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A Study of the Microbiological Reduction of α,β-Unsaturated Carbonyl *ent*-Kaurenes by *Gibberella fujikuroi*

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Abstract: The incubation of 18-hydroxy-15-oxo-*ent*-kaur-16-ene with the fungus *Gibberella fujikuroi* gave 16α,17-dihydro-15-oxo derivatives, whilst the feeding of 3-oxo-15α,16α-epoxy-*ent*-kaur-1-ene afforded a series of products, which conserve the 1,2-double bond. These results indicated that the hydrogenation of α,β-unsaturated carbonyl compounds with an *ent*-kaurene skeleton by this fungus is a stereospecific and regiospecific reduction, that does not depend on the biosynthetic route of gibberellins and kaurenolides.

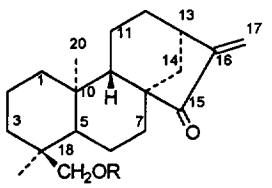
In a previous work we have obtained 15-oxo-dihydro-gibberellins by chemical and microbiological methods. In the latter form 15-oxo-*ent*-kaur-16-ene was mainly transformed by the fungus *Gibberella fujikuroi* into the 15-oxo-16α,17-dihydro derivatives of GA_{12}, GA_{24}, GA_{25} and GA_7.[1] Thus, the presence in the substrate of the 15-oxo-group favours the stereospecific reduction of the 16,17-double bond. This fact is very interesting, because in the biosynthetic pathway of gibberellins there is no analogous reduction step. Recently, a similar hydrogenation by another fungus, *Cephalosporium aphidicola*, has also been observed.[2]

The aim of the first part of this work was to confirm the generality of the biohydrogenation by *G. fujikuoi* of other 15-oxo-*ent*-kaur-16-ene derivatives and to determine in what step of the gibberellin biosynthesis this reduction occurs. The objective of the second part has been to study the regioselectivity of this hydrogenation. Thus, we have firstly incubated with this fungus, 18-hydroxy-15-oxo-*ent*-kaur-16-ene (**1**) and then 3-oxo-15α,16α-epoxy-*ent*-kaur-1-ene (**32**).

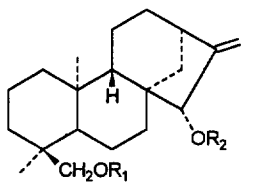
The substrate **1** was chosen because its metabolism by the fungus must be slow, permitting a better study of the initial biosynthetic post-kaurene steps. Thus, the presence of the 18-hydroxyl hinders the oxidation at C-19, typical of the gibberellin route, and although the 19-acid could be formed slowly, the 18-OH prevents the contraction of the ring B to form gibberellins.[3]

Compound **1** had been isolated from the liverwort *Porella densifolia*,[4,5] and we have synthesized it starting from candidiol, which had been isolated from species of the genus *Sideritis*.[6,7] Thus, partial acetylation

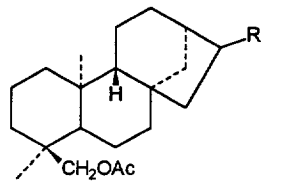
of candidiol (**3**) gave the two monoacetates and the diacetate. The monoacetate **5** was oxidized with pyridinium dichromate to afford as the major compound the 15-oxo-derivative **2**, and as minor products the aldehyde **6**, the epoxy-aldehyde **7**, the epoxy-alcohol **8** and the keto-epoxide **9**. The hydrolysis of **2** led to the target diterpene **1**.[8]



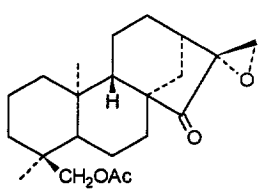
1 R = H
2 R = Ac



3 $R_1 = R_2 = H$
4 $R_1 = H$ $R_2 = Ac$
5 $R_1 = Ac$ $R_2 = H$



6 $\Delta^{15(16)}$ R = CHO
7 15α,16α-epoxy R = CHO
8 15α,16α-epoxy R = CH_2OH



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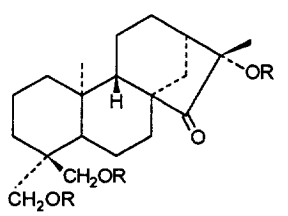
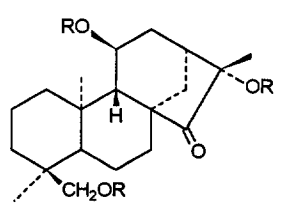


