therefore the structure of **10** was established as 7α,9*β*,18-trihydroxy-*ent*-kaur-16-ene.

The second metabolite obtained in this feeding was identified as 7α,15α,18-trihydroxy-*ent*-kaur-16-ene (canditriol) (**11**). This triol had been described previously as a natural product from *Sideritis infernalis*,[25] and comparison of spectroscopic data confirmed its identity. We have now assigned the ^{1}H and ^{13}C NMR spectra utilizing 2D NMR data (COSY, HSQC, and HMBC).

The most polar compound, **12**, of this bioconversion was obtained as its triacetate **13** and its tetraacetate **14** by acetylation and chromatography of the fractions containing it. Comparison of the spectroscopic data of **13** with those of **8** indicated that those compounds were very similar. Thus, the absence in their ^{1}H NMR spectrum of vinylic hydrogen signals at C-17, the appearance of a pair of doublets at δ 4.44 and 4.82 (J = 12.4 Hz) of the 17-acetoxymethylene group, and the quaternary carbinol at C-16 resonating at δ 79.2 confirmed this assertion. The HRMS of **13** showed the molecular ion at *m*/*z* 464.2786 [M]$^+$ and was in accordance with the molecular formula $C_{26}H_{40}O_7$. The stereochemistry at C-16 was established as *R* on the basis of the same arguments described above for **10**. On the other hand, the NMR spectrum of the tetraacetate **14** showed signals similar to those observed for **9**. Therefore, the original alcohol was assigned the structure 7α,16α,17,18-tetrahydroxy-*ent*-kaurane (**12**).

Several conclusions can be obtained from these microbiological transformations:

1. A spatial change in the orientation of the hydroxyl group at C-7 from *axial* in epicandicandiol (**1**) to *equatorial* in candicandiol (**2**) affected the way in which these kaurenes bind to the oxidative enzymes affording a different hydroxylation pattern in the A and B rings. Thus, the main difference in both feedings was the formation of a 3α-hydroxylated derivative of **1** in the first and a 9*β*-hydroxylated one of **2** in the second.

2. Incubations of both candicandiols **1** and **2** with *M. plumbeus* afforded mainly **7** and **12** in 15.0% and 22.7% yield, respectively. These 16,17-dihydroxylated compounds can be formed by enzymatic epoxidation of the exocyclic double bond to give the corresponding epoxides, followed by opening of these epoxides in the medium. In this context, we have reported that 16α,17-dihydroxy derivatives were formed when 16α,17-epoxy-*ent*-kauranes were added without the fungus to a medium prepared for growing *Gibberella fujikuroi*.[24]

3. The formation of sideritriol (**5**) in the incubation of **1** and of canditriol (**11**) in that of **2** should be due to the same enzyme. Thus, **5** can be formed by enzymatic abstraction of a hydrogen at C-15 in **1** with formation of a carbonium ion, migration of the double bond to the 15,16-position, and neutralization of the cation at C-17 by a −OH group, probably originating from water. The presence of the 7*β*-OH in the substrate can anchimerically assist this process. Sideritriol (**5**) can also be formed by enzymatic opening of the epoxide **15** and concomitant formation of the 15,16-double bond. We think that this is not the mechanism of formation in our case, because analogously in the biotransformation of candicandiol (**2**) this process must also imply the formation of the 7α-epimer of the diterpene sideritriol (**5**), which was not isolated therefrom.

4. The hydroxylations produced in these incubations of the substrates **1** and **2** with *M. plumbeus* resemble those observed in the *Sideritis* genus, where these compounds were transformed into the metabolites **3** and **11**, respectively, and from which compound **5** was also obtained.

Experimental Section

General Experimental Procedures. Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. IR spectra were recorded in a Perkin-Elmer 1600 FT. ^{1}H NMR spectra were recorded in $CDCl_3$ solutions at 500.13 MHz with a Bruker AMX2-500 spectrometer. ^{13}C NMR spectra were run in $CDCl_3$ at 50.32 and 125.13 MHz with a Bruker AC-200 or a Bruker AMX2-500, respectively. Chemical shifts are given in ppm (δ). Mass spectra and HRMS were taken at 70 eV in a Micromass Autospec spectrometer. HPLC was performed using a Beckman System Gold 125P. Purification by HPLC was achieved using a Si gel column (Ultrasphere Si 5 μm, 10 × 250 mm). Dry column chromatographies were made on Si gel Merck 0.2−0.065 mm. Epicandicandiol (**1**) and candicandiol (**2**) were obtained from *Sideritis candicans* (Labiatae), a species endemic to Tenerife (Canary Islands).[15,16] The acetylated compounds were prepared with Ac_2O−pyridine (2:1) at room temperature overnight.

