The Microbiological Transformation of Two 15*β*-Hydroxy-*ent*-kaurene Diterpenes by *Gibberella fujikuroi*

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The incubation of 15*β*-hydroxy-3-oxo-*ent*-kaur-16-ene (**1**) with the fungus *Gibberella fujikuroi* afforded 11*β*-hydroxy-3,15-dioxo-*ent*-kaurane (**6**), 11*β*,15*β*-dihydroxy-3-oxo-*ent*-kaur-16-ene (**8**), 7*β*,11*β*,15*β*-trihydroxy-3-oxo-*ent*-kaur-16-ene (**9**), 7α,11*β*-dihydroxy-3,15-dioxo-*ent*-kaurane (**7**), and 7α,11*β*,15*β*-trihydroxy-3-oxo-*ent*-kaur-16-ene (**10**). The incubation of 15*β*-hydroxy-*ent*-kaur-2,16-diene (**3**) with the same fungus yielded 7α,11*β*-dihydroxy-15-oxo-*ent*-kaur-2-ene (**12**), 7α,11*β*,15*β*-trihydroxy-*ent*-kaur-2,16-diene (**13**), 7*β*,-15*β*-dihydroxy-*ent*-kaur-2,16-dien-19,6-olide (**14**), 1*β*,7*β*,15*β*-trihydroxy-*ent*-kaur-2,16-dien-19-oic acid (**15**), 7α,11*β*,16α-trihydroxy-15-oxo-*ent*-kaur-2-ene (**17**), and 7α,15*β*,17-trihydroxy-11*β*,16*β*-epoxy-*ent*-kaur-2-ene (**19**). These results indicated that a 3-oxo group in *ent*-kaur-16-ene derivatives inhibits the oxidation at C-19, typical of the biosynthetic pathway of gibberellins and kaurenolides, while a 2,3-double bond or a 15*β*-OH does not. In both substrates a 15*β*-alcohol directs hydroxylations at C-11(*β*) and C-7(α), while in those with a 2,3-double bond the functionalization of C-1(*β*) is favored.

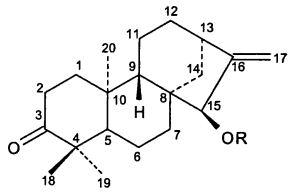
For several years we have studied the biotransformation of *ent*-kaurene diterpenes by the fungus *Gibberella fujikuroi*. The main aims of these works have been to prepare new gibberellin analogues, to study the biosynthetic pathway of these plant hormones, and to obtain information about the substrate specificity of the enzymes involved in the biosynthesis of gibberellins and kaurenolides. We have shown that in *ent*-kaurene derivatives a 15α-hydroxyl group,[1,2] a 16α-alcohol,[3] or a 15α,16α-epoxide[4] directs hydroxylation at C-11(*β*) and C-7(α) and inhibits oxidation at C-19. This oxidation is typical of the biosynthetic route of gibberellins.[5] We have also studied the microbial transformation of 3α,15*β*-dihydroxy derivatives by this fungus.[6] In the latter neither gibberellins nor kaurenolides were obtained due to the presence of the 3α-hydroxyl group in the molecule, which also inhibits the oxidation at C-19. We have also shown that *ent*-kaurene diterpenes with a 15-oxo[7] or a 15*β*,18-dihydroxyl[8] group can be oxidized at C-19 at the acid level and biotransformed all along the gibberellin and kaurenolide pathway. Here, we report the results obtained in the incubation with *G. fujikuroi* of two 15*β*-hydroxy-kaurene diterpenes, **1** and **3**, which are not hydroxylated at C-19 and possess a 3-oxo group and a 2,3-double bond, respectively.

Results and Discussion

The substrates **1** and **3** were prepared as follows: (a) 3α-Hydroxy-15*β*-angeloxy-*ent*-kaur-16-ene (**5**) was oxidized with Jones reagent to give **2**, which was hydrolyzed with methanolic potassium hydroxide, affording 3-oxo-15*β*-hydroxy-*ent*-kaur-16-ene (**1**). (b) Dehydration of **5** with phosphorus oxychloride led to **4**, which was hydrolyzed, giving 15*β*-hydroxy-*ent*-kaur-2,16-diene (**3**).[9] The starting compound in both preparations (**5**) had been isolated from *Elaseolinum tenuifolium*.[9,10]

The biotransformations with the fungus were carried out in the presence of AMO 1618, a compound that inhibits the production of *ent*-kaur-16-ene without affecting the post-kaurene metabolism.[11,12] The substrates were incubated with *G. fujikuroi* in two different experiments, and the cultures were extracted following the usual procedure.

Chromatography of the extract obtained in the incubation of **1** afforded **6**−**10**. The least polar metabolite **6** showed a molecular ion at *m*/*z* 318.2185 in accordance with



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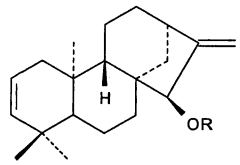
2 R = OAng



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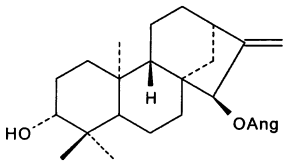
4 R = OAng



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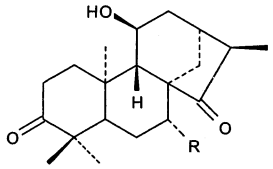
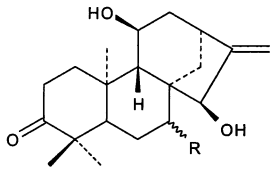


6 R = H
7 R = OH



8 R = H
9 R = β-OH
10 R = α-OH

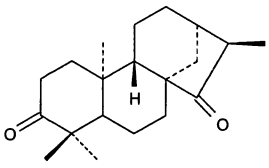


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Table 1. ^{13}C NMR Data (δ) for Compounds **1** and **6**−**10**

