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Microbial transformation of two 15α-hydroxy-*ent*-kaur-16-ene diterpenes by *Mucor plumbeus*

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ABSTRACT

The microbiological transformation of candidiol (15α,18-dihydroxy-*ent*-kaur-16-ene) by *Mucor plumbeus* led to 3β,15α,18-trihydroxy-*ent*-kaur-16-ene, 6α,15α,18-trihydroxy-*ent*-kaur-16-ene, 3α,15α,18-trihydroxy-*ent*-kaur-16-ene, 11β,15α,18-trihydroxy-*ent*-kaur-16-ene and 15α,17,18-trihydroxy-11β,16β-epoxy-*ent*-kaurane, whilst the incubation of 15α,19-dihydroxy-*ent*-kaur-16-ene gave 9β,15α,19-trihydroxy-*ent*-kaur-16-ene, 3α,15α,19-trihydroxy-*ent*-kaur-16-ene, 11β,15α,19-trihydroxy-*ent*-kaur-16-ene, 6α,15α,19-trihydroxy-*ent*-kaur-16-ene, 15α,17,19-trihydroxy-11β,16β-epoxy-*ent*-kaurane, 19-(β-D-glucopyranosyl)-15α-hydroxy-*ent*-kaur-16-ene and 19-(β-D-glucopyranosyl)-15-oxo-*ent*-kaur-16-ene. An interesting rearrangement in dilute acid medium of 9β,15α,19-trihydroxy-*ent*-kaur-16-ene into 16-oxo-19-hydroxy-*ent*-abiet-8(9),15-diene, is also described in this work.

1. Introduction

Over the last few years, we have been interested in the study of the microbiological transformation of diterpenes by the fungus *Mucor plumbeus*, which possesses a broad specificity in the substrate. The aim of these studies has been to develop models to explain the hydroxylation of these compounds by this microorganism. Firstly, we carried out the biotransformation with this fungus of labdane[1] and labdane oxide derivatives, of the *normal* and *enantio* series.[2,3] Later, we incubated dehydroabietene,[4] stemodane[5] and *ent*-kaur-16-ene diterpenes[6] with this fungus.

In this work, we describe the results of the microbiological transformation by *M. plumbeus* of two *ent*-kaurene derivatives, 15α,18-dihydroxy-*ent*-kaur-16-ene (**1**) and 15α,19-dihydroxy-*ent*-kaur-16-ene (**8**), which are epimeric at C-4. In this way, we can also determine whether a spatial change in the orientation of the hydroxymethylene group at this carbon, from β-equatorial in the former to α-axial in the latter, causes any effect on the biotransformation.

15α,18-Dihydroxy-*ent*-kaur-16-ene (candidiol) (**1**) was isolated for the first time from *Sideritis candicans*[7] and later from other species of this genus,[8,9] whilst 15α,19-dihydroxy-*ent*-kaur-16-ene (**8**) was obtained by lithium aluminium hydride reduction of grandiflorolic acid methyl ester. This alcohol **8** had been prepared by reduction of the corresponding acid, 15α-hydroxy-*ent*-kaur-16-en-19-oic acid, isolated from *Espeletia grandiflora*.[10]

2. Results and discussion

The incubation of candidiol (**1**) with *M. plumbeus* led to five hydroxylated products **2**–**6** (Scheme 1). The last metabolite was obtained as the 18-monoacetate (**7a**) and 17-monoacetate (**7b**) by acetylation and chromatography of the fractions containing it.

The least polar metabolite **2** showed in the HRMS the molecular ion at *m*/*z* 320.2341, in accordance with the molecular formula $C_{20}H_{32}O_3$, indicating that a new oxygen was introduced into the molecule. This must be a part of a secondary hydroxyl group, because a new geminal hydrogen to an alcoholic group was observed at δ 3.70 (br s) in the ^{1}H NMR spectrum. This form of resonance is characteristic of an α-equatorial proton at C-1, C-3 or C-12, or a β-equatorial at C-6. The corresponding alcohol was located at C-3 because it affected the resonances of the hydroxymethylene hydrogens (δ 3.42 and 3.55, each doublet) in comparison with those of the substrate **1** (δ 3.11 and 3.43). Moreover, in the ^{13}C NMR spectrum β or γ-*gauche* effects, due to the 3β-alcohol in **2**, were

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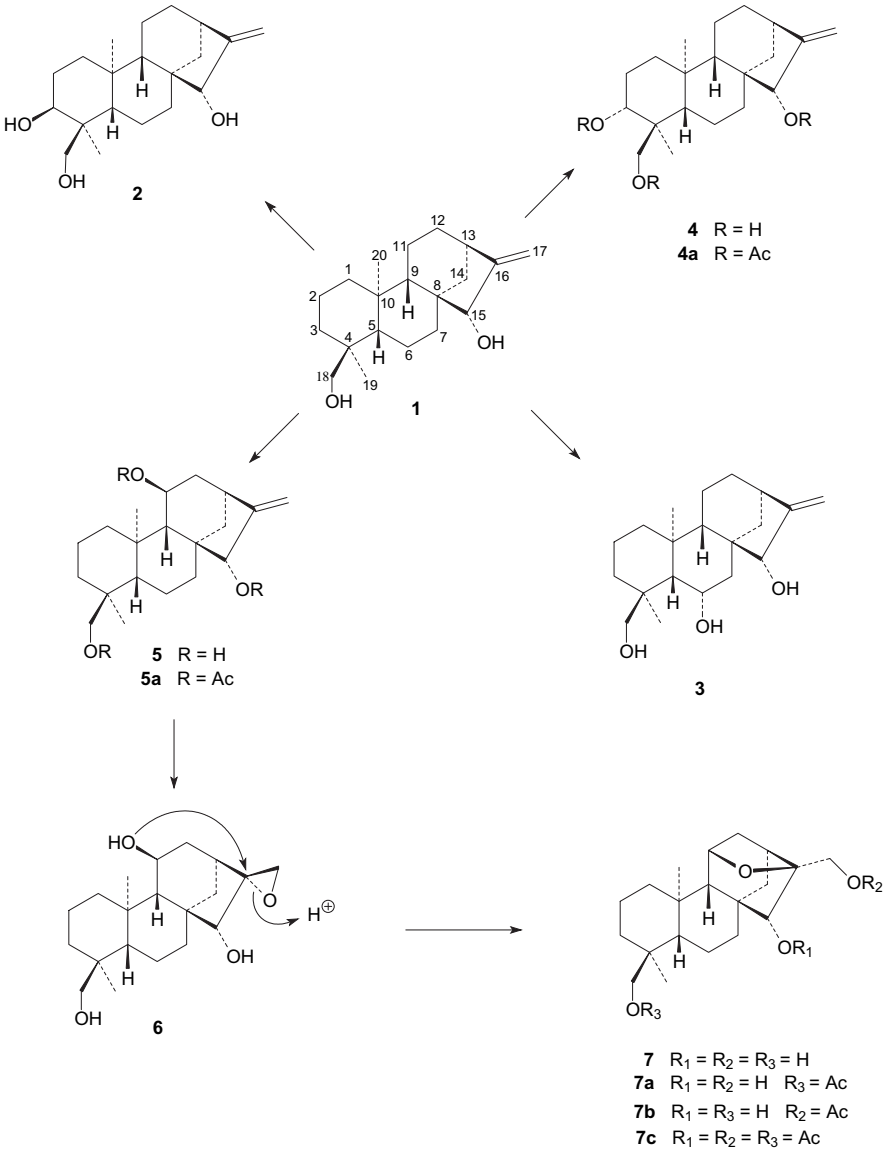


Scheme 1.

observed in the chemical shifts of C-1, C-2, C-4 and C-5, δ 33.0, 26.3, 40.5 and 42.8, in comparison with the corresponding carbons of the substrate, δ 39.9, 17.9, 37.5 and 49.1, respectively. In addition, a new methine was also observed in this spectrum at δ 76.4 (Table 1). This fact was confirmed by study of the 2D NMR spectra. Thus, in the HMBC experiment correlations of H-3 with C-1 and C-5, and of H-18 and H-19 with C-3, were observed. Consequently, the structure of this metabolite was determined as 3β,15α,18-trihydroxy-*ent*-kaur-16-ene (**2**).