10 R = H
11 R = Ac



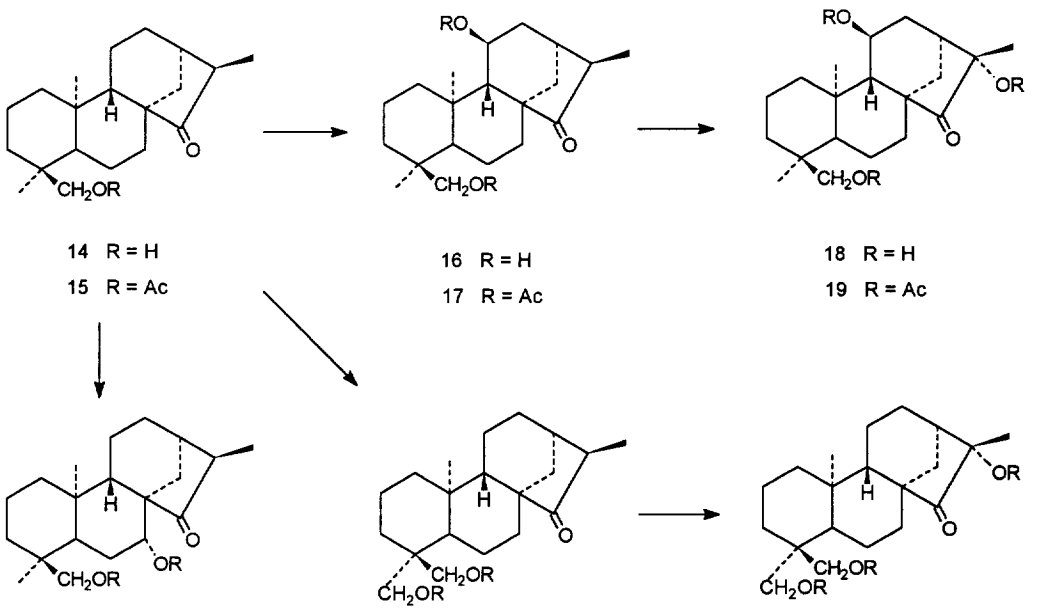
12 R = H
13 R = Ac

The incubation of **1** with the fungus was carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene without perturbing the post-kaurene metabolism.[9,10] The fermentation was carried out for a period of 6 days, and the combined broth and mycelium extract separated into neutral and acid fractions. The neutral fraction was chromatographed and the following substances were isolated: 18-hydroxy-15-oxo-*ent*-(16S)-kaurane (**14**), 11β,18-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**16**), 18,19-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**20**), 7α,18-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**24**), and a mixture of compounds which was resolved by acetylation and chromatography (see below). No metabolites were isolated from the acidic fraction.

The high resolution mass spectrum of the least polar substance was in accordance with the formula $C_{20}H_{32}O_2$ (m/z 304.2430) and possessed two hydrogen atoms more than the substrate **1**. The ^1H NMR spectrum indicated that the exocyclic double bond had been hydrogenated, its typical protonic signals disappearing and being substituted by a methyl doublet at δ 1.10 (J = 7 Hz). In the ^{13}C NMR C-16 and C-17

resonate at δ 47.7 and 10.0, respectively. Thus, the structure **14** was assigned to this compound, considering also that in this type of biological reduction the C-17 β-stereochemistry is formed.[1] We also obtained this compound by catalytic hydrogenation[4] of **1**.

Another compound isolated in this incubation was identified as 11β,18-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**16**) as follows: Its molecular formula $C_{20}H_{32}O_3$ had one oxygen more than compound **14**. The ^1H and ^{13}C NMR spectra indicated that this oxygen was introduced in the molecule as a secondary hydroxyl. The geminal proton to this group appears in the ^1H NMR spectrum of **16** at δ 3.94 as a doublet (J = 5.7 Hz). This form of resonance and the chemical shifts of this hydrogen, in **16** and in its acetate **17**, are typical of the geminal hydrogens at these two oxygen functions at C-11(β).[11,12]



14 R = H
15 R = Ac

16 R = H
17 R = Ac

18 R = H
19 R = Ac

24 R = H
25 R = Ac

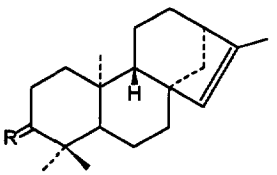
20 R = H
21 R = Ac

22 R = H
23 R = Ac

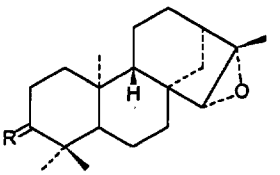
Scheme 1

The third compound obtained in this incubation was identified as 18,19-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**20**). Its ^1H NMR spectrum showed only two methyl resonances and an additional hydroxymethylene group, which resonates as two doublets at δ 3.76 and 3.92 (J = 11 Hz). The location of the new hydroxyl

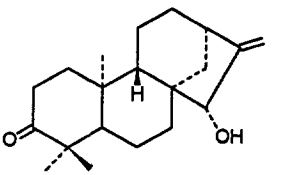
group at C-19 rather than C-20 followed from the ^{13}C NMR spectrum of its diacetate **21** (Table 1). This fact is also in accordance with biogenetic considerations, because in the gibberellin and kaurenolide pathway the first step after the formation of *ent*-kaur-16-ene is the hydroxylation at C-19.