C	1	6	7	8	9	10
1	39.2	38.1	37.7	38.8	38.9	38.5
2	34.1	33.5[a]	33.8[a]	33.8	33.7[a]	33.7
3	218.6	217.0	216.4	217.7	217.8	216.9
4	47.1	47.1	46.9	47.0	46.4[b]	46.8
5	53.9	53.4	50.3	53.54	44.1	50.6
6	21.1	19.8	29.0	21.0	28.1	26.9
7	37.8	33.6[a]	71.0	37.8	76.9	71.1
8	45.5	50.7	56.7	44.4	46.6[b]	49.7
9	45.3	62.0	61.1	53.4	49.2	52.4
10	38.1	37.3	36.9	36.6	36.4	36.7
11	18.6	65.3	65.3	67.1	66.4	67.0
12	33.0	33.2[a]	33.4[a]	43.0	43.1	43.5
13	40.0	34.6	34.0	38.8	38.9	38.5
14	36.0	36.9	28.3	35.4	33.6[a]	30.3
15	82.2	222.3	221.7	82.3	82.0	75.6
16	157.9	49.5	51.0	157.9	155.7	157.4
17	105.1	11.1	11.1	106.3	106.8	106.7
18	27.2	27.3	27.3	27.5	27.4	27.4
19	21.0	20.8	20.8	20.8	20.8	20.8
20	17.7	17.8	18.0	18.0	18.3	18.0

[a,b] These values can be interchanged.

the molecular formula $C_{20}H_{30}O_3$. In its ^1H and ^{13}C NMR spectra it was observed that the exocyclic double bond and the 15β-alcohol of the substrate had disappeared, being replaced by a carbonyl (δ_C 222.3) and a methyl group (δ_H 1.27, d, J = 6.8 Hz, δ_C 11.1). Thus, the formation of a 15-oxo,17-methyl derivative had occurred during the fermentation. The ^1H NMR spectrum also showed a new hydrogen signal at δ 3.94 (d, J = 5.5 Hz). Its chemical shift, the form of the signal, and the coupling constant were characteristic of a hydrogen geminal to an alcohol group at C-11(β) in *ent*-kaur-16-ene diterpenes.[1,2] The results of a 2D NMR study (COSY, HSQC, and HMBC) were in accordance with these assertions and permitted us to assign the structure of 11β-hydroxy-3,15-dioxo-*ent*-(16*S*)-kaurane (**6**) to this substance. The formation of this 15-oxo,17-methyl derivative implies a rearrangement, produced by protonation of the 16,17-double bond with formation of a 16-carbocation and a 15,16-hydride shift, because this type of rearrangement occurs very readily in acid medium.[13] Another possibility is that compound **6** could be formed by oxidation of the 15β-OH and subsequent hydrogenation of the 15,16-double bond, because we have shown that *G. fujikuroi* transforms 15-oxo-*ent*-kaur-16-ene derivatives into the corresponding dihydroderivatives.[7] The β-stereochemistry assigned to the 17-methyl was given considering that both reactions, 15,16-hydride shift and hydrogenation of the 16,17-double bond, take place by the α-face.[7,13,14]

The second metabolite isolated (**8**) had a molecular formula $C_{20}H_{30}O_3$ as determined by HRMS, which indicated that a new oxygen had been introduced into the molecule during the fermentation. Its ^1H NMR spectrum showed a proton geminal to a new alcohol group resonating as a doublet at δ 4.00 (J = 4.8 Hz), which was assigned to C-11(β). This was confirmed by its ^{13}C NMR spectrum (Table 1), which was unambiguously assigned by two-dimensional NMR analysis. Therefore, the structure of this compound was determined to be 11β,15β-dihydroxy-3-oxo-*ent*-kaur-16-ene (**8**). This compound had been isolated from *Rabdosia inflexa* and named inflexarabdonin J.[15]

The third substance obtained from this incubation was **9**. Its HRMS was in accordance with the formula $C_{20}H_{30}O_4$. The ^1H NMR spectrum was very similar to that of **8**, but now the signal of a proton geminal to a new alcohol appeared at δ 3.81 (t, J = 3 Hz). Its coupling constant indicated that the hydroxyl group must be axial and situated at C-1(β) or C-7(β). The latter position was chosen considering its HMBC spectrum, where correlations of H-7 with C-5 and C-15 were observed. Thus, the structure 7β,-11β,15β-trihydroxy-3-oxo-*ent*-kaur-16-ene (**9**) was assigned to this compound.

Another metabolite was identified as 7α,11β,15β-trihydroxy-3-oxo-*ent*-kaur-16-ene (**10**) as follows. The molecular ion at 334.2139 indicated a $C_{20}H_{30}O_4$ molecular formula. Its ^1H NMR spectrum, in comparison with that of the substrate, showed two new hydrogens at δ 3.72 (dd, J = 11.6 and 4.3 Hz) and 4.00 (d, J = 4.9 Hz). The coupling constants for the first of these signals were typical of a proton geminal to an equatorial alcohol group at C-1(α) or C-7(α). The 7α-position was chosen considering the ^{13}C NMR data (Table 1). The second of these signals was assigned to the proton geminal to an 11β-hydroxyl by comparison with the ^1H and ^{13}C NMR spectra of compounds such as **8** and **9**. A study of the HMQC and HMBC spectra confirmed the structure of **10**.