Organism. The fungal strain *Mucor plumbeus* CMI 116688 was a gift from Dr. J. R. Hanson, School of Chemistry, Physics and Environmental Sciences, University of Sussex, U.K.

Incubation Experiments. The fungus *M. plumbeus* was grown in shake cultures at 25 °C in conical flasks (250 mL), each containing 50 mL of a sterile medium comprising (per liter) glucose (80 g), NH_4NO_3 (0.48 g), KH_2PO_4 (5 g), $MgSO_4$ (1 g), and trace elements solution (2 mL) containing (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 dissolved in EtOH was equally distributed among 40 conical flasks, and the fermentation was continued for a further 6 days before the mycelium was filtered and the broth extracted with ethyl acetate. The extracts were dried over Na_2SO_4 and the solvent was evaporated to give a residue, which was chromatographed on a Si gel column using a petroleum ether−EtOAc gradient. Fractions collected were purified further by HPLC when necessary.

Epicandicandiol (1): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, H-19), 0.85 (1H, td, J = 13, 3.3 Hz, H-1*β*), 1.06 (1H, s, H-20), 1.17 (2H, m, H-3 and H-14), 1.82 (3H, m, H-1α, H-5 and H-14), 2.24 (2H, br s, H-15), 2.68 (1H, br s, H-13), 2.96 and 3.47 (each 1H, d, J = 11 Hz, H-18), 3.59 (1H, t, J = 2.8 Hz, H-7), 4.80 and 4.82 (each 1H, s, H-17); EIMS *m*/*z* (rel int) 304 (1), 286 (14), 255 (100), 241 (14), 213 (12), 199 (11), 185 (9), 173 (11), 159 (7), 145 (10); HREIMS *m*/*z* 304.2409 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Candicandiol (2): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72 (1H, td, J = 13, 4.2 Hz, H-1*β*), 0.74 (3H, s, H-19), 1.05 (3H, s, H-20), 1.08 (1H, d, J = 7.1 Hz, H-9), 1.24 (1H, dd, J = 11, 2 Hz, H-5), 1.26 (1H, m, H-3), 1.78 (1H, dt, J = 13, 2 Hz, H-1α), 1.93 (1H, d, J = 16.4 Hz, H-15), 2.63 (1H, dt, J = 16.9 Hz, H-15), 2.67 (1H, br s, H-13), 3.09 and 3.41 (each 1H, d, J = 10.8 Hz, H-18), 3.50 (1H, br d, J = 10.7 Hz, H-7), 4.75 and 4.81 (each 1H, s, H-17); EIMS *m*/*z* (rel int) 304 (84), 286 (78), 268 (66), 255 (100), 240 (26), 225 (26), 211 (18), 199 (29), 185 (27), 169 (28), 157 (39), 145 (43); HREIMS *m*/*z* 304.2409 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Incubation of Epicandicandiol (1). The substrate **1** (140 mg) dissolved in EtOH (8 mL) was equally distributed among 40 conical flasks. After 6 days the mycelium was filtered and the broth extracted with ethyl acetate. The solvent was evaporated to give a residue (715 mg), which was chromatographed on a Si gel column, using a *n*-hexane−EtOAc−MeOH gradient as eluent, obtaining four fractions, which showed biotransformation products F-1 (7:3:0, 15.4 mg), F-2 (3:7:0, 33.9 mg), F-3 (0:9:1, 58.6 mg), and F-4 (0:85:15, 206.3 mg). Fraction

1 contained starting material **1** (1.5 mg). Fraction 2 was purified by column chromatography on Si gel, using a *n*-hexane−EtOAc gradient. Fractions eluted with *n*-hexane−EtOAc (9:1) were purified by HPLC using a gradient of 35% EtOAc to 55% in *n*-hexane, giving foliol **3** (4.8 mg). Fractions eluted with *n*-hexane−EtOAc (85:15) were acetylated and purified by HPLC using EtOAc as isocratic eluent to give foliol triacetate (**4**) (2.3 mg). Chromatographic separation of the acetylated fraction 3 afforded **4** (3.1 mg) and sideritriol triacetate **6** (1.3 mg). Fraction 4 was acetylated and purified by chromatography on a Si gel column with a gradient of ethyl acetate−hexane (4:1 to 1:1, v/v) and then by HPLC with a gradient of 30% EtOAc to 50% in *n*-hexane to give the tetraacetate **9** (1 mg) and the triacetate **8** (19.2 mg).