26 R = α-OAc, H
27 R = α-OH, H
28 R = O



29 R = α-OH, H
30 R = O



31

Table 1. ^{13}C NMR data

C	1	2	14	15	16	17	21	25
1	39.1	39.0	38.9	38.8	39.1	39.1	39.0	38.6
2	17.6	17.5	17.6	17.5	18.0	17.8	17.5	17.4
3	35.0	35.5	35.1	35.6	35.4	35.9	31.0	35.6
4	37.4	36.3	37.5	36.4	38.0	36.9	39.6	36.2
5	48.4	48.9	48.7	49.0	49.9	49.4	49.9	45.9
6	18.2	18.3	18.4	18.6	18.6	17.8	19.3	24.4
7	36.5	36.5	37.4	37.3	37.7	37.4	37.3	73.6
8	52.4	52.2	52.5	52.5	51.4	51.4	52.6	56.2
9	52.4	52.3	52.3	52.2	64.1	59.2	52.5	51.7
10	39.8	39.8	39.5	39.6	38.6	38.8	40.2	39.1
11	18.1	18.1	18.0	18.0	65.4	67.7	18.3	17.8
12	32.3	32.2	24.7	24.7	34.4	32.0	24.8	24.9
13	38.0	38.0	34.9	34.9	35.1	34.8	35.1	34.2
14	33.1	33.1	33.8	33.7	33.5	34.3	34.4	29.7
15	210.8	209.9	224.5	224.8	223.4	221.8	224.6	n.o.
16	149.4	149.4	47.7	47.7	48.8	49.1	47.9	47.7
17	114.4	114.4	10.0	10.1	11.5	11.1	10.2	9.8
18	71.9	72.5	72.2	72.6	72.3	72.7	69.2	71.8
19	17.3	17.4	17.2	17.3	17.7	18.6	64.7	17.5
20	17.9	17.9	17.9	18.0	18.3	18.7	18.1	18.1

Compound **24** was also obtained in this fermentation. In the MS the molecular ion appears at m/z 320 indicating that it possesses one oxygen more than compound **14**, and in consequence is isomeric with **16** and **20**. Its ^{1}H NMR spectrum was similar to that of **14**, except that a new hydrogen geminal to a hydroxyl group appeared in the spectrum at δ 3.98, as a double doublet with coupling constants of 11 and 4 Hz, which is

typical of an equatorial substituent at C-1, C-3 or C-7. Positions 1 and 3 were excluded considering the ^{13}C NMR data (Table 1). Thus, the structure of 7α,18-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**24**) was assigned to this product.

The mixture of substances obtained in this biotransformation and named above was resolved by acetylation and chromatography giving two pure compounds, 11β,16α,18-triacetoxy-15-oxo-*ent*-(16S)-kaurane (**19**), and 16α,18,19-triacetoxy-15-oxo-*ent*-(16S)-kaurane (**23**). These two metabolites possess a tertiary hydroxyl group which was assigned to C-16(α). In both cases the ^1H NMR spectrum showed the disappearance of the exocyclic double bond and the presence of a methyl over an oxygen function. These spectra also permitted the assignment of the 11β-acetoxy group in (**19**) [H-11(α), δ 5.08, d, J = 5 Hz] and the CH_2OAc in **23** [H-19, δ 3.96 and 4.04, each d, J = 11 Hz] by comparison with those of the acetates **17** and **21**, described above. The corresponding triols, **18** and **22**, formed in the biotransformation can be derived from **16** and **20**, also isolated from this incubation, by α-hydroxylation at C-16. A hydration of the substrate **1** can be discarded, because this reaction must form the 17-hydroxy derivative by a Michael addition. On the other hand, the α-stereochemistry was given because it is known that in these *ent*-kaurane derivatives the 16-hydroxyl enters by the less hindered α-face.[13]

The results of this incubation indicate that the stereospecific hydrogenation of the 16,17-double bond by *G. fujikuroi* can occur in a general manner with 15-oxo-*ent*-kaur-16-ene derivatives. The isolation of the hydrogenated substrate is proof that the hydrogenation occurs as a first step of the biotransformation.

At this point we decided to establish the regioselectivity of this bioreduction by incubating an *ent*-kaurene derivative having an α,β-unsaturated carbonyl group in another part of the molecule. Thus, we decided to choose as substrate a product with a 3-oxo-$\Delta^{1(2)}$ function, such as **32**. We introduced the 15α,16α-epoxidic function to inhibit the oxidation of C-19 to a carboxylic acid,[14] which is a characteristic step in the biosynthesis of gibberellins and kaurenolides. A possible subsequent decarboxylation could thus be avoided.

The substrate was prepared as follows: The acetate **26**, prepared from linearol,[15] was hydrolyzed to give the alcohol **27**. Epoxidation of this compound with MCPB acid gave **29**, which by oxidation with pyridinium dichromate afforded **30**. Alternatively, this last substance was directly prepared by oxidation of **27** with Jones reagent. In this reaction compound **28**, as expected, was the main product, but **31** was also obtained, by acid opening of the oxirane ring in **30**. On the other hand, the oxidation of **27** with pyridinium dichromate only led to the 3-oxo-derivative **28**. The epoxidation of **28** led to a further amount of **30**, dehydrogenation of which with benzeneselenic anhydride[16] gave the required substrate **32**.