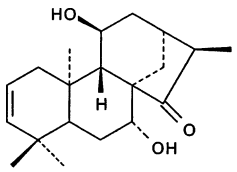
An isomer of **10**, with structure **7**, was also isolated from this incubation. Its ^1H NMR spectrum showed, as in the case of **6**, that during the incubation a rearrangement of ring D had occurred. Thus, the proton geminal to the 15β-OH and the hydrogens of the 16,17-double bond of the substrate were not observed, being substituted by a C-17 methyl (δ_H 1.26, d, J = 6.9 Hz; δ_C 11.1) and a 15-oxo group (δ 221.7). The hydrogen geminal to the 7α- and 11β-alcohol appeared at δ 3.93 (dd, J = 11.6 and 4.3 Hz) and 4.00 (d, J = 4.9 Hz), respectively. The chemical shifts and splitting patterns of these protons were similar to those of its isomer **10** and to those of other compounds with these oxygen functions.[7] Correlations between the H-7 and the 15-oxo group and of H-11 with C-9 were observed in the HMBC spectrum. Therefore, the structure of this compound was determined to be 7α,11β-dihydroxy-3,15-dioxo-*ent*-(16S)-kaurane (**7**).

The second incubation with *G. fujikuroi* was carried out with substrate **3**. In comparison with **1**, the 3-oxo group of **1**, inhibitor of the oxidation at C-19, has been substituted by a 2,3-double bond in **3**. This biotransformation yielded the metabolites **12**−**15**, **17**, and **19**. A compound hydroxylated at C-15(β) and C-19 had been previously incubated with this fungus, giving 1β- and 6β-hydroxy derivatives with C-19 in the form of an acid group.[16]

The arguments used above in the assignment of the alcohol groups at C-7(α) and C-11(β) in metabolites **7** and **10** were the same ones utilized to locate them in substances **12** and **13**, respectively. Compound **18** was obtained when **13** was left in $CDCl_3$ in a NMR tube for four weeks. Substance **18** appears to be formed from **13** by protonation of the 16,17-double bond in the acid medium and neutralization of the ion created at C-16 by 11β-OH. Their ^1H and ^{13}C NMR spectra showed H-11 at δ 4.24 (br s) and 84.2, respectively. These chemical shifts and forms of resonance are characteristic of this type of compound.[3,6]

Another substance obtained from this experiment was 7β,15β-dihydroxykaurenolide (**14**) ($C_{20}H_{24}O_3$). The ^1H NMR spectrum showed that ring B contained the vicinal 6α,7β-oxygen functions characteristic of similar lactones (δ 4.42, d, J = 6.5 Hz, H-7; 4.77, t, J = 6.5 Hz, H-6; 2.28, d, J = 6.5 Hz, H-5). The H-15 signal appeared as a broad singlet at δ 4.68. ^{13}C NMR showed the corresponding carbons at 71.4 (C-7), 83.3 (C-6), 50.6 (C-5), and 73.2 (C-15), and the oxo group of C-19 appeared at δ 178.0.

Compound **15** was obtained as its triacetate methyl ester **16a** by methylation of the extract and subsequent acetylation of the chromatographic fractions containing it. Its ^1H NMR spectrum showed, in comparison with those of the



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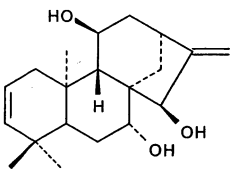

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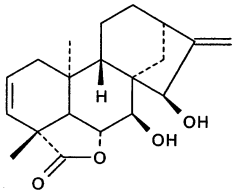

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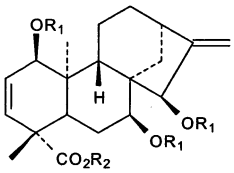
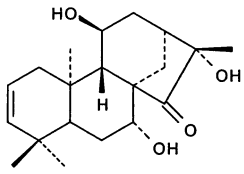


15 $R_1 = R_2 = H$
16 $R_1 = H$ $R_2 = Me$
16a $R_1 = Ac$ $R_2 = Me$



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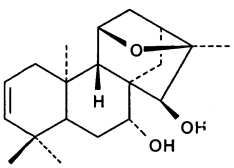

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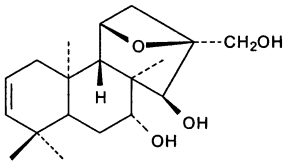

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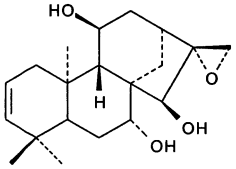

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substrate, the signals of a methyl ester at δ 3.61 and of two new geminal protons to acetoxy groups at δ 4.78 (br s) and 5.07 (d, J = 5.4 Hz). The last one was assigned to C-1(β) by a double radiation experiment. Irradiation of H-1 at δ 5.07 converted the double doublet of H-2 at 5.76 into a clean doublet. Alternatively, irradiation of this last signal transformed the doublet of H-1 into a singlet. Unambiguous assignment of the ^{13}C NMR data (Table 2), using HMQC and HMBC spectra, confirmed the position of this acetoxy group and permitted us to place the other acetate at C-7(β) and the methyl ester at C-19. The β-stereochemistry for the acetate at C-1 was determined considering the coupling constant (5.4 Hz) observed between H-1(α) and H-2 in the conformer of less energy, which was obtained by MM+ molecular mechanics calculations. Therefore, the structure of the corresponding compound formed in the incubation was determined to be 1β,7β,15β-trihydroxy-*ent*-kaur-2,16-diene-19-oic acid (**15**).