The second of the products isolated from this feeding was **3**, an isomer of **2**. In comparison with the substrate **1** a new signal at δ 4.46 (br s) appeared in the ^{1}H NMR spectrum, which is typical of an equatorial proton geminal to a hydroxyl group. In this spectrum shifting of the H-19 and H-20 methyls to lower field was also observed, which permitted this alcohol group to be assigned to C-6(α). The 2D NMR experiments confirmed this and allowed the assignment of the ^{1}H and ^{13}C NMR spectra. Thus, the structure of this compound was determined as 6α,15α,18-trihydroxy-*ent*-kaur-16-ene (**3**).

The structure 3α,15α,18-trihydroxy-*ent*-kaur-16-ene (**4**) was assigned to another metabolite obtained in this incubation, on the

Table 1
^{13}C NMR data of compounds **1**–**5**, **4a**, **5a** and **7a**

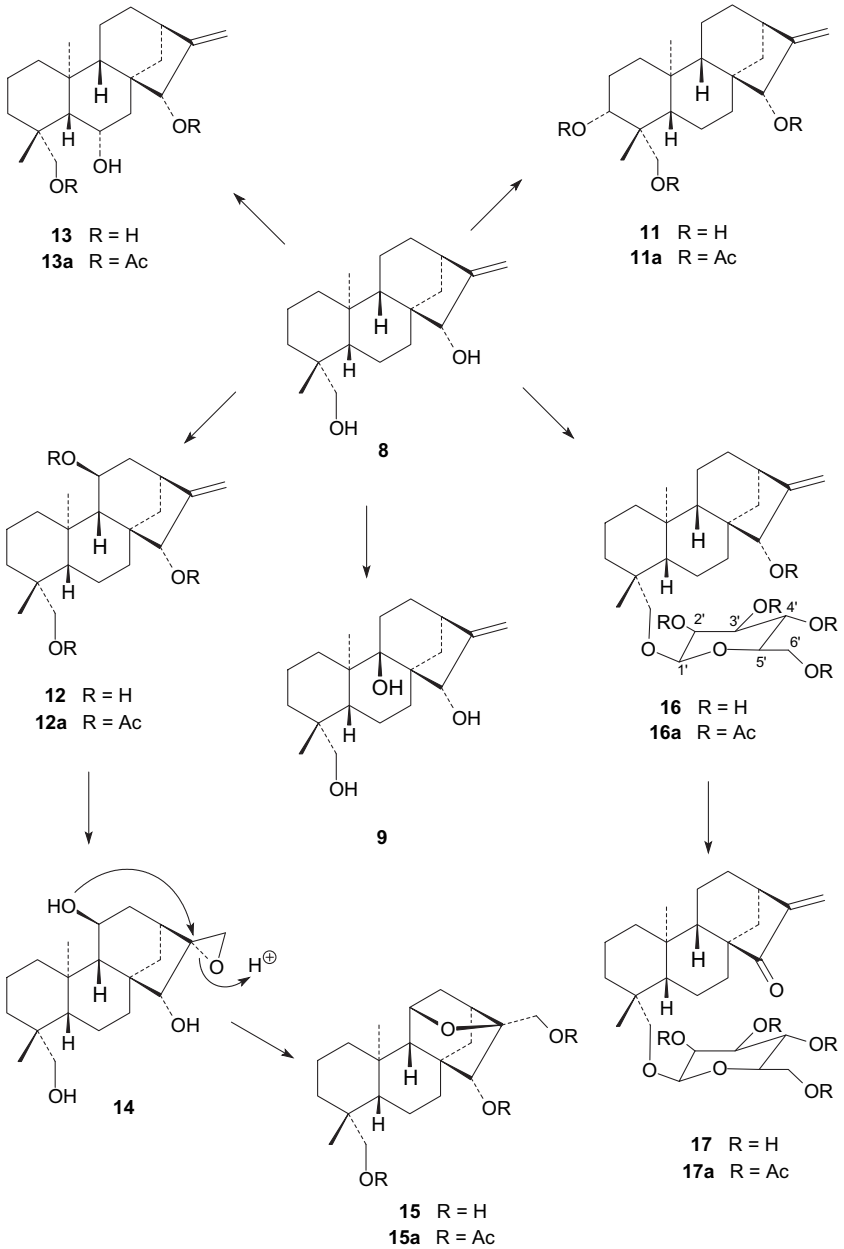
Carbon	1	2	3	4	4a	5	5a	7a
1	39.9	33.0	42.4	38.4	37.9	39.4	39.3	40.7
2	17.9	26.3	18.1	26.9	23.0	17.9	17.6	17.6
3	35.2	76.4	37.2	76.5	74.2	35.1	35.4	35.5
4	37.5	40.5	38.4	41.9	40.5	38.1	38.4	36.7
5	49.1	42.8	50.3	49.6	47.3	49.0	49.3	49.8
6	19.1	18.8	67.6	19.3	18.6	18.9	18.9	19.3
7	34.8	34.6	42.9	34.9	34.2	34.3	33.8	32.1
8	47.6	47.5	45.5	47.4	47.1	46.9	46.4	48.4
9	54.2	53.9	54.2	54.0	53.4	63.9	60.1	59.5
10	39.3	39.1	38.9	39.0	39.0	37.6	36.5	36.3
11	18.0	18.1	18.1	18.1	18.3	66.6	68.7	77.0
12	32.8	32.8	32.7	32.7	32.8	43.0	39.6	40.4
13	42.3	42.3	43.0	42.2	42.4	40.6	40.7	40.9
14	36.3	36.3	37.6	36.2	37.1	35.7	36.6	38.0
15	82.9	82.9	83.6	82.8	83.0	82.2	82.2	86.2
16	160.2	160.0	160.6	160.2	155.3	160.6	155.3	90.1
17	108.3	108.4	108.1	108.4	110.2	109.4	109.4	63.5
18	72.1	71.3	72.4	72.0	65.2	72.0	72.5	72.8
19	17.5	17.8	19.7	11.4	13.2	17.6	17.6	17.8
20	18.1	17.8	19.6	18.0	18.2	17.7	18.9	19.3

following basis. The HRMS of this compound indicated that it was an isomer of **2** and **3** ($C_{20}H_{32}O_3$). The geminal hydrogen to the new hydroxyl group in C-3 resonates in the ^{1}H NMR spectrum at δ 3.63, as a triplet with a coupling constant of 8.2 Hz. This alcohol also affected the resonance of the AB system of the two H-18. The carbon bearing the new alcohol appeared in the ^{13}C NMR spectrum at δ 76.5. This signal showed cross-peaks with H-2, H-18 and H-19 in the HMBC experiment. Compound **4** has been isolated from *Suregada multiflora*, showing appreciable anti-allergic activity.[11]