The second incubation was also made in the presence of the inhibitor AMO 1618, and the substrate **32** was very well metabolised by the fungus *G. fujikuroi*. The EtOAc extracts of the broth and mycelium were

combined and then chromatographed giving four mixtures of products, which were separately resolved by acetylation and chromatography.

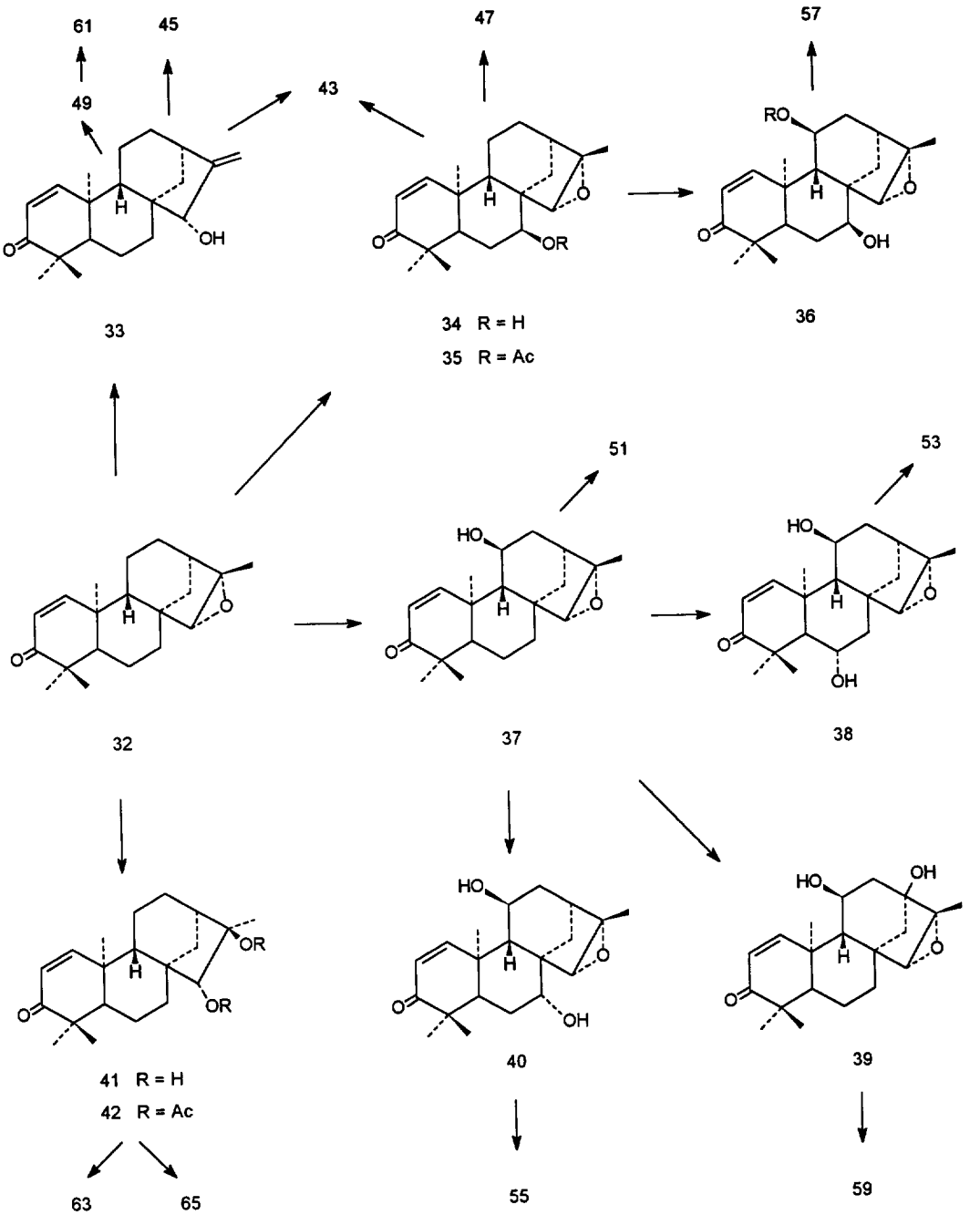
The high resolution MS of the acetate **35** was in accordance with the formula $C_{22}H_{30}O_4$, which indicated that a new hydroxyl group had been introduced in the substrate during the feeding. The presence in its ^{1}H NMR spectrum of a hydrogen geminal to an acetoxy group resonating as a broad singlet at δ 4.89 confirmed this assert. The form of the signal permitted this acetoxy function to be assigned to C-7 (axial), which was also confirmed by assignment of its ^{13}C NMR spectrum (Table 2). Therefore, the structure of the alcohol formed in the biotransformation must be 3-oxo-7β-hydroxy-15α,16α-epoxy-*ent*-kaur-1-ene (**34**).