The structure of 7α,11β,16α-trihydroxy-15-oxo-*ent*-kaur-2-ene (**17**) was assigned to another substance from this fermentation. Its ^{1}H NMR spectrum was very similar to that of **12**, except that in **17** the C-17 methyl appeared as a singlet at δ 1.33, indicating the presence in C-16 of a hydroxyl group, which was also in accordance with the corresponding carbon resonance (δ 77.7) and with the molecular formula $C_{20}H_{30}O_4$ (*m*/*z* 334.2137). The stereochemistry assigned to the 16α-alcohol was given considering that in this kind of molecule the hydroxylation normally occurs by the α-face.[17]

Table 2. ^{13}C NMR Data for Compounds **3**, **12**, **14**, **16a**, and **17**−**19**

C	3	12	14	16a	17	18	19
1	41.2	40.0	39.5	70.7	40.1	40.9	40.9
2	121.4	120.4	122.7	122.1	120.3	120.8	120.7
3	137.7	138.0	127.6	136.1	138.1	137.4	137.4
4	34.7	34.6	43.6	44.9	34.7	34.3	34.3
5	51.3	48.9	50.6	39.9	48.9	49.2[a]	48.9
6	20.8	28.4[a]	83.3	25.9	28.2	29.6	29.5
7	35.9	71.1	71.4	78.2	70.8	69.7	69.3
8	45.7	57.1	47.4	47.3	57.0	50.0	50.3
9	44.9	61.3	43.8	32.5	63.2	48.8[a]	49.8
10	37.6	36.5	33.9	40.5	36.7	35.0	35.0
11	18.4	65.2	16.6	17.8	65.4	76.5[b]	76.8
12	33.3	33.7	33.1	33.2	36.4	39.1	39.5
13	40.1	34.1	36.0	40.5	40.9	41.3	38.2
14	37.9	28.6[a]	28.9	35.4	25.1	29.6	29.5
15	82.5	222.0	73.2	79.0	220.0	76.6[b]	73.7
16	158.1	50.4	161.4	153.2	77.7	84.2	86.7
17	104.9	11.1	107.6	107.4	19.5	20.2	63.7
18	31.8	31.6	24.7	26.9	31.7	31.8	31.8
19	22.5	22.3	178.0	175.1	22.4	22.6	22.6
20	17.9	18.2	18.2	16.0	18.3	18.8	18.8

[a],[b] These values can be interchanged.

The ^{1}H NMR spectrum of compound **19** ($C_{20}H_{30}O_4$) showed three methyls, a hydroxymethylene group, and three protons geminal to oxygenated functions. The disappearance of the exocyclic double-bond hydrogens in this spectrum and the absence of the oxo carbon resonances indicated the existence of an ether bridge between C-11(β) and C-16(β). This was confirmed by the resonance of H-11 at δ 4.32, whose chemical shift and splitting pattern are characteristic of this grouping.[3] The other two geminal hydrogens were overlapped in the ^{1}H NMR spectrum. One was assigned to C-15(β) considering that it is present in the substrate and the other at C-7(α) considering its ^{13}C resonance at δ 69.3, which was similar to that observed in **18** (δ 69.7). The hydroxymethylene group was located at C-17 observing the chemical shift of C-16, δ 84.2 and 86.7, in **18** and **19**, respectively. Thus, **19** was determined to be 7α,15β,17-trihydroxy-11β,16β-epoxy-*ent*-kaur-2-ene. This was confirmed by the correlations observed in the HMBC spectrum of H-17 with C-16 and C-13, of H-13 with C-14 and C-11, and of H-12 with C-14 and C-16. Substance **19** must be an artifact, probably formed in the medium from **20** by acid opening of the 16α,17α-epoxide with attack over C-16 of the 11β-OH. The oxirane ring in **20** has an α-stereochemistry because the microbiological epoxidation of *ent*-kaurene derivatives occurs via the α-face.[2,18] The β-stereochemistry to the ether bridge in **19** was assigned considering the 11β-alcohol attack at C-16 by the β-face, also favored by the α-opening of the epoxy ring.

The results of these two biotransformations may be summarized as follows:

1. The existence of a 15β-hydroxyl group in the molecule does not inhibit the oxidation at C-19, typical of the biosynthetic pathway of gibberellins and kaurenolides, in contrast with our previous studies that showed that its epimer, a 15α-alcohol, inhibits oxidation thereof.

2. The presence of a 15β-alcohol directs the hydroxylation at C-11(β) and C-7(α). Similar results were obtained by us with 15α-alcohols[1,2] and 15-oxo *ent*-kaur-16-ene[7] derivatives.

3. A 3-oxo group in the molecule inhibits oxidation at C-19. This had also occurred when there was a 3α-alcohol[1,2] but not when a 3β-OH was present.[19]

4. The presence of a 2,3-double bond does not affect the oxidation at C-19.

5. In the biotransformation of substrates with a 15β-hydroxyl and a 2,3-double bond such as **3**, there are two competitive processes, the oxidation at C-19 and the hydroxylation at C-11(β) and C-7(α). Thus, the amount of substrate that is oxidized in C-19 to an acid group, a prerequisite to be transformed into kaurenolides and gibberellins, is low. Moreover, we have also observed that there is a preference for the formation of kaurenolides with respect to gibberellins.

6. Another competitive biotransformation is the hydroxylation at C-1(β) in the substrate **3** to give **15**, which is directed by the presence of the 2,3-double bond.

7. Isomerization of the 15β-hydroxy to the 15-oxo derivatives seems not to be produced in the medium by acid rearrangement because compound **11**, which should be obtained from rearrangement of substrate **1**, was not isolated from the fermentation. Thus, this is a biotransformation produced by the fungus by a garryfoline-cuauchichicine type rearrangement[13] or by oxidation of the 15β-alcohol and subsequent hydrogenation of the 16,17-double bond.

8. The occurrence of 11β-hydroxylated *ent*-kaur-16-ene diterpenes with oxygen functions of the 15α-OH, 15β-OH, or 15-oxo type in liverworts[20,21] and higher plants[22,23] seems to indicate that the 11β-hydroxylation should be directed by the presence in the molecule of a 15-oxygenated substituent, as occurs in the microbiological transformations with the fungus *G. fujikuroi*.