Foliol (3): colorless crystals; mp 194−197 °C (lit.[20] 198−200 °C); IR ($CHCl_3$) ν_{max} 3350, 2930, 1045, 1020, 875, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.75 (3H, s, H-19), 0.97 (1H, dd, J = 12.7, 7.6, H-14β), 1.04 (3H, s, H-20), 1.15 (1H, dd, J = 11.4, 4.7 Hz, H-1β), 1.43 (1H, br s, H-9), 2.21 (2H, br s, H-15), 2.66 (1H, br s, H-13), 3.58 (1H, br s, H-7), 3.35 and 3.59 (each 1H, J = 11.1 Hz, H-18), 3.68 (1H, dd, J = 8.9 and 7.7 Hz, H-3), 4.77 and 4.81 (each 1H, s, H-17); EIMS m/z (rel int) 320 $[M]^+$ (3), 302 (21), 284 (1), 272 (100), 253 (59), 211 (20), 199 (24), 157 (24); HREIMS m/z 320.23363 (calcd for $C_{20}H_{32}O_3$ 320.23514).

Triacetate (4): ^1H NMR ($CDCl_3$, 500 MHz) δ 0.80 (3H, s, H-19), 1.06 (1H, m, H-1β), 1.08 (3H, s, H-20), 1.24 (1H, dd, J = 11.6, 5.3 Hz, H-14), 1.48 (1H, d, J = 6.8 Hz, H-9), 1.99 and 2.01 (each 3H, s, OAc), 2.09 (1H, dt, J = 17, 3 Hz, H-15), 2.17 (1H, d, J = 17 Hz, H-15), 2.68 (1H, br s, H-13), 3.51 and 3.90 (each 1H, d, J = 11.6 Hz, H-18), 4.73 (1H, dd, J = 12, 5.3 Hz, H-3), 4.74 (1H, s, H-17), 4.78 (1H, d, J = 2 Hz, H-17); EIMS m/z (rel int) 446 $[M]^+$ (0.6), 386 (6), 344 (7), 326 (57), 311 (5), 284 (9), 266 (100), 251 (69), 238 (12), 225 (21), 223 (21); HREIMS m/z 446.2672 (calcd for $C_{26}H_{38}O_6$, 446.2668).

Sideritriol (5). Acetylation and chromatography of the fraction containing this metabolite gave the triacetate **6**: ^1H NMR ($CDCl_3$, 500 MHz) δ 0.79 (3H, s, H-19), 0.81 (1H, dd J = 13, 2 Hz, H-1β), 1.06 (3H, s, H-20), 1.29 (1H, dd, J = 10.3, 3.6 Hz, H-3β), 1.36 (1H, d, J = 6 Hz, H-9), 1.47 (1H, m, H-12), 1.56 (1H, d, J = 12.2 Hz, H-6β), 1.6 (1H, m, H-5), 1.75 (1H, dd, J = 12.2, 3.1 Hz, H-6α), 1.80 (1H, dt, J = 13, 3 Hz, H-1α), 2.00 (1H, d, J = 8.2 Hz, H-14), 2.03, 2.05 and 2.06 (each 3H, s, OAc), 2.58 (1H, br s, H-13), 3.63 and 3.70 (each 1H, J = 12.0 Hz, H-18), 4.55 and 4.65 (each 1H, J = 14.0 Hz, H-17), 4.73 (1H, br s, H-7), 5.56 (1H, s, H-15); EIMS m/z (rel int) 446 $[M]^+$ (1), 404 (3), 386 (13), 371 (7), 344 (74), 326 (86), 313 (21), 284 (16), 266 (69), 253 (100), 238 (21), 225 (30), 223 (29), 209 (18), 197 (23), 183 (25), 169 (23), 157 (26), 145 (41); HREIMS m/z 446.2666 (calcd for $C_{26}H_{38}O_6$, 446.2668).

7β,16α,17,18-Tetrahydroxy-*ent*-kaurane (7). This compound was obtained as the triacetate **8** and tetraacetate **9** by acetylation and chromatography of the fraction containing it.