The ^{1}H NMR spectrum of the diacetate **44** revealed that the oxirane ring of the substrate had disappeared, showing the hydrogens of an exocyclic double bond at δ 5.12 and 5.26, and the geminal protons to two acetoxy groups resonating at δ 5.45 (s) and 5.06 (br s), which were due to the presence of a 15α- and a 7β-acetoxy group, respectively. These positions were assigned considering the form of resonance of their geminal hydrogens and its ^{13}C NMR spectrum. Thus we assigned the structure **43** (3-oxo-7β,15α-dihydroxy-ent-kaur-1,16-diene) to the corresponding alcohol, which must be formed in the feeding by hydroxylation of **33**, or by opening of the epoxidic ring in **34** with concomitant formation of the 16,17-double bond (Scheme 2).

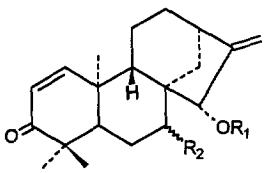
Compound. **46** was isomeric with **44**, their ^{1}H NMR spectra being very similar. Thus the main difference was that the broad singlet of the equatorial H-7 in **44** had been substituted by a double doublet at δ 5.07, with coupling constants of 10.6 and 4.5 Hz. This form of resonance is typical of an axial hydrogen, which was assigned also to C-7, but with the β-stereochemistry, by comparison with the resonance of this hydrogen in **25** . Thus, the corresponding alcohol formed in the biotransformation was 3-oxo-7α,15β-dihydroxy-*ent*-kaur-1,16-diene (**45**), which derived from **33** by hydroxylation at C-7(α), or alternatively by hydroxylation of **32** and then opening of the oxirane ring.

The diacetate **50** showed spectral data characteristic of the 15α-OAc and 16,17-double bond, also observed in **44**. A geminal hydrogen to an acetoxy group appears also in its ^{1}H NMR spectrum, but now resonating at δ 5.24 (br s). This acetyl function was assigned to C-11(β) on the basis of its ^{13}C NMR spectrum. Therefore, the structure of this compound was determined as **50**, and that of the diol found in the feeding as **49**, which must be formed by hydroxylation of **33** (Scheme 2).

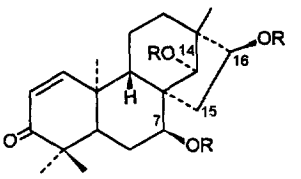
The substance **47** is also formed from **34**, but in the opening of the oxirane ring, the cation formed at C-16 rearranged to another cation with a *ent*-beyerane skeleton,[14] which is neutralized by the β-face with a hydroxyl group of water origin, to give **47**. As the other metabolites of this latter incubation, this substance was isolated in the acetylated form **48**. In the ^{1}H NMR spectrum the geminal hydrogens to the acetoxyl groups at C-7, C-14 and C-16 appeared at δ 4.99 (br s), 5.05 (s) and 4.83 (br s), respectively.


61
45
49
43
47
57
RO
OH
OR
33
34 R = H
35 R = Ac
36
51
53
HO
32
37
38
41 R = H
42 R = Ac
40
39
63
65
55
59

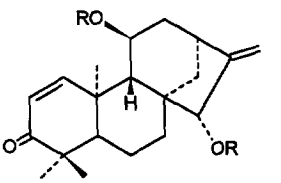
Scheme 2



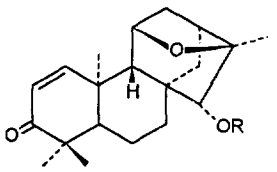
43 R_1 = H R_2 = β-OH
44 R_1 = Ac R_2 = β-OAc
45 R_1 = H R_2 = α-OH
46 R_1 = Ac R_2 = α-OAc



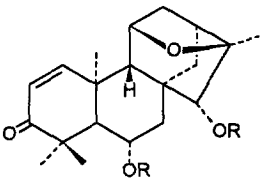
47 R = H
48 R = Ac



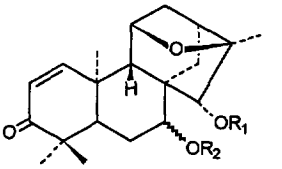
49 R = H
50 R = Ac



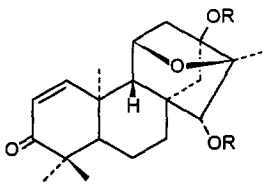
51 R = H
52 R = Ac



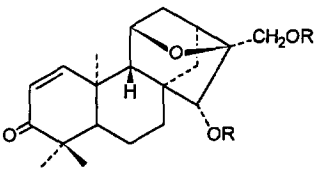
53 R = H
54 R = Ac



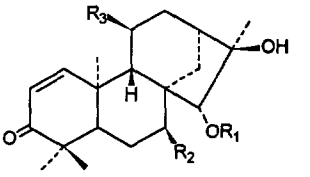
55 R_1 = H R_2 = α-OH
56 R_1 = Ac R_2 = α-OAc
57 R_1 = H R_2 = β-OH
58 R_1 = Ac R_2 = β-OAc