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 $CDCl_3$ solution at 200.13 and 500.13 MHz with a Bruker AC-200 or a Bruker AMX-500 spectrometer, respectively, and the ^{13}C NMR at 125.03 MHz in a Bruker AMX-500, except those of **3** and **6**−**10**, which were recorded at 50 MHz in a Bruker AC-200. Mass spectra were taken at 70 eV (probe) in a Shimadzu Q2000, and high-resolution mass spectra in a Micromass Autospec spectrometer. Optical rotations have been measured at 25 °C in a Perkin-Elmer 343 Plus. HPLC was performed using a Beckman System Gold 125P. Purification by HPLC was achieved using a silica gel column (Ultrasphere Si 5 μm, 10 × 250 mm). Dry column chromatography was made on Merck 0.2−0.065 mm silica gel. Molecular mechanics calculations were carried out with the program Hyperchem 7.0 (Hypercube).

Microorganism. The fungal strain was *Gibberella fujikuroi* IMI 58289 and was a gift from Dr. J. R. Hanson, School of Chemistry, Physics and Environmental Science (University of Sussex, UK).

Preparation of 15β-Hydroxy-3-oxo-*ent*-kaur-16-ene (1). 3β-Hydroxy-15β-angeloxy-*ent*-kaur-16-ene (**5**) (350 mg), dissolved in acetone (20 mL) at 5 °C, was oxidized with Jones reagent to afford **2**.[9] The crude extract, dissolved in methanolic KOH (5%, 15 mL), was heated at 50 °C for 5 h. Usual workup and column chromatography of the crude mixture, eluting with petroleum ether−EtOAc (10−1), afforded **1** (210 mg, 76% overall): colorless crystals; mp 135−136 °C (petroleum ether−EtOAc); IR ($CHCl_3$) ν_{max} 3593, 3463, 2936, 1698, 1458, 1386 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.03 (3H, s, H-19), 1.07 (1H, m, H-14), 1.08 (3H, s, H-18), 1.09 (3H, s, H-20), 1.38 (1H, m, H-7), 1.96 (1H, d, J 12.0 Hz, H-14), 2.05 (1H, dt, J = 13.3 and 6.5 Hz, H-1), 2.47 (2H, dd, J = 8.5 and 6.4 Hz, H-2), 2.69 (1H, br s, H-13), 3.80 (1H, t, J = 2 Hz, H-15), 4.98 and 5.10 (each 1H, br s, H-17); EIMS m/z (rel int) 302 [M]$^+$ (51), 284 (88), 269 (68), 251 (20), 244 (47), 227 (26), 199 (29); HREIMS m/z [M]$^+$ 302.2250 (calcd for $C_{20}H_{30}O_2$, 302.2246); *anal.* C 79.07%, H 9.66%, calcd for $C_{20}H_{30}O_2$, C 79.42%, H 9.99%.

Preparation of 15β-Hydroxy-*ent*-kaur-2,16-diene (3). To 3α-hydroxy-15β-angeloxy-*ent*-kaur-16-ene (**5**) (175 mg), dissolved in dry pyridine (3 mL), was added phosphorus oxychloride (250 mg). The mixture was stirred overnight at room temperature and then heated to 80 °C for 4 h. Usual workup led to **4**,[9] which was dissolved in methanolic KOH (5%, 7 mL) and heated to 50 °C for 7 h. Purification of the crude mixture by column chromatography, eluting with petroleum ether−EtOAc (12:1), afforded **3**[9] (75 mg, 58% overall): ^{1}H NMR (200 MHz) δ 0.89, 0.96 and 1.09 (each 3H, s), 1.99 (1H, d, J = 11.9 Hz, H-14), 2.15 (1H, dd, J = 16.9 and 5.3 Hz, H-1), 2.69 (1H, br s, H-13), 3.78 (1H, br s, H-15), 4.98 and 5.10 (each 1H, br s, H-17), 5.42 (2H, m, H-2 and H-3); EIMS m/z (rel int) 286 [M]$^+$ (6), 271 (3), 253 (8), 228 (6), 204 (4), 171 (5); HREIMS m/z [M]$^+$ 286.2282 (calcd for $C_{20}H_{30}O$, 286.2297).

Incubation of 15β-Hydroxy-3-oxo-*ent*-kaur-16-ene (1). The fungus *G. fujikuroi*, inhibited with 5 × 10^{-5} M AMO 1618, was grown on shake culture at 25 °C for 2 days in 55 conical flasks (250 mL), each containing 50 mL of sterile medium comprising (per dm^3) 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** (195 mg) dissolved in EtOH (11 mL) was evenly distributed between the flasks and the incubation allowed to continue for a further 6 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, dried over Na_2SO_4, and concentrated to yield a syrup (450 mg), which was methylated with diazomethane. Chromatography on silica gel, eluting with petroleum ether−ethyl acetate gradient, gave starting material (**1**) (4 mg), 11β-hydroxy-3,15-dioxo-*ent*-(16S)-kaurane (**6**) (16 mg), 11β,15β-dihydroxy-3-oxo-*ent*-kaur-16-ene (**8**) (10 mg) (inflexarabdonin J), 7β,11β,15β-trihydroxy-3-oxo-*ent*-kaur-16-ene (**9**) (12 mg), 7α,11β-dihydroxy-3,15-dioxo-*ent*-(16S)-kaurane (**7**) (26 mg), and 7α,11β,15β-trihydroxy-3-oxo-*ent*-kaur-16-ene (**10**) (11 mg). The metabolites **6**, **8** and **7**, **10** were obtained as a pair of mixtures, which were resolved by chromatography on silica gel impregnated with $AgNO_3$ (15%) eluting with petroleum ether−EtOAc (1:1) and EtOAc, respectively.