Triacetate 8: colorless crystals; mp 155−158 °C; IR ($CHCl_3$) ν_{max} 3495, 2935, 1735, 1370, 1250, 1040, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.78 (3H, s, H-19), 0.79 (1H, td, J = 13.3, 3 Hz, H-1β), 1.04 (3H, s, H-20), 1.28 (1H, dd, J = 11, 3.5 Hz, H-14), 1.41 (1H, d, J = 7.02 Hz, H-9), 1.57 (2H, m, H-15), 1.83 (1H, br d, J = 11 Hz, H-14), 2.01, 2.08 and 2.14 (each 3H, s, OAc), 2.06 (1H, m, H-13), 3.61 and 3.68 (each 1H, J = 11.2 Hz, H-18), 4.18 (2H, s, H-17), 4.79 (1H, br s, H-7α); EIMS m/z (rel int) 464 $[M]^+$ (6), 446 (15), 404 (23), 344 (90), 331 (79), 313 (29), 284 (26), 271 (100), 253 (42), 225 (26), 145 (19), 119 (21), 109 (32); HREIMS m/z 464.27869 (calcd for $C_{26}H_{40}O_7$, 464.27740).

Tetraacetate 9: ^1H NMR ($CDCl_3$, 500 MHz) δ 0.78 (3H, s, H-19) 0.84 (1H, td, J = 13, 3.3 Hz, H-1β), 1.04 (3H, s, H-20), 1.29 (1H, m, H-3), 1.44 (1H, d, J = 6.8 Hz, H-9), 1.52 (1H, dd, J = 11, 4.6 Hz, H-14), 1.70 (1H, dd, J = 16.2, 3.8 Hz, H-15), 1.79 (1H, dd, J = 13, 3 Hz, H-1α), 1.86 (1H, br d, J = 11 Hz, H-14), 1.96, 2.01, 2.02, 2.04 (each 3H, s, OAc), 1.98 (1H, m, H-15), 2.53 (1H, d, J = 2.8 Hz, H-13), 3.60 and 3.69 (each 1H, d, J = 11.1 Hz, H-18), 4.43 and 4.86 (each 1H, d, J = 12.0 Hz, H-17), 4.70 (1H, br s, H-7α); EIMS m/z (rel int) 446 $[M - AcOH]^+$ (6), 404 (15), 386 (49), 344 (85), 326 (92), 313 (62), 266 (58), 253 (100), 225 (40), 197 (19), 157 (25); HREIMS m/z 446.2726 $[M - AcOH]^+$ (calcd for $C_{26}H_{38}O_6$, 446.26683).

Biotransformation of Candicandiol (2). Candicandiol (7α,18-dihydroxy-*ent*-kaur-16-ene) (**2**) (230.8 mg) dissolved in EtOH (8 mL) and 3 drops of Tween-80 was distributed among 40 conical flasks and incubated for a further 6 days. The metabolites were isolated as above and chromatographed on Si gel. Elution with *n*-hexane−EtOAc (3:2) gave starting material **2** (84.6 mg). Further elution with *n*-hexane−EtOAc (1:1) gave 7α,9β,18-trihydroxy-*ent*-kaur-16-ene (**10**) (2.5 mg). Fractions eluted with *n*-hexane−EtOAc (1:4) were purified by HPLC using EtOAc as isocratic eluent to give canditriol (**11**) (3.4 mg). Acetylation of fractions eluted with EtOAc−MeOH (1:1) and further chromatography on a Si gel column eluted with *n*-hexane−EtOAc mixtures gave the tetraacetate **14** (9.1 mg) and the triacetate **13** (25.7 mg).

7α,9β,18-Trihydroxy-*ent*-kaur-16-ene (10): colorless prism; mp 196−198 °C; IR ($CHCl_3$) ν_{max} 3400, 2920, 1460, 1380, 1290, 1055, 1025, 1005, 935, 860 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.76 (3H, s, H-19), 1.17 (3H, s, H-20), 1.26 (1H, m, J = 14 Hz, H-14), 1.50 (1H, m, H-1), 1.67 (2H, m, H-2 and H-12), 1.73 (2H, m, H-11), 1.89 (1H, dd, J = 12.9, 1.9 Hz, H-5), 1.98 (1H, dd, J = 14.3, 5.7 Hz, H-14), 2.43 (1H, dt, J = 17.6, 3 Hz, H-15β), 2.46 (1H, d, J = 17.6 Hz, H-15α), 2.67 (1H, br s, H-13), 3.11 and 3.42 (each 1H, J = 10.9 Hz, H-18), 3.81 (1H, dd, J = 12, 4.3 Hz, H-7), 4.78 and 4.81 (each 1H, s, H-17); EIMS m/z (rel int) 302 $[M - H_2O]^+$ (3), 290 (2), 284 (2), 271 (11), 253 (9), 163 (30), 123 (62), 109 (100), 93 (17), 81 (18); HREIMS m/z 302.2195 $[M - H_2O]^+$ (calcd for $C_{20}H_{30}O_2$, 302.2246).