59 R = H
60 R = Ac



61 R = H
62 R = Ac



63 R_1 = R_3 = H R_2 = OH
64 R_1 = Ac R_2 = OAc R_3 = H
65 R_1 = R_2 = H R_3 = OH
66 R_1 = Ac R_2 = H R_3 = OAc

The acetate **52** has a molecular formula $C_{22}H_{30}O_4$. Its ^1H NMR spectrum showed two geminal protons to oxygenated functions, one of which was an acetoxy group. One of these hydrogens, δ 4.82 (br s), was assigned to the geminal proton to the 15α-OAc considering the form of resonance. The disappearance of the double bond in the substrate and the resonance of the second geminal proton at δ 4.51 as a broad singlet, led us to locate an oxygen bridge between C-11(β) and C-16(β). In the ^{13}C NMR spectrum these two carbons resonate at δ 75.9 and 87.8, respectively, and the C-17 at 18.2. Thus, the compound isolated from the

incubation was 3-oxo-15α-hydroxy-11β,16β-epoxy-*ent*-kaur-1-ene (**51**). This substance is probably formed in the feeding, or in the extraction procedure, from the product **37**, occurring via protonation and opening of the oxirane ring, and then neutralisation of the cation formed at C-16 by attack of the 11β-OH.

The ^{1}H NMR spectrum of compound **54** was similar to that of **52**, indicating an oxygen bridge between C-11(β) and C-16(β). The main difference between both is that in the latter there appears a new geminal proton to an acetoxy group. This hydrogen resonates as a broad singlet at δ 5.37 in its ^{1}H NMR spectrum which indicates an equatorial stereochemistry. Considering its ^{13}C NMR spectrum (Table 3) this acetoxy group was assigned to C-6(α). Therefore, the product isolated from the fermentation was the alcohol (**53**), which must be formed from **38** in the same manner as indicated above for **51** (from **37**).

Other compounds of this type with an oxygen bridge were **55** and **57**. These metabolites possess a new hydroxyl group, which had been introduced during the incubation, and which we have assigned to the C-7(α) and C-7(β) positions, respectively. The resonance of the geminal proton to the corresponding acetoxy groups in **56** and **58** appear as a double doublet and a broad singlet at δ 5.05 (J = 10.8 and 5 Hz) and 4.98, respectively. The ^{13}C NMR data (Table 3) also confirmed these structures. The alcohols **55** and **57** should proceed from **40** and **36**, respectively, by opening of the epoxidic ring.

The diacetate **60** was isomeric with **56** and **58** ($C_{34}H_{32}O_6$), but only shown in its ^{1}H NMR spectrum one geminal proton (δ 4.77, s) to an acetoxy at C-15(α), indicating that the second acetate was tertiary. We assigned this group to C-13 considering its ^{13}C NMR spectrum (Table 3) and for biosynthetic reasons, this is the unique tertiary carbon hydroxylated in the gibberellin biosynthetic pathway. The corresponding alcohol **59** can be formed by rearrangement of **39**, which must be originated by hydroxylation of **37** (Scheme 2).

Compound **61** also possesses an oxygen bridge between C-11 and C-16, but in this case the new hydroxyl group has been introduced at C-17. Thus, in the diacetate **62** there appears a pair of doublets at δ 4.03 and 4.56 (J = 12 Hz), which correspond to the H-17 protons, and two singlets at δ 4.60 and 5.03, characteristic of H-11 and H-15, respectively. The diol **61** must be formed in the biotransformation from **49** by 16α,17-epoxidation and opening of the epoxide with concomitant attack of the 11β-hydroxyl group over C-16.

Finally, other metabolites isolated from this incubation were **41**, **63** and **65**. Compound **41** is formed by opening of the oxirane ring of the substrate and neutralization of the cation formed with a hydroxyl group. In the ^{1}H NMR spectrum of the diacetate **42** the geminal proton to the 15α-OAc appears as a singlet at δ 4.72. The hydroxylation of **41** at C-7(β) and C-11(β) led to **63** and **65**, respectively. The resonance of the geminal protons to the new secondary acetoxy groups, which appeared at δ 4.88 (br s) and 5.47 (t) in the triacetates **64** and **66**, respectively, permitted these oxygenated functions to be assigned at 7β and 11β in the respective molecules. The first was assigned by comparison with the H-7 in the corresponding spectrum of **58** (δ 4.98, br s) and the latter by study of its ^{13}C NMR spectrum, which is given in Table 3.