11β-Hydroxy-3,15-dioxo-*ent*-(16*S*)-kaurane (6): colorless crystals; mp 185−187 °C (petroleum ether−EtOAc), $[\alpha]_D$ −82° (*c* 0.16, $CHCl_3$); ^{1}H NMR (500 MHz) δ 1.04 (3H, s, H-19), 1.05 (3H, s, H-20), 1.12 (3H, s, H-18), 1.25 (1H, br s, H-9), 1.27 (3H, d, J = 6.8 Hz, H-17), 1.65 (1H, dt, J = 13.5 and 8.5 Hz, H-1β), 2.07 (1H, ddd, J = 13.5, 7.7 and 5.3 Hz, H-1α), 2.30 (1H, quint, J = 6.7 Hz, H-16), 2.34 (1H, dd, J = 12 and 1.1 Hz, H-14), 2.46 (1 H, ddd, J = 16, 8.5 and 7.7 Hz, H-2), 2.48 (1H, br s, H-13), 2.55 (1H, ddd, J = 16, 8.5 and 5.3 Hz, H-2), 3.94 (1H, d, J = 5.5 Hz, H-11); EIMS m/z (rel int) 318 [M]$^+$ (12), 303 (7), 300 (55), 285 (35), 267 (22), 257 (21), 243 (23), 227 (15), 209 (19), 201 (19), 185 (19); HREIMS m/z [M]$^+$ 318.2185 (calcd for $C_{20}H_{30}O_3$, 318.2195).

7α,11β-Dihydroxy-3,15-dioxo-*ent*-(16*S*)-kaurane (7): colorless needles; mp 229−232 °C (petroleum ether−EtOAc); $[\alpha]_D$ −111° (*c* 0.53, $CHCl_3$); ^{1}H NMR (500 MHz) δ 1.05 (6H, s, H-19 and H-20), 1.13 (3H, s, H-18), 1.22 (1H, s, H-9), 1.26 (3H, d, J = 6.9 Hz, H-17), 1.50 (1H, dt, J = 12 Hz, H-6α), 1.62 (1H, dt, J = 13.3 and 8.5 Hz, H-1), 1.67 (1H, dd, J = 12 and 2.4 Hz, H-5), 1.77 (1H, ddd, J = 12, 4.2, and 2.4 Hz, H-6β), 2.27 (1H, quint, J = 6.8 Hz, H-16), 2.47 (1H, dt, J = 16 and 8.5 Hz, H-2), 2.52 (1H, br s, H-13), 2.55 (1H, ddd, J = 16, 8.5, and 5.2 Hz, H-2), 3.93 (1H, d, J = 5.1 Hz, H-11), 4.00 (1H, dd, J = 12 and 4.2 Hz, H-7); EIMS m/z (rel int) 334 [M]$^+$ (12), 316 (21), 298 (8), 288 (24), 260 (65), 243 (30), 231 (12), 225 (14), 213 (14), 196 (48), 178 (68); HREIMS m/z [M]$^+$ 334.2140 (calcd for $C_{20}H_{30}O_4$, 334.2144).

11β,15β-Dihydroxy-3-oxo-*ent*-kaur-16-ene (inflexarabdonin J) (8): colorless crystals; mp 170−172 °C (petroleum

ether−EtOAc); $[\alpha]_D$ −80° (*c* 0.22, $CHCl_3$); ^1H NMR (500 MHz) δ 0.99 (each 3H, s, H-20), 1.04 (3H, s, H-19), 1.11 (3H, s, H-18), 1.14 (1H, m, H-14), 1.68 (1H, br s, H-9), 1.94 (1H, d, J = 12.1 Hz, H-14), 2.04 (1 H, ddd, J = 14.6, 5.0, and 2.8 Hz, H-12), 2.09 (1 H, ddd, J = 13.4, 7.9, and 5.5 Hz, H-1), 2.45 (1H, dt, J = 16 and 7.9 Hz, H-2), 2.58 (1 H, ddd, J = 16, 8.4, and 5.5 Hz, H-2), 2.68 (1 H, br s, H-13), 3.82 (1H, br s, H-15), 4.00 (1H, d, J = 4.8 Hz, H-11), 5.05 and 5.14 (each 1H, br s, H-17); EIMS *m/z* (rel int) 318 $[M]^+$ (8), 300 (48), 285 (37), 267 (22), 243 (11), 229 (8), 215 (14), 201 (13), 197 (14), 183 (16); HREIMS *m/z* $[M]^+$ 318.2215 (calcd for $C_{20}H_{30}O_3$, 318.2195).

7β,11β,15β-Trihydroxy-3-oxo-*ent*-kaur-16-ene (9): a gum; ^1H NMR (500 MHz) δ 0.98 (3H, s, H-20), 1.04 (3H, s, H-19), 1.12 (3H, s, H-18), 1.20 (1H, dd, J = 12 and 4 Hz, H-14), 1.59 (1 H, dt, J = 12 and 2 Hz, H-6α), 1.69 (1H, dt, J = 12 and 3 Hz, H-6β), 1.82 (1H, d, J = 12 Hz, H-14), 2.37 (1H, dd, J = 12 and 3 Hz, H-5), 2.42 (1H, dt, J = 15 and 7.8 Hz, H-2), 2.66 (1H, ddd, J = 15, 9, and 5.8 Hz, H-2), 2.69 (1H, br s, H-13), 3.81 (1H, t, J = 3 Hz, H-7), 4.00 (1H, d, J = 4.7 Hz, H-11), 4.29 (1H, br s, H-15), 5.09 and 5.15 (each 1H, br s, H-17); EIMS *m/z* (rel int) 334 $[M]^+$ (79), 316 (50), 303 (96), 298 (81), 283 (44), 270 (28), 265 (24), 255 (27), 227 (22), 209 (24); HREIMS *m/z* $[M]^+$ 334.2142 (calcd for $C_{20}H_{30}O_4$, 334.2144).