The fourth product **5** showed the molecular ion in its HRMS in accordance with the formula $C_{20}H_{32}O_3$. The ^{1}H NMR spectrum was very similar to that of the substrate **1**, the main difference being the appearance at δ 3.90 (d, J=5.4 Hz) of a geminal proton to a new hydroxyl group. This chemical shift and form of resonance are typical of a hydrogen geminal to an 11β-hydroxyl group.[12,13] Thus, we assigned to this metabolite the structure of 11β,15α,18-trihydroxy-*ent*-kaur-16-ene (**5**). We had obtained this product by biotransformation of the same substrate **1** by the fungus *Gibberella fujikuroi*.[12]

The most polar compound **7** was obtained by acetylation with Ac_2O–pyridine, at room temperature, of the fractions containing it. In this way, two monoacetates **7a** and **7b**, the former in better yield, were isolated with the molecular formula $C_{22}H_{34}O_5$. Later, compound **7a** was acetylated under reflux affording the triacetate **7c**. This product was identical with another one, which had been prepared by acetylation of a triol, formed in a feeding of candidiol (**1**) with *G. fujikuroi*.[14] Compound **7** may be an artefact formed during the isolation procedure from the true biotransformed metabolite **6**. Thus, opening of the oxirane ring and neutralization of the carbocation formed at C-16, by attack of the 11β-hydroxyl group, led to the alcohol **7**.

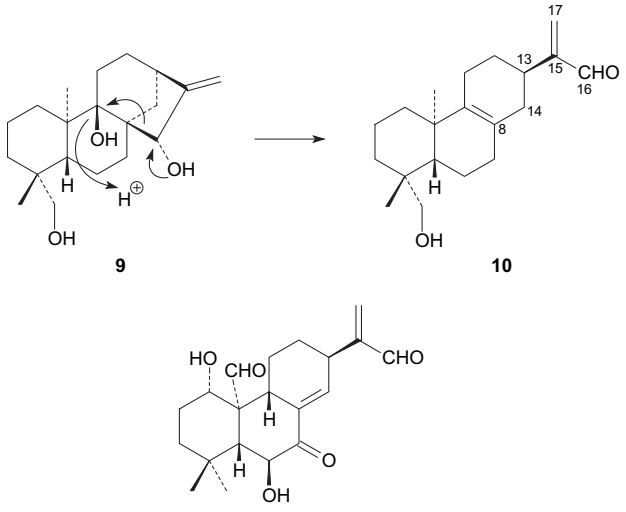
The incubation of 15α,19-dihydroxy-*ent*-kaur-16-ene (**8**) with *M. plumbeus* led to the isolation of the metabolites **9**, **11**–**13** and **15**–**17** (Scheme 2). The structure of the least polar compound was determined to be 9β,15α,19-trihydroxy-*ent*-kaur-16-ene (**9**). Its HRMS showed the molecular ion at m/z 320.2361 ($C_{20}H_{32}O_3$). In comparison with the substrate, no new signal appeared in the ^{1}H NMR



Scheme 2.

spectrum. We observed that this product rearranges into **10** in the NMR tube when $CDCl_3$ is used as solvent (see below); consequently, we ran the ^{13}C NMR spectrum and the 2D experiments in C_6D_6. In the first one, a new singlet appeared at δ 77.1, which is typical of a carbon bearing a tertiary alcohol. This was assigned to C-9 considering the observed shift of H-15 to lower field (δ 4.57) and of C-15 to higher field (δ 77.6). This location was confirmed in the HMBC experiment by a correlation of the methyl group protons of H-20 with C-9. The β-stereochemistry of the 9-OH was determined considering the H-15 resonance, which was identical with that observed in the methyl ester of 9β,15α-dihydroxy-*ent*-kaur-16-en-19-oic acid.[15,16]

The molecular formula of **10**, formed by rearrangement of **9**, was found to be $C_{20}H_{30}O_2$. Its ^1H NMR spectrum showed, in comparison with that of **9**, the signal of an aldehyde group at δ 9.52 (s), the downfield shift of the two H-17 at δ 5.99 and 6.17, due to the conjugation with the oxo group of the aldehydic function, and the disappearance of the geminal proton to the hydroxyl group at C-15. In its ^{13}C NMR spectrum two new carbons of a tetrasubstituted double bond appeared at δ 124.9 and 137.8, whilst the carbon of an aldehyde group resonated at δ 194.8 (d). Signals of secondary carbons bearing hydroxyl groups were not displayed in this spectrum. In the HMBC experiment correlations of H-14 with C-8, of H-17 with C-13, C-15 and C-16, and of H-20 with C-9, were observed. Therefore, the structure of this rearranged compound was determined as 16-oxo-19-hydroxy-*ent*-abiet-8(9),15-diene (**10**). The possible mechanism of formation of this 8,15-*seco*-*ent*-kaurene diterpene is shown in Scheme 3. A compound of this type, named hebeiabinin A (**18**), has been isolated from *Isodon rubescens*.[17]



Scheme 3.

The following product in polarity order was **11**, which was obtained as its triacetate **11a** by acetylation and chromatography of the fractions containing it. This compound possesses one more oxygen than the substrate, considering the molecular formula of its triacetate, $C_{26}H_{38}O_6$. In the ^1H and ^{13}C NMR spectra of **11a** new signals at δ 4.53 (dd, J=10.0 and 6.7 Hz) and δ 80.1, respectively, were observed. These resonances correspond to a geminal hydrogen to an α-equatorial acetoxy group, which was located at C-3, taking into consideration its correlations with C-18 and C-19 in the HMBC spectrum. Therefore, the structure of the original alcohol, formed in the incubation, was determined as 3α,15α,19-trihydroxy-*ent*-kaur-16-ene (**11**).

Another compound isolated from this fermentation was **12**, which is also characterized by the introduction in the molecule of a new oxygen atom. Thus, in the ^1H NMR spectrum the resonance of a new geminal hydrogen to an alcohol group was located at δ 3.89 (d, J=5.6 Hz). This chemical shift and coupling were similar to those observed in compound **5**, indicating that the new hydroxyl group must be situated at C-11(β), which was confirmed in the HMBC spectrum with cross-peaks of H-11 with C-8 (δ 46.9) and C-13 (δ 40.6), and of H-9 with C-11 (δ 66.6). Thus, the structure 11β,15α,19-trihydroxy-*ent*-kaur-16-ene (**12**) was assigned to this substance.

Compound **13** was obtained as its diacetyl derivative **13a** by acetylation of the fractions containing it. Its HRMS spectrum showed the molecular ion at m/z 404.2552 ($C_{24}H_{36}O_5$), which corresponds to a triol of molecular formula $C_{20}H_{32}O_3$. Consequently, it is an isomer of **12**. The ^1H NMR spectrum of the diacetate **13a** showed the signal of a new geminal proton to a secondary hydroxyl group at δ 4.44 as a broad singlet, which is typical of an equatorial hydroxyl group, whose corresponding carbon resonates at δ 66.5. This relatively low chemical shift is characteristic of carbons, such as C-6 or C-12, situated between a methylene and a methine group. The existence of correlations of H-6 with C-4 and C-8 permitted to this hydroxyl group to be assigned to the position C-6. Therefore, the structure of the alcohol formed in the biotransformation was determined as 6α,15α,19-trihydroxy-*ent*-kaur-16-ene (**13**). The non-acetylation of the 6α-alcohol can be explained by the steric hindrance due to the C-20 methyl.