Table 2. ^{13}C NMR data

C	26	27	28	29	30	32	35	42	44
1	39.3	39.4	39.4	38.8	39.3	159.6	158.6	157.6	158.7
2	23.6	27.3	34.2	27.2	34.0	126.0	125.9	125.5	126.1
3	81.0	79.0	218.1	78.9	217.6	205.4	204.7	204.8	204.9
4	39.1	39.1	47.3	38.9	47.3	44.5	44.0	44.6	44.0
5	55.0	54.8	54.4	54.9	54.4	53.5	40.3	53.1	41.8
6	19.0[a]	19.0[a]	20.5[a]	18.8[a]	20.2[a]	19.9[a]	24.1	20.6[a]	24.5
7	38.4	38.9	38.5	32.1	31.8	32.9	74.1	34.2	73.7
8	49.0	48.9	48.9	43.5	43.5	44.3	46.7	37.5	50.1
9	48.7	48.8	47.8	50.4	49.3	45.1	44.5	44.6	44.5
10	37.8	38.8	38.6	38.9	38.2	41.3	40.7	40.0	41.1
11	18.8[a]	18.7[a]	19.2[a]	18.1[a]	18.5[a]	18.7[a]	17.9	18.4[a]	18.1
12	25.0	25.0	24.7	27.0	26.8	27.0	26.8	22.6	32.7
13	44.8	44.9	44.7	39.2	39.0	39.2	38.6	34.8	42.6
14	43.8	43.7	43.6	35.6	34.8	35.3	31.3	22.8	35.8
15	135.3	135.3	134.8	68.3	67.8	67.8	62.8	83.5	79.7
16	142.6	142.5	142.8	61.3	61.2	61.5	61.4	86.1	154.0
17	15.3	15.4	15.4	14.5	14.5	14.9	14.3	17.8	111.4
18	28.2	28.2	27.0	28.3	27.1	28.5	27.7	27.5	28.1
19	16.5	15.4	21.0	15.4	21.0	21.7	21.1	21.5	21.0
20	17.7	17.7	17.5	17.4	17.4	21.4	21.2	18.8	14.1

Table 3. ^{13}C NMR data

C	48	50	52	54	56	58	60	62	66
1	156.7	158.3	157.1	156.7	155.7	156.4	156.4	156.6	156.8
2	126.1	126.4	126.1	125.3	126.6	126.3	126.3	126.3	125.6
3	204.5	203.7	204.7	203.8	203.9	204.1	204.5	204.5	205.5
4	44.4	44.6	44.5	45.2	44.3	43.9	44.6	44.6	44.5
5	44.6	52.7	53.1	52.5	48.9	44.5	52.8	52.8	52.5
6	25.2	19.6	19.6	68.2	26.4	25.0	19.5	19.6	19.3
7	74.9	36.9	32.2	36.1	72.6	70.9	32.1	32.0	32.0
8	50.5	46.6	48.9	47.2	51.7	51.4	47.6	49.0	44.1
9	52.7	54.0	52.8	52.9	52.8	49.6	52.1	52.9	53.3
10	39.0	39.9	38.2	38.2	38.0	38.2	38.1	38.2	39.9
11	19.4	68.4	75.9	75.8	75.6	75.6	74.7	77.2	70.4
12	34.1	38.9	39.4[a]	40.6[a]	40.6	40.4	43.9[a]	40.2	29.7[a]
13	48.1	40.4	44.3	44.8	43.1	44.3	88.3	41.4	27.3
14	85.9	33.4	40.2[a]	40.4[a]	33.8	38.4	44.2[a]	38.9	30.3[a]
15	40.4	81.6	87.4	87.6	86.5	82.8	85.7	83.9	85.3
16	76.6	154.3	87.8	87.4	87.9	87.9	87.8	89.1	85.3
17	18.2	110.2	18.2	18.1	18.2	18.1	15.3	63.2	15.7
18	28.0	28.5	28.7	29.1	28.6	28.2	28.7	28.7	27.2
19	21.6	21.7	21.5	21.7	21.7	21.8	21.9	21.5	21.4
20	12.7	21.2	20.9	21.0	21.1	21.0	20.9	20.9	20.1

[a] These values can be interchanged

The results of these biotransformations indicate that: 1.- The stereospecific hydrogenation of the 16,17-double bond by *G. fujikuroi* can occur in a general manner with 15-oxo-*ent*-kaur-16-ene derivatives. 2.- The hydrogenation occurs as an initial step of the incubation and does not depend on the biosynthetic route of gibberellins and kaurenolides, probably being due to the addition of hydride from an NADH-depending enzyme. 3.- The reaction is regiospecific. Whilst in 15-oxo-$\Delta^{16(17)}$ derivatives the double bond is hydrogenated, in compounds with a 3-oxo-$\Delta^{1(2)}$ group the double bond is not reduced. This regioespecificity can be due more to the facility of the 15-oxo-16,17-double bond to undergo nucleophilic attack than to a special enzymatic characteristic.[17] 4.- The position and stereochemistry of the alcoholic group introduced in the biotransformation of **32** confirm our previous conclusion that the presence in the substrate of a 15α,16α-epoxy group inhibits the hydroxylation at C-19 and mainly directs the hydroxylation at the 11β–, 7α– and 7β– positions. 5.- While in the incubation of **32** the same group, the 15α,16α-epoxy, is the inhibitor and the directing group of the hydroxylation, we believe that in the similar functionalization at C-11(β) and C-7(α) produced in the feeding of the 18-hydroxy-15-oxo derivative **1**, there exists a combined effect, the 18-hydroxyl partially inhibits the 19-oxidation and the 15-oxo group directs the hydroxylation at these carbons. In this last conclusion we have considered that *ent*-kaurenes having these two functions separately such as 18-hydroxy-derivatives[3,18] or 15-oxo-derivatives[1] are not hydroxylated at C-7 or C-11 by this fungus.