7α,11β,15β-Trihydroxy-3-oxo-*ent*-kaur-16-ene (10): colorless needles; 190−195 °C (EtOAc); $[\alpha]_D$ −85° (*c* 0.07, $CHCl_3$); ^1H NMR (500 MHz) δ 1.01 (3H, s, H-20), 1.06 (3H, s, H-19), 1.13 (3H, s, H-18), 1.56 (1H, t, J = 12.4 Hz, H-6α), 1.09 (2H, m, H-1 and H-2), 2.46 (1H, ddd, J = 15.8 and 7.9 Hz, H-2), 2.59 (1H, ddd, J = 15.8, 8.5, and 5.5 Hz, H-2), 2.73 (1H, br s, H-13), 3.72 (1H, dd, J = 11.6 and 4.3 Hz, H-7), 4.00 (1H, d, J = 4.9 Hz, H-11), 4.54 (1H, br s, H-15), 5.08 and 5.16 (each 1H, br s, H-17); EIMS *m/z* (rel int) 334 $[M]^+$ (25), 316 (34), 298 (64), 288 (25), 283 (44), 280 (36), 265 (23), 255 (20), 241 (18), 213 (41), 195 (44), 181 (46), 165 (48); HREIMS *m/z* $[M]^+$ 334.2139 (calcd for $C_{20}H_{30}O_4$, 334.2144).

Incubation of 15β-Hydroxy-*ent*-kaur-2,16-diene (3). *G. fujikuroi* was grown as above in 55 conical flasks (250 mL). Substrate **3** (215 mg) dissolved in ethanol (11 mL) was evenly distributed between the flasks. After 6 days the broth was filtered and treated as above for **1** to yield a syrup (540 mg). This extract was treated with diazomethane before purification.

Chromatography on silica gel, eluting with petroleum ether−EtOAc gradient, gave starting material (**3**) (10 mg), 7α,-11β-dihydroxy-15-oxo-*ent*-(16*S*)-kaur-2-ene (**12**) (14 mg), 7α,-11β,15β-trihydroxy-*ent*-kaur-2,16-diene (**13**) (3 mg), 7β,15β-dihydroxy-*ent*-kaur-2,16-dien-19,6-olide (**14**) (2 mg), 1β,7β,15β-trihydroxy-*ent*-kaur-2,16-diene-19-methylester (**16**) (4 mg), 7α,11β,16α-trihydroxy-15-oxo-*ent*-kaur-2-ene (**17**) (7 mg), and 7α,15β,17-trihydroxy-11β,16β-epoxy-*ent*-kaur-2-ene (**19**) (4 mg). The metabolite **16** was purified as its triacetate **16a**.

7α,11β-Dihydroxy-15-oxo-*ent*-(16*S*)-kaur-2-ene (12): colorless crystals; 242−244 °C; $[\alpha]_D$ −91° (*c* 0.30, $CHCl_3$); ^1H NMR (500 MHz) δ 0.90 (3H, s, H-19), 0.98 (3H, s, H-18), 1.02 (3H, s, H-20), 1.15 (1H, s, H-9), 1.27 (3H, d, J = 6.8 Hz, H-17), 1.35 (1H, dd, J = 12.5 and 2.0 Hz, H-5), 1.44 (1H, dt, J = 12.5 Hz, H-6α), 1.71 (1H, d, J = 16.8 Hz, H-1), 1.79 (1H, ddd, J = 12.5, 4, and 2 Hz, H-6β), 1.95 (1H, dt, J = 12 and 4 Hz, H-12), 1.99 (1H, br d, J = 12 Hz, H-12), 2.01 (2H, br s, H-14), 2.13 (1 H, m, H-1), 2.26 (1H, quint, J = 6.8 Hz, H-16), 2.49 (1H, br s, H-13), 3.94 (1H, d, J = 5.4 Hz, H-11), 4.02 (1H, dd, J = 11.7 and 4.1 Hz, H-7), 5.42 (2H, s, H-2 and H-3); EIMS *m/z* (rel int) 318 $[M]^+$ (41), 300 (34), 285 (16), 272 (9), 267 (5), 244 (24), 227 (18), 211 (14), 196 (23), 182 (75); HREIMS *m/z* $[M]^+$ 318.2185 (calcd for $C_{20}H_{30}O_3$, 318.2195).

7α,11β,15β-Trihydroxy-*ent*-kaur-2,16-diene (13): ^1H NMR (200 MHz, $CDCl_3$) δ 0.91 (3H, s, H-19), 0.99 (6H, s, H-18 and H-20), 2.71 (1H, br s, H-13), 3.68 (1H, dd, J = 12 and 4.6 Hz, H-7), 4.03 (1H, d, J = 4.8 Hz, H-11), 4.55 (1H, br s, H-15), 5.08 and 5.44 (each 1H, br s, H-17), 5.44 (2H, br s, H-2 and H-3). After four weeks in a NMR tube this compound was transformed into **19**.

7α,15β-Dihydroxy-11β,16β-epoxy-*ent*-kaur-2,16-diene (19): ^1H NMR (500 MHz, $CDCl_3$) δ 0.90, 0.96, 1.09, and 1.33 (each 3H, s), 1.43 (1H, dt, J = 12.4 Hz, H-6α), 2.01 (1H, dd, J = 16.6 and 6 Hz, H-1α), 3.45 (2H, m, H-7 and H-15), 4.24 (1H, br s, H-11), 5.38 (1H, dd, J = 10 and 2.5 Hz, H-3), 5.45 (1H, ddd, J = 10, 6, and 1.8 Hz, H-2); EIMS *m/z* (rel int) 318 $[M]^+$ (29), 300 (3), 282 (3), 256 (6), 208 (5).