Another substance obtained from this feeding was **15**, which was isolated in the form of its triacetate **15a**, an isomer of **7c**, indicating that two new oxygens had been introduced in the molecule of the substrate **8**. The disappearance in the ^1H NMR spectrum of **15a** of the double bond resonances, the presence of signals from a new $-CH_2OAc$ group and of a geminal proton to an oxygen function, indicated that the C and D rings of **15a** and **7c** should have a similar structure. This was confirmed in the HMBC experiment where correlations of C-16 with H-12, H-14, H-15 and H-17, of C-15 with H-9, H-14 and H-7, of C-11 with H-9 and H-13, and of C-17 with H-13, were observed. Consequently, the structure of the corresponding alcohol was determined as 15α,17,19-trihydroxy-11β,16β-epoxy-*ent*-kaurane (**15**). As in the case of **7**, this alcohol **15**, corresponding to the triacetate **15a**, must be an artefact formed during the isolation procedure and, consequently, the true biotransformed product of **8** must be the epoxide **14** (Table 2).

The most polar compounds of this feeding were two diterpene glucosides, **16** and **17**, which were separated as their peracetates **16a** and **17a**, respectively, by acetylation of the fractions containing them. The first of them, with a molecular formula of $C_{36}H_{52}O_{12}$, showed in its ^1H NMR spectrum signals of five acetoxy groups. In the ^{13}C NMR spectrum signals at 62.1 (t), 68.7 (d), 71.3 (d), 71.6 (d), 72.9 (d) and 101.2 (d) indicated the presence in the molecule of an acetylated glucose. A correlation of H-19 with C-1′ of the glucose showed the union of C-19 with the anomeric carbon. The β-configuration of this carbon was indicated by the H-1′ coupling constant (J=7.9 Hz). Therefore, the structure 19-(β-D-glucopyranosyl)-15α-hydroxy-*ent*-kaur-16-ene (**16**)was assigned to the original metabolite formed in the incubation.

The second of the acetylated glucosides (**17a**) showed in the HRMS the molecular ion to m/z 632.3174, which loses a molecule of acetylated glucose to give a peak at m/z 284. The ^1H NMR spectrum was very similar to compound **16a**, but now the disappearance of the H-15 and the downfield shift of the two H-17 were observed. These facts indicated that the hydroxyl group at C-15 had been oxidized to an oxo group, being now conjugated with the 16,17-double bond. The carbon of this new oxo group appeared at δ 210.5. Thus, the structure of the original compound formed in the

Table 2
^{13}C NMR data of compounds **8**–**10**, **11a**–**13a**, **12** and **15a**–**17a**

Carbon	**8**	**8**[c]	**9**[c]	**10**	**11a**	**12**	**12a**	**13a**	**15a**	**16a**	**17a**
1	40.4	40.6	32.4	37.1	38.4	40.0	39.8	43.0	41.0	40.2	39.6
2	18.0[a]	18.3[a]	18.6	18.7[a]	23.6	18.2	18.0	18.7[a]	18.0	18.2	18.0[a]
3	35.5[b]	35.9[b]	35.5	35.5	80.1	35.1	36.0	39.3	36.0	36.2	36.1
4	38.6	38.8	38.8	38.7	41.1	37.9	37.0	39.6	36.9[a]	37.7	37.7
5	56.7	56.7	49.0	52.7	55.4	56.5	56.0	57.5	56.3	56.1	55.7
6	19.6	19.9	19.9	19.0[a]	20.1	19.4	19.2	66.5	19.3	19.6	18.9
7	35.6[b]	35.4[b]	30.9	32.9	35.0	35.4[a]	36.5	41.8	32.7	35.1	36.6
8	47.6	47.9	52.4	124.9	47.2	46.9	46.5	45.8	48.6	47.3	52.4
9	54.4	54.6	77.1	137.8	53.6	64.0	60.2	54.5	59.2	54.0	57.7
10	39.4	39.5	44.4	37.5	39.0	38.7	38.3	38.2	36.7[a]	39.4	40.0
11	18.2[a]	18.7[a]	29.1	21.1	18.4	66.6	68.6	18.8[a]	77.4	18.2	18.2[a]
12	32.6	33.0	34.4	26.8	32.7	43.0	39.5	33.1	40.1	32.8	32.4
13	42.3	42.8	41.4	30.8	42.4	40.6	40.7	43.5	41.7	42.5	38.1
14	36.2	36.4	36.7	35.3	36.9	35.5[a]	34.5	38.9	38.6	37.2	33.9
15	82.8	82.8	77.6	153.9	82.9	82.1	82.2	84.4	84.7	83.1	210.5
16	160.2	161.0	160.0	194.8	155.2	160.6	155.2	155.3	88.6	155.4	149.4
17	108.3	107.8	108.0	133.4	110.2	109.4	109.4	110.2	63.4	110.1	114.5
18	27.0	27.3	27.4	26.7	22.8	27.0	27.5	27.9	27.8	27.5	27.5
19	65.5	65.2	65.4	65.5	65.3	65.6	67.0	69.4	67.0	73.2	73.0
20	18.2	18.3	19.9	20.4	17.5	17.8	18.0	19.6	19.4	18.1	18.0
1′										101.2	101.2
2′										71.3	71.4
3′										72.9	72.9
4′										68.7	68.8
5′										71.6	71.7
6′										62.1	62.1

[a] These values can be interchanged.
[b] These values can be interchanged.
[c] Solvent C_6D_6.

biotransformation was determined as 19-(β-D-glucopyranosyl)-15-oxo-*ent*-kaur-16-ene (**17**), which was confirmed by a study of the 2D NMR spectra. Diterpene glucosides derived from *ent*-kaurenoic acid have been shown to possess trypanocidal activity.[18]

Several conclusions can be obtained from the results of these biotransformations:

1. Hydroxylations at C-3(α), C-6(α) or C-11(β) occur in both biotransformations. This means that a change in the spatial orientation of the hydroxymethylene group at C-4, from *equatorial* in **1** to *axial* in **8**, does not affect the way in which these *ent*-kaurenes bind to the oxidative enzymes. The preference for an equatorial hydroxylation at C-3 by this fungus had been observed with other diterpenic substrates.[4,5,19]
2. Epoxidation of the exocyclic double bond also occurred in both fermentations. This type of reaction had also been observed in the incubation of other diterpenes of the *enantio* and *normal* series with this fungus.[2,3,6]
3. The 3β-axial hydroxylation, together with the 3α named above, was also produced in the incubation of **1**, which indicates a loss of stereoselectivity at C-3 in this feeding. This fact had also been observed at positions such as C-2, C-3, C-6, C-7 and C-11 in other diterpenic biotransformations carried out with this microorganism.
4. A C-9(β) hydroxylation was produced in the incubation of **8**. This reaction had also been observed in the feeding of candicandiol (7α,18-dihydroxy-*ent*-kaur-16-ene) with this fungus.[6] Consequently, a 15α,19-diol is equivalent to a 7α,18-diol in the molecular recognition of the substrate by the enzyme involved in this reaction.
5. Glucosides were formed in the feeding of **8** (α-axial CH_2OH), but not in that of **1** (β-equatorial CH_2OH), this being the first time that *ent*-kaurene derivatives of this type are formed in a biotransformation by a *Mucor* species. Other glucosyl derivatives had been obtained in the feeding of the mycotoxin zearalenone[20] and the steroid resibufogenin[21] with other species of *Mucor*.
6. The rearrangement in dilute acid medium of 9β,15α,19-trihydroxy-*ent*-kaur-16-ene (**9**) to form the aldehyde **10**, which possesses an abietane skeleton, is now described for the first time. This is an interesting reaction, because it permits the preparation of abiet-8-ene derivatives functionalized at the isopropyl group.