EXPERIMENTAL

M.p.s. were determined with a Kofler hot-plate apparatus and are uncorrected. The recrystallization solvent was petrol-EtOAc. IR spectra were run on a Perkin Elmer 257 and UV spectra were taken on a Jasco V-560. The NMR spectra were run on a Bruker WP200 SY and a Bruker AMX 400, for solutions in $CDCl_3$. The ^{13}C NMR spectra were taken at 50.3 MHz, except those of **16**, **17** and **48**, which were determined at 100.6 MHz. MS were taken at 70eV (probe) on a Hewlett-Packard 5930A and a Shimadzu QP 2000, and HRMS on a VG-Micromass ZAB-2F. Silica gel Merck (0.05-0.2 mm) was used for column chromatography.

Part 1

Partial acetylation of candidiol (3).- Compound **3** (1.2 g) in pyridine (10 ml) was treated with Ac_2O at 0° for 1.5 h. Usual work-up and chromatography of the residue, using petrol-EtOAc (8:2) as eluent afforded, besides starting material (120 mg), a mixture of the two monoacetates (760 mg) and the diacetate (140 mg). This mixture was chromatographed on a dry column of silica gel impregnated with 15% $AgNO_3$. Elution with petrol-EtOAc (8:2) gave **15α-acetoxy-18-hydroxy-*ent*-kaur-16-ene (4)** (220 mg)[11]. Further elution afforded **18-acetoxy-15α-hydroxy-*ent*-kaur-16-ene (5)** (510 mg), m.p. 108-110° C; ^1H NMR (200 MHz): δ 0.81 and 1.04 (each 3H, s), 2.03 (each 3H, s), 2.71 (1H, br s, H-13), 3.60 and 3.85 (each 1H, d, J = 11 Hz, H-18), 3.79 (1H, s, H-15), 5.05 and 5.18 (each 1H, s, H-17); EIMS *m/z* (rel. int.): 346 $[M]^+$ (6), 331 (3), 328 (10), 313 (26), 288 (100), 286 (51), 273 (26), 271 (86), 268 (17), 255 (42), 253 (39), 228 (46), 203 (21), 199 (14).

Oxidation of 5 with PDC.- The monoacetate **5** (500 mg) in CH_2Cl_2 (10 ml) was treated with pyridinium dichromate (800 mg) at room temp. for 10 h. The soln was diluted with Et_2O, filtered and evapd. The residue was chromatographed, eluting with petrol-EtOAc (9:1) to afford **18-acetoxy-15-oxo-*ent*-kaur-16-**

ene (2) (390 mg), m.p. 139-141° C; ^{1}H NMR (400 MHz): δ 0.83 and 1.12 (each 3H, s), 2.09 (each 3H, s), 3.04 (1H, br s, H-13), 3.64 and 3.86 (each 1H, d, J = 11 Hz, H-18), 5.25 and 5.94 (each 1H, s, H-17); EIMS *m/z* (rel. int.): 344 $[M]^+$ (14), 329 (3), 284 (56), 271 (22), 269 (35), 256 (9), 253 (13), 241 (14), 227 (8), 215 (6), 199 (11). Further elution gave a mixture of the aldehyde (**6**) and the epoxy-aldehyde (**7**). Further elution with petrol-EtOAc (8:2) afforded **18-acetoxy-16α,17α-epoxy-15-oxo-*ent*-kaurane (9)** (31 mg), ^{1}H NMR (200 MHz): δ 0.84 and 1.16 (each 3H, s), 2.09 (3H, s), 2.22 (1H, br s, H-13), 2.96 and 3.24 (each 1H, d, J = 6 Hz, H-17), 3.64 and 3.87 (each 1H, d, J = 11 Hz, H-18), ; EIMS *m/z* (rel. int.): 360 $[M]^+$ (7), 345 (2), 327 (2), 300 (24), 287 (13), 285 (21), 282 (15), 267 (23), 259 (15), 257 (15), 241 (12), 225 (8), 199 (9), and **18-acetoxy-15α,16α-epoxy-17-hydroxy-*ent*-kaurane (8)** (24 mg), ^{1}H NMR (200 MHz): δ 0.82 and 1.04 (each 3H, s), 2.07 (3H, s), 2.29 (1H, br s, H-13), 2.96 (1H, s, H-15), 3.65 and 3.85 (each 1H, d, J = 11 Hz, H-18), 3.79 and 4.04 (each 1H, d, J = 11 Hz, H-17); EIMS *m/z* (rel. int.): 362 $[M]^+$ (3), 344 (2), 331 (2), 302 (3), 289 (22), 288 (30), 271 (32), 269 (17), 253 (12), 243 (9), 228 (13), 213 (14), 201 (9), 199 (8), 189 (8).

Hydrolysis of 2.- The monoacetate **2** (350 mg) in MeOH was treated with methanolic KOH (5%) (12 ml) at room temp. and left overnight. Usual work up and chromatography with petrol-EtOAc (8:2) gave **18-hydroxy-15-oxo-*ent*-