7β,15β-Dihydroxy-*ent*-kaur-2,16-dien-19,6-olide (14): colorless crystals; 253−255 °C ($CHCl_3$); ^1H NMR (500 MHz) δ 0.85 (3H, s, H-20), 1.32 (3H, s, H-18), 1.43 and 2.15 (each 1H, m, H-12), 1.46 (1H, d, J = 11.9 Hz, H-14), 1.66 (1H, dd, J = 11.9 and 5.3 Hz, H-14), 1.70 (1H, br d, J = 16.5 Hz, H-1), 1.96 (1H, dd, J = 16.5 and 6.4 Hz, H-1), 2.28 (1H, d, J = 6.5 Hz, H-5), 2.64 (1H, t, J = 7 Hz, H-13), 4.42 (1H, d, J = 6.5 Hz, H-7), 4.68 (1H, br s, H-15), 4.77 (1H, t, J = 6.5 Hz, H-6), 5.00 and 5.10 (each 1H, br s, H-17), 5.73 (1H, ddd, J = 9.8, 6.4, and 1.6 Hz, H-2), 5.78 (1H, dd, J = 9.8 and 2.5 Hz, H-3); EIMS *m/z* (rel int) 312 $[M]^+$ (58), 284 (41), 269 (15), 240 (19), 235 (17), 207 (8), 195 (16), 169 (16); HREIMS *m/z* $[M]^+$ 312.1724 (calcd for $C_{20}H_{24}O_3$, 312.1725).

1β,7β,15β-Trihydroxy-*ent*-kaur-2,16-diene-19-acid (15). This compound was obtained as the triacetate methyl ester **16a** by methylation of the extract and subsequent acetylation (Ac_2O−py; 80 °C, 12 h) and chromatography of the fractions containing it, eluting with petroleum ether−EtOAc (2:1).

Triacetate methyl ester (16a): a gum; ^1H NMR (500 MHz) δ 1.00 (3H, s, H-20), 1.22 (3H, s, H-18), 1.33 (1H, m, H-14), 1.38 (1H, m, H-11), 1.70 (2H, m, H-11 and H-1), 1.89 (1H, d, J = 12 Hz, H-14), 1.96, 2.05 and 2.09 (each 3H, s, −OAc), 2.07 (1H, dd, J = 9 and 3 Hz, H-5), 2.46 (1H, d, J = 6.9 Hz, H-9), 2.69 (1H, br s, H-13), 3.61 (3H, s, −OMe), 4.78 (1H, br s, H-7), 4.87 and 4.95 (each 1H, br s, H-17), 5.07 (1H, d, J = 5.4 Hz, H-1), 5.45 (1H, br s, H-15), 5.76 (1H, dd, J = 10.1 and 5.4 Hz, H-2), 5.88 (1H, d, J = 10.1 Hz, H-3); EIMS *m/z* (rel int) 488 $[M]^+$ (0.2), 428 (1), 386 (1), 368 (2), 308 (6), 265 (11), 249 (42), 221 (27), 207 (27), 169 (32); HREIMS *m/z* $[M]^+$ 488.2425 (calcd for $C_{27}H_{36}O_8$, 488.2410).

7α,11β,16α-Trihydroxy-15-oxo-*ent*-(16S)-kaur-2-ene (17): colorless plates; mp 231−234 °C (petroleum ether−EtOAc); $[\alpha]_D$ −88° (*c* 0.52, $CHCl_3$); ^1H NMR (500 MHz) δ 0.88 (3H, s, H-19), 0.97 (3H, s, H-18), 1.01 (3H, s, H-20), 1.54 (3H, s, H-17), 1.24 (1H, br s, H-9), 1.33 (1H, dd, J = 12.4 and 2.0 Hz, H-5), 1.42 (1H, dt, J = 12.4 Hz, H-6α), 1.69 (1H, d, J = 16.5 Hz, H-1), 1.83 (1H, ddd, J = 12.4, 4.3, and 2.0 Hz, H-6β), 2.05 (2H, br s, H-12), 2.11 (1H, dd, J = 16.6 and 4.8 Hz, H-1), 2.29 (1H, dd, J = 7 and 4 Hz, H-13), 2.48 (1H, dd, J = 11.9 and 4.4 Hz, H-14), 3.96 (1H, d, J = 4.8 Hz, H-11), 3.99 (1H, dd, J = 11.6 and 4.2 Hz, H-7), 5.41 (2H, br s, H-2 and H-3); EIMS *m/z* (rel int) (334) $[M]^+$ (1), 316 (3), 306 (8), 298 (3), 288 (59), 270 (52), 255 (30), 227 (37), 211 (24); HREIMS *m/z* $[M]^+$ 334.2137 (calcd for $C_{20}H_{30}O_4$, 334.2144).

7α,15β,17-Trihydroxy-11β,16β-epoxy-*ent*-kaur-2-ene (19): a gum; ^1H NMR (500 MHz) δ 0.88 (3H, s, H-19), 0.94 (3H, s, H-18), 1.08 (3H, s, H-20), 1.24 (1H, br d, J = 12.8 Hz, H-5), 1.41 (1H, quint, J = 12.3 Hz, H-6α), 1.55 (1H, br s, H-9), 1.60 (1H, d, J = 12 Hz, H-14), 1.66 (1H, dd, J = 12 and 6.4 Hz, H-14), 1.76 (1H, dd, J = 12, 4.3, and 1.9 Hz, H-6β), 1.88 (1H, m, H-12), 2.03 (1H, dd, J = 16.5 and 5.7 Hz, H-1), 2.13 (1H, d, J = 11.4 Hz, H-12), 2.51 (1H, t, J = 6 Hz, H-13), 3.60 (2H, complex signal, H-7 and H-15), 3.65 and 3.80 (each 1H, d, J = 11.8 Hz, H-17), 4.32 (1H, br s, H-11), 5.37 (1H, dd, J = 10 and 2.6 Hz, H-3), 5.43 (1 H, ddd, J = 10, 6 and 1.4 Hz, H-2); EIMS *m/z* (rel int) 334 $[M]^+$ (23), 316 (6), 303 (40), 288 (12), 279 (7), 255 (5), 227 (10), 185 (11); HREIMS *m/z* $[M]^+$ 334.2152 (calcd for $C_{20}H_{30}O_4$, 334.2144).

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