3. Experimental

3.1. General experimental details

Mps were determined with a Reichert Thermovar apparatus and are uncorrected. IR spectra were taken in a Thermo Nicolet Avatar 360 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ soln at 500.13 and 125.77 MHz, respectively, with a Bruker AMX2-500 spectrometer. Chemical shifts are given in parts per million (δ). Mass spectra were taken at 70 eV (probe) in a Micromass Autospec spectrometer. Semipreparative HPLC was carried out with a Beckman System Gold 125P. Dry column chromatographies were made on silica gel Merck 0.015–0.04 mm. The substances were crystallised from petrol–EtOAc except where otherwise indicated.

3.2. Organism

The fungal strain, *M. plumbeus* CMI 116688, was a gift from Prof. J.R. Hanson, Department of Chemistry, University of Sussex, UK.

3.3. Substrates

*3.3.1. 15α,18-Dihydroxy-ent-kaur-16-ene (candidiol) (**1**).* ^{1}H NMR (500 MHz) δ 0.76 (1H, td, *J*=12.8 and 4.0 Hz, H-1β), 0.77 (3H, s, H-19), 1.07 (3H, s, H-20), 1.07 (1H, d, *J*=7.9 Hz, H-9), 1.13 (1H, dd, *J*=12.1 and 1.7 Hz, H-5), 1.82 (1H, dt, *J*=12.8 and 3.3 Hz, H-1α), 1.92 (1H, dd, *J*=11.5 and 1.6 Hz, H-14), 2.74 (1H, br s, H-13), 3.11 and 3.43 (each 1H, d, *J*=10.8 Hz, H-18), 3.81 (1H, s, H-15), 5.07 and 5.20 (each

1H, s, H-17); EIMS *m*/*z* (rel int.) 304 $[M]^+$ (14), 289 (17), 286 (11), 273 (100), 255 (54), 246 (40), 229 (8), 221 (9), 203 (9), 189 (10), 173 (18), 159 (15), 147 (22), 135 (24), 123 (42); HREIMS *m*/*z* 304.2354 (calcd for $C_{20}H_{32}O_2$, 304.2402).

*3.3.2. 15α,19-Dihydroxy-ent-kaur-16-ene (**8**).* This substrate was obtained by lithium aluminium hydride reduction of grandiflorolic acid methyl ester. ^{1}H NMR (500 MHz) δ 0.77 (1H, td, *J*=13.0 and 3.9 Hz, H-1β), 0.93 (2H, m, H-3β and H-5), 0.96 (3H, s, H-18), 1.00 (3H, s, H-20), 1.02 (1H, d, *J*=8.5 Hz, H-9), 1.33 (1H, m, H-14), 1.40 (1H, m, H-6), 1.45 (1H, m, H-12), 1.56 (1H, m, H-11), 1.63 (1H, ddd, *J*=12.7, 5.7 and 2.2 Hz, H-12), 1.72 (1H, m, H-6), 1.77 (1H, br d, *J*=13.6 Hz, H-3α), 1.84 (1H, br d, *J*=12.8 Hz, H-1α), 1.86 (1H, dd, *J*=12.0 and 1.4 Hz, H-14), 2.72 (1H, br s, H-13), 3.44 and 3.73 (each 1H, d, *J*=11.0 Hz, H-19), 3.79 (1H, s, H-15), 5.06 and 5.19 (each 1H, s, H-17); ^{1}H NMR (500 MHz, C_6D_6) δ 0.59 (1H, td, *J*=13.1 and 4.0 Hz, H-1β), 0.77 (1H, dd, *J*=12.3 and 1.8 Hz, H-5), 0.84 (3H, s, H-18), 0.94 (3H, s, H-20), 1.73 (1H, dd, *J*=11.8 and 1.7 Hz, H-14), 1.77 (1H, dt, *J*=13.5 and 2.3 Hz, H-1α), 1.85 (1H, dtd, *J*=13.5, 3.4 and 1.6 Hz, H-3α), 2.60 (1H, br s, H-13), 3.23 (1H, dd, *J*=10.8 and 1.2 Hz, H-19), 3.53 (1H, d, *J*=10.8 Hz, H-19), 3.73 (1H, br s, H-15), 4.98 and 5.16 (each 1H, br s, H-17); EIMS *m*/*z* (rel int.) 304 $[M]^+$ (24), 289 (29), 286 (12), 274 (22), 273 (68), 271 (16), 256 (21), 255 (94), 246 (33), 229 (8), 215 (8), 203 (8); HREIMS *m*/*z* 304.2410 (calcd for $C_{20}H_{32}O_2$, 304.2402).

3.4. Fermentation and isolation conditions

M. plumbeus was grown in shake culture at 25 °C for two days in 20–22 conical flasks (250 ml), each containing sterile medium[6] (50 ml). The substrate (see below) in EtOH (4–4.5 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The mycelium was filtered and the broth extracted with EtOAc in the usual way. The solvent was evaporated to give a residue, which was chromatographed on a silica gel column using a petroleum ether–EtOAc gradient. Fractions collected were purified further by HPLC when necessary.

3.5. Incubation of 15α,18-dihydroxy-*ent*-kaur-16-ene (1)

The biotransformation of **1** (253 mg) gave 3β,15α,18-trihydroxy-*ent*-kaur-16-ene (**2**) (14 mg), 6α,15α,18-trihydroxy-*ent*-kaur-16-ene (**3**) (2 mg), 3α,15α,18-trihydroxy-*ent*-kaur-16-ene (**4**) (20 mg), 11β,15α,18-trihydroxy-*ent*-kaur-16-ene (**5**) (5 mg) and 15α,17,18-trihydroxy-11β,16β-epoxy-*ent*-kaurane (**7**), which was obtained as its 18-acetate (**7a**) (1.6 mg) and its 17-acetate (**7b**) (0.5 mg) by acetylation and chromatography of the fractions containing it.

*3.5.1. 3β,15α,18-Trihydroxy-ent-kaur-16-ene (**2**).* Mp 210–212 °C; ^{1}H NMR (500 MHz) δ 0.71 (3H, s, H-19), 1.07 (3H, s, H-20), 1.18 (1H, d, *J*=8.3 Hz, H-9), 1.26 (1H, td, *J*=13.6 and 3.6 Hz, H-1β), 1.39 (1H, dd, *J*=11.8 and 5.9 Hz, H-14), 1.50 (1H, m, H-2), 1.70 (2H, m, H-5 and H-7), 1.91 (1H, d, *J*=11.8 Hz, H-14), 1.96 (1H, m, H-2), 2.75 (1H, br s, H-13), 3.42 and 3.55 (each 1H, d, *J*=11.4 Hz, H-18), 3.70 (1H, br s, H-3), 3.84 (1H, s, H-15), 5.08 and 5.22 (each 1H, s, H-17); EIMS *m*/*z* (rel int.) 320 $[M]^+$ (20), 302 (36), 287 (31), 272 (36), 271 (66), 269 (30), 253 (26), 229 (16), 213 (16), 185 (18), 159 (27), 145 (37); HREIMS *m*/*z* 320.2341 (calcd for $C_{20}H_{32}O_3$, 320.2351).