Canditriol (11): ^1H NMR ($CDCl_3$, 500 MHz) δ 0.71 (1H, td, J = 13.2, 3.4 Hz, H-1β), 0.74 (H-19), 1.04 (1H, d, J = 9 Hz, H-9), 1.29 (1H, dd, J = 12.6, 1.0 Hz, H-5), 1.63 (1H, dd, J = 2 Hz, H-14), 1.79 (1H, dt, J = 13.2, 3 Hz, H-1α), 2.03 (1H, dd, J = 12.1, 5.3 Hz, H-14), 2.82 (1H, br s, H-13), 3.06 and 3.47 (each 1H, d, J = 11.0 Hz, H-18), 3.90 (1H, dd, J = 11.1, 4.5 Hz, H-7), 4.08 (1H, br s, H-15), 5.08 and 5.15 (each 1H, s, H-17); EIMS m/z (rel int) 320 $[M]^+$ (87), 302 (63), 287 (29), 275 (32), 271 (61), 262 (61), 257 (27), 253 (19), 201 (16), 162 (35), 152 (28), 147 (30), 145 (27), 131 (26), 129 (21), 123 (66), 109 (100); HREIMS m/z 320.2351 (calcd for $C_{20}H_{30}O_3$, 320.2351).

7α,16α,17,18-Tetrahydroxy-*ent*-kaurane (12). This compound was obtained as its triacetate **13** and its tetraacetate **14** by acetylation and chromatography of the fraction containing it (see above).

Triacetate 13: colorless crystals; mp 139−141 °C; IR ($CHCl_3$) ν_{max} 3500, 2935, 1735, 1375, 1250, 1040, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.71 (1H, td, J = 13.3, 4.3 Hz, H-1β), 0.78 (3H, s, H-19), 1.05 (3H, s, H-20), 1.07 (1H, d, J = 7.6 Hz, H-9), 1.25 (1H, d, J = 12.1 Hz, H-5), 1.61 (2H, m, H-14 and H-15), 1.74 (1H, dt, J = 12.9, 2.8 Hz, H-1α), 2.01 (1H, m, H-13), 2.13 (1H, dd, J = 11.9, 5.2 Hz, H-14), 3.55 and 3.79 (each 1H, J = 11 Hz, H-18), 4.64 (1H, dd, J = 11.4, 4.3 Hz, H-7), 4.13 and 4.22 (each 1H, J = 11.5 Hz, H-17); EIMS m/z (rel int) 446 (1), 386 (24), 344 (100), 326 (65), 284 (71), 269 (29), 266 (76), 253 (96), 237 (20); 223 (31); HREIMS m/z 446.2706 (calcd for $C_{26}H_{38}O_6$, 446.2668).

Tetraacetate 14: colorless needles; mp 169−171 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.73 (1H, td, J = 13.3, 3.8 Hz, H-1β), 0.77 (3H, s, H-19), 1.04 (3H, s, H-20), 1.10 (1H, d, J = 8.1 Hz, H-9), 1.24 (1H, d, J = 14.8 Hz, H-5), 1.52 and 2.08 (each 1H, d, J = 14.8 Hz, H-15), 1.74 (1H, dt, J = 12.9, 2 Hz, H-1α), 1.94 (1H, dd, J = 14.7 and 4.3 Hz, H-14), 1.96, 2.00, 2.03, 2.05 (each 3H, s, −OAc), 2.53 (1H, d, J = 2.9 Hz, H-13), 3.54 and 3.80 (each 1H, J = 11.0 Hz, H-18), 4.58 (1H, d, J = 11.1, 4.0 Hz, H-7β), 4.44 and 4.82 (each 1H, J = 12.4 Hz, H-17); EIMS m/z (rel int) 446 $[M - H_2O]^+$ (3), 404 (5), 386 (80), 373 (12), 344 (72), 326 (100), 313 (45), 284 (30), 266 (68), 253 (74), 225 (21), 197 (14), 157 (29); HREIMS m/z 446.26379 $[M - AcOH]^+$ (calcd for $C_{26}H_{38}O_6$, 446.26683).

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