*3.5.2. 6α,15α,18-Trihydroxy-ent-kaur-16-ene (**3**).* Mp 246–248 °C; ^{1}H NMR (500 MHz) δ 0.76 (1H, td, *J*=12.8 and 3.5 Hz, H-1β), 1.14 (1H, d, *J*=8.0 Hz, H-9), 1.16 (3H, s, H-19), 1.20 (1H, m, H-3), 1.42 (3H, s, H-20), 1.51 (1H, m, H-14), 1.78 (2H, m, H-1α and H-7), 2.28 (1H, d, *J*=11.5 Hz, H-14), 2.77 (1H, br s, H-13), 3.21 and 3.59 (each 1H, d, *J*=10.8 Hz, H-18), 3.84 (1H, s, H-15), 4.46 (1H, br s, H-6), 5.09 and 5.22 (each 1H, s, H-17); EIMS *m*/*z* (rel int.) 320 $[M]^+$ (11), 305 (6), 302 (26), 289 (31), 287 (17), 272 (23), 271 (58), 269 (34), 257 (18), 253 (16), 201 (10), 187 (10), 175 (11), 159 (16), 151 (23), 145 (22); HREIMS *m*/*z* 320.2365 (calcd for $C_{20}H_{32}O_3$, 320.2351).

*3.5.3. 3α,15α,18-Trihydroxy-ent-kaur-16-ene (**4**).* Mp 174–176 °C; ^{1}H NMR (500 MHz) δ 0.88 (3H, s, H-19), 0.89 (1H, m overlapped with H-19 and H-5, H-1β), 0.92 (1H, d, *J*=10.8 Hz, H-5), 1.01 (1H, d, *J*=8.4 Hz, H-9), 1.07 (3H, s, H-20), 1.37 (1H, m, H-14), 1.56 (1H, dd, *J*=14.9 and 5.7 Hz, H-11), 1.64 (2H, m, H-2 and H-12), 1.70 (1H, m, H-7), 1.86 (1H, dt, *J*=13.2 and 3.4 Hz, H-1α), 1.89 (1H, d, *J*=11.8 Hz, H-14), 2.74 (1H, br s, H-13), 3.41 and 3.71 (each 1H, d, *J*=10.3 Hz, H-18), 3.63 (1H, t, *J*=8.2 Hz, H-3), 3.79 (1H, s, H-15), 5.07 and 5.20 (each 1H, s, H-17); EIMS *m*/*z* (rel int.) 320 $[M]^+$ (5), 305 (7), 302 (23), 287 (31), 284 (18), 272 (14), 271 (42), 269 (16), 253 (13), 242 (26), 229 (13), 185 (19), 145 (30). *Triacetate* (**4a**) HREIMS *m*/*z* 446.2674 (calcd for $C_{26}H_{38}O_6$, 446.2668); ^{1}H NMR (500 MHz) δ 0.82 (3H, s, H-19), 1.00 (1H, td, *J*=13.3 and 3.5 Hz, H-1β), 1.09 (3H, s, H-20), 1.86 (1H, dt, *J*=13.3 and 3.6 Hz, H-1α), 1.93 (1H, d, *J*=11.7 Hz, H-14), 2.80 (1H, br s, H-13), 3.71 and 3.80 (each 1H, d, *J*=12.0 Hz, H-18), 4.76 (1H, dd, *J*=11.9 and 5 Hz, H-3), 5.09 and 5.13 (each 1H, s, H-17), 5.26 (1H, s, H-15); EIMS *m*/*z* (rel int.) 446 $[M]^+$ (2), 404 (4), 386 (72), 371 (20), 344 (31), 326 (100), 311 (58), 284 (60), 269 (30), 266 (57), 253 (46), 251 (56), 246 (30); HREIMS *m*/*z* 320.2347 (calcd for $C_{20}H_{32}O_3$, 320.2351).

*3.5.4. 11β,15α,18-Trihydroxy-ent-kaur-16-ene (**5**).* ^{1}H NMR (500 MHz) δ 0.77 (3H, s, H-19), 0.96 (3H, s, H-20), 1.04 (1H, td, *J*=13.1 and 3.6 Hz, H-1β), 1.22 (1H, dd, *J*=12.0 and 1.1 Hz, H-5), 1.41 (1H, br s, H-9), 1.45 (1H, m, H-14), 1.65 (1H, qt, *J*=13.8 and 3.6 Hz, H-2α), 1.79 (1H, dt, *J*=12.8 and 2.9 Hz, H-7), 1.84 (1H, dd, *J*=11.7 and 1.8 Hz, H-14), 1.92 (2H, m, H-1α and H-12α), 2.01 (1H, ddd, *J*=14.7, 5.7 and 2.9 Hz, H-12β), 2.78 (1H, br s, H-13), 3.14 and 3.44 (each 1H, d, *J*=10.8 Hz, H-18), 3.90 (1H, d, *J*=5.4 Hz, H-11), 4.28 (1H, s, H-15), 5.24 and 5.27 (each 1H, s, H-17); EIMS *m*/*z* (rel int.) 320 $[M]^+$ (12), 302 (23), 289 (13), 287 (13), 272 (26), 271 (62), 269 (13), 253 (21), 239 (12), 215 (12), 213 (10), 189 (12), 177 (13), 167 (15), 159 (14), 149 (53), 145 (21); HREIMS *m*/*z* 320.2361 (calcd for $C_{20}H_{32}O_3$, 320.2351). *Triacetate* (**5a**) ^{1}H NMR (500 MHz) δ 0.82 (3H, s, H-19), 0.92 (1H, td, *J*=13.0 and 3.6 Hz, H-1β), 1.02 (3H, s, H-20), 1.16 (1H, d, *J*=11.4 Hz, H-5), 1.49 (1H, br s, H-9), 1.50 (2H, m overlapped with H-9, H-2 and H-14), 1.61 (1H, m, H-2), 1.68 (1H, dd, *J*=10.4 and 3.2 Hz, H-7α), 1.95, 2.07 and 2.08 (each 3H, s), 2.77 (1H, br s, H-13), 3.62 and 3.85 (each 1H, d, *J*=11.0 Hz, H-18), 5.04 and 5.09 (each 1H, s, H-17), 5.08 (1H, m, H-11), 5.64 (1H, s, H-15); EIMS *m*/*z* (rel int.) 446 $[M]^+$ (0.4), 404 (5), 386 (48), 371 (8), 344 (56), 326 (81), 313 (37), 311 (100), 284 (36), 269 (19), 266 (31), 253 (80), 251 (45), 211 (13); HREIMS *m*/*z* 446.2675 (calcd for $C_{26}H_{38}O_6$, 446.2668).

*3.5.5. 18-Acetoxy-15α,17-dihydroxy-11β,16β-epoxy-ent-kaurane (**7a**).* IR ($CHCl_3$) ν_{max} 3500, 1730, 1241 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.87 (3H, s, H-19), 1.17 (3H, s, H-20), 1.50 (1H, d, *J*=3 Hz, H-9), 1.90 (1H, d, *J*=3.1 Hz, H-14), 2.38 (1H, br s, H-13), 3.62 and 3.87 (each 1H, d, *J*=11.0 Hz, H-18), 3.68 (1H, s, H-15), 3.81 and 3.92 (each 1H, d, *J*=11.7 Hz, H-17), 4.36 (1H, t, *J*=3.3 Hz, H-11); EIMS *m*/*z* (rel int.) 378 $[M]^+$ (3), 360 (2), 347 (17), 332 (26), 329 (13), 318 (19), 305 (27), 301 (32), 287 (37), 272 (42), 269 (36), 257 (34), 229 (42); HREIMS *m*/*z* 378.2388 (calcd for $C_{22}H_{34}O_5$, 378.2406).

*3.5.6. 17-Acetoxy-15α,18-dihydroxy-11β,16β-epoxy-ent-kaurane (**7b**).* ^{1}H NMR (500 MHz) δ 0.86 (3H, s, H-19), 1.16 (3H, s, H-20), 2.29 (1H, t, *J*=6.3 Hz, H-13), 3.10 and 3.39 (each 1H, d, *J*=11.2 Hz, H-18), 3.49 (1H, s, H-15), 4.01 and 4.74 (each 1H, d, *J*=11.8 Hz, H-17), 4.40 (1H, m, H-11); EIMS *m*/*z* (rel int.) 378 $[M]^+$ (3), 360 (2), 347 (17), 332 (26), 329 (13), 318 (19), 305 (27), 301 (32), 287 (37), 272 (42), 269

(36), 257 (34), 229 (42); HREIMS *m/z* 378.2403 (calcd for $C_{22}H_{34}O_5$, 378.2406).

3.6. Incubation of 15α,19-dihydroxy-*ent*-kaur-16-ene (8)

The biotransformation of **8** (338 mg) gave starting material (102 mg), 9β,15α,19-trihydroxy-*ent*-kaur-16-ene (**9**) (5 mg), 3α,15α,19-trihydroxy-*ent*-kaur-16-ene (**11**) (2 mg), 11β,15α,19-trihydroxy-*ent*-kaur-16-ene (**12**) (2 mg), 6α,15α,19-trihydroxy-*ent*-kaur-16-ene (**13**) (5 mg), 15α,17,19-trihydroxy-11β,16β-epoxy-*ent*-kaurane (**15**) (3 mg), 19-(β-D-glucopyranosyl)-15α-hydroxy-*ent*-kaur-16-ene (**16**) (10 mg), 19-(β-D-glucopyranosyl)-15-oxo-*ent*-kaur-16-ene (**17**) (1 mg). Compound **9** rearranges into aldehyde **10** in the NMR tube. Compounds **11**, **13** and **15**–**17** were characterized as their acetates **11a**, **13a** and **15a**–**17a**, by acetylation and chromatography of the fractions containing them.

*3.6.1. 9β,15α,19-Trihydroxy-ent-kaur-16-ene (**9**).* ^{1}H NMR (400 MHz) δ 1.01 (3H, s, H-18) 1.14 (3H, s, H-20), 2.74 (1H, br s, H-13), 3.49 and 3.76 (each 1H, d, *J*=10.7 Hz, H-19), 4.55 (1H, br s, H-15), 5.10 and 5.22 (each 1H, s, H-17); ^{1}H NMR (C_6D_6, 500 MHz) δ 0.89 (3H, s, H-20), 0.92 (3H, s, H-18), 1.01 (1H, ddd, *J*=14.6, 13.3 and 7.8 Hz, H-11), 1.41 (2H, m, H-5 and H-14), 1.59 (1H, m, H-6), 1.67 (1H, dd, *J*=14.6 and 2.3 Hz, H-11), 1.68 (1H, m, H-6), 1.80 (1H, br d, *J*=13.5 Hz, H-3α), 1.84 (1H, dd, *J*=12.0 and 1.5 Hz, H-14), 2.53 (1H, br s, H-13), 3.22 and 3.51 (each 1H, d, *J*=10.7 Hz, H-19), 4.57 (1H, s, H-15), 4.97 and 5.14 (each 1H, s, H-17); EIMS *m/z* (rel int.) 320 $[M]^+$ (2), 302 (4), 289 (42), 271 (38), 253 (17),161 (40), 149 (22), 123 (100), 109 (51), 95 (47); HREIMS *m/z* 320.2361 (calcd for $C_{20}H_{32}O_3$, 320.2351).

*3.6.2. 16-Oxo-19-hydroxy-ent-abiet-8(9),15-diene (**10**).* ^{1}H NMR (500 MHz) δ 0.96 (3H, s, H-20), 1.00 (3H, s, H-18), 1.30 (1H, dd, *J*=9.0 and 7.3 Hz, H-5), 1.68 (1H, m, H-12), 1.94 (2H, m, H-7 and H-11), 2.08 (1H, dd, *J*=17.0 and 6.0 Hz, H-14), 2.82 (1H, br s, H-13), 3.48 and 3.79 (each 1H, d, *J*=10.9 Hz, H-19), 5.99 and 6.17 (each 1H, s, H-17), 9.52 (1H, s, H-16); EIMS *m/z* (rel int.) 302 $[M]^+$ (48), 287 (36), 284 (14), 272 (27), 271 (100), 269 (25), 254 (9), 253 (30), 251 (10), 239 (9), 211 (7), 201 (13), 189 (12); HREIMS *m/z* 302.2249 (calcd for $C_{20}H_{30}O_2$, 302.2246).

*3.6.3. 3α,15α,19-Triacetoxy-ent-kaur-16-ene (**11a**).* Mp 143–145 °C; ^{1}H NMR (500 MHz) δ 1.00 (1H, d, *J*=12.8 Hz, H-5), 1.00 (3H, s, H-18), 1.04 (1H, m, H-1β), 1.06 (3H, s, H-20), 1.20 (1H, d, *J*=6.7 Hz, H-9), 1.27 (1H, m, H-7), 1.44 (2H, m, H-6 and H-14), 1.50 (1H, m, H-12), 1.58 (1H, m, H-11), 1.69 (3H, m, H-6, H-7 and H-12), 1.91 (2H, m, H-1α and H-14), 2.80 (1H, br s, H-13), 4.13 and 4.33 (each 1H, d, *J*=11.7 Hz, H-19), 4.53 (1H, dd, *J*=10.0 and 6.7 Hz, H-3) 5.10 and 5.11 (each 1H, s, H-17), 5.25 (1H, s, H-15); EIMS *m/z* (rel int.) 446 $[M]^+$ (8), 404 (17), 386 (71), 371 (24), 344 (31), 329 (16), 326 (67), 311 (43), 284 (45), 269 (26), 266 (74), 253 (46), 251 (74), 211 (18), 209 (27); HREIMS *m/z* 446.2675 (calcd for $C_{26}H_{38}O_6$, 446.2668).

*3.6.4. 11β,15α,19-Trihydroxy-ent-kaur-16-ene (**12**).* Mp 229–231 °C; ^{1}H NMR (500 MHz) δ 0.91 (3H, s, H-20), 0.98 (1H, m, H-5), 1.00 (3H, s, H-18), 1.08 (1H, m, H-1β), 1.38 (1H, s, H-9), 1.44 (1H, m, H-2), 1.49 and 1.80 (each 1H, m, H-14), 1.89 (1H, m, H-12), 1.96 (1H, br d, *J*=12.5 Hz, H-1α), 2.01 (1H, ddd, *J*=14.7, 5.7 and 2.9 Hz, H-12), 2.78 (1H, br s, H-13), 3.47 and 3.72 (each 1H, d, *J*=10.9 Hz, H-19), 3.89 (1H, d, *J*=5.6 Hz, H-11), 4.27 (1H, s, H-15), 5.24 and 5.27 (each 1H, s, H-17); EIMS *m/z* (rel int.) 320 $[M]^+$ (42), 302 (18), 289 (19), 287 (8), 275 (25), 271 (64), 259 (14), 253 (12), 246 (30), 229 (7), 215 (12), 213 (9), 187 (11); HREIMS *m/z* 320.2353 (calcd for $C_{20}H_{32}O_3$, 320.2351). *Triacetate* (**12a**) ^{1}H NMR (500 MHz) δ 0.95 (3H, s, H-18), 0.95 (1H, m, H-1β), 0.99 (3H, s, H-20), 1.02 (1H, m, H-3β), 1.04 (1H, br s, H-5), 1.33 (2H, m, H-6 and H-14), 1.46 (2H, m, H-2 and H-7), 1.48 (1H, s, H-9), 1.71 (3H, m, H-3, H-6 and H-14), 1.90 (3H, m, H-1α, H-7 and H-12), 1.99 (1H, m, H-12), 2.78 (1H, br s, H-13), 3.88 and 4.17 (each 1H, d, *J*=11.1 Hz, H-19), 5.03 and 5.10 (each 1H, s, H-17), 5.07 (1H, d, *J*=4.2 Hz, H-11), 5.64 (1H, s, H-15); EIMS *m/z* (rel int.) 446 $[M]^+$ (0.9), 404 (5), 386 (29), 344 (30), 326 (36), 313 (26), 311 (25), 284 (12), 271 (11), 266 (13), 253 (46), 251 (14); HREIMS *m/z* 446.2658 (calcd for $C_{26}H_{38}O_6$, 446.2668).

*3.6.5. 15α,19-Diacetoxy-6α-hydroxy-ent-kaur-16-ene (**13a**).* Mp 162–164 °C; ^{1}H NMR (500 MHz) δ 0.82 (1H, td, *J*=13.0 and 3.9 Hz, H-1β), 0.99 (3H, s, H-18), 1.05 (1H, s, H-5), 1.09 (1H, td, *J*=13.7 and 4.4 Hz, H-3β), 1.30 (1H, d, *J*=7.6 Hz, H-9), 1.39 (3H, s, H-20), 1.44 (1H, m, H-2), 1.56 (1H, dd, *J*=14.4 and 3.4 Hz, H-7), 1.61 (1H, dd, *J*=6.7 and 2.0 Hz, H-14), 1.68 (1H, m, H-3α), 1.74 (1H, m, H-12), 1.79 (1H, dd, *J*=14.4 and 3.4 Hz, H-7), 1.83 (1H, br d, H-1α), 2.34 (1H, dd, *J*=12.0 and 1.5 Hz, H-14), 2.81 (1H, br s, H-13), 4.37 and 4.49 (each 1H, d, *J*=11.6 Hz, H-19), 4.44 (1H, br s, H-6), 5.11 and 5.12 (each 1H, s, H-17), 5.30 (1H, s, H-15); EIMS *m/z* (rel int.) 404 $[M]^+$ (7), 386 (5), 362 (9), 344 (25), 326 (12), 311 (9), 302 (6), 284 (35), 269 (19), 253 (16), 251 (22); HREIMS *m/z* 404.2552 (calcd for $C_{24}H_{36}O_5$, 404.2563).

*3.6.6. 15α,17,19-Triacetoxy-11β,16β-epoxy-ent-kaurane (**15a**).* IR ($CHCl_3$) ν_{max} 3430, 1733, 1248 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.94 (3H, s, H-18), 0.98 (1H, d, *J*=11.9 Hz, H-5), 1.03 (1H, m, H-3β), 1.12 (3H, s, H-20), 1.13 (1H, td, *J*=12.7 and 3.7 Hz, H-1β), 1.21 (1H, m, H-6), 1.43 (1H, m, H-2), 1.49 (2H, m, H-7), 1.63 (1H, m, H-6), 1.66 (1H, br s, H-9), 1.72 (1H, br d, *J*=13.4 Hz, H-3α), 1.80 (2H, m, H-1α and H-14), 1.88 (1H, m, H-12), 1.94 (1H, dd, *J*=12.0 and 1.3 Hz, H-14), 2.15 (1H, d, *J*=11.5 Hz, H-12), 2.39 (1H, t, *J*=6.4 Hz, H-13), 3.87 and 4.24 (each 1H, d, *J*=11.0 Hz, H-19), 3.99 and 4.53 (each 1H, d, *J*=11.9 Hz, H-17), 4.42 (1H, br s, H-11), 4.99 (1H, d, *J*=1.3 Hz, H-15); EIMS *m/z* (rel int.) 462 $[M]^+$ (7), 444 (6), 420 (11), 402 (14), 384 (12), 360 (18), 342 (70), 329 (30), 300 (9), 287 (12), 282 (20), 269 (34); HREIMS *m/z* 462.2631 (calcd for $C_{26}H_{38}O_7$, 462.2618).

*3.6.7. 19-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-15α-acetoxy-ent-kaur-16-ene (**16a**).* ^{1}H NMR (500 MHz) δ 0.78 (1H, td, *J*=13.2 and 3.7 Hz, H-1β), 0.89 (3H, s, H-18), 0.92 (2H, m, H-3β and H-5), 1.00 (3H, s, H-20), 1.20 (1H, d, *J*=7.2 Hz, H-9), 1.26 (2H, m, H-6 and H-7), 1.39 (1H, dd, *J*=11.0 and 4.4 Hz, H-14), 1.52 (2H, m, H-2 and H-12), 1.59 (1H, m, H-7), 1.64 (1H, br d, *J*=11.9 Hz, H-12), 1.72 (1H, br d, *J*=13.3 Hz, H-3α), 1.83 (1H, br d, *J*=13.2 Hz, H-1α), 1.91 (1H, dd, *J*=11.8 and 1.1 Hz, H-14), 2.78 (1H, br s, H-13), 3.20 and 3.93 (each 1H, d, *J*=9.3 Hz, H-19), 3.67 (1H, ddd, *J*=10.0, 4.9 and 2.6 Hz, H-5′), 4.13 (1H, dd, *J*=12.2 and 2.6 Hz, H-6′), 4.27 (1H, dd, *J*=12.2 and 5.0 Hz, H-6′), 4.41 (1H, d, *J*=7.9 Hz, H-1′), 4.99 (1H, dd, *J*=9.7 and 7.9 Hz, H-2′), 5.07 (1H, t, *J*=9.6 Hz, H-4′), 5.08 and 5.09 (each 1H, s, H-17), 5.19 (1H, t, *J*=9.6 Hz, H-3′), 5.25 (1H, s, H-15); EIMS *m/z* (rel int.) 676 $[M]^+$ (0.5), 661 (0.4), 643 (0.4), 616 (0.8), 556 (0.4), 331 (52), 328 (7), 269 (14), 255 (9), 169 (100), 109 (36), 91 (7); HREIMS *m/z* 676.3478 (calcd for $C_{36}H_{52}O_{12}$, 676.3459).

*3.6.8. 19-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-15-oxo-ent-kaur-16-ene (**17a**).* Mp 128–130 °C; ^{1}H NMR (500 MHz) δ 0.79 (1H, td, *J*=13.3 and 4.0 Hz, H-1β), 0.92 (3H, s, H-18), 0.93 (1H, m, H-3β), 1.07 (3H, s, H-20), 2.36 (1H, d, *J*=11.6 Hz, H-14), 3.04 (1H, br s, H-13), 3.19 and 3.96 (each 1H, d, *J*=9.4 Hz, H-19), 3.68 (1H, ddd, *J*=10.0, 4.9 and 2.6 Hz, H-5′), 4.14 (1H, dd, *J*=12.3 and 2.8 Hz, H-6′), 4.27 (1H, dd, *J*=12.3 and 4.9 Hz, H-6′), 4.42 (1H, d, *J*=7.9 Hz, H-1′), 5.00 (1H, dd, *J*=9.6 and 7.9 Hz, H-2′), 5.08 (1H, t, *J*=9.7 Hz, H-4′), 5.20 (1H, t, *J*=9.5 Hz, H-3′), 5.25 and 5.94 (each 1H, s, H-17); EIMS *m/z* (rel int.) 632 $[M]^+$ (15), 331 (43), 284 (26), 271 (31), 211 (3), 169 (100), 127 (12), 109 (40), 91 (31); HREIMS *m/z* 632.3174 (calcd for $C_{34}H_{48}O_{11}$, 632.3197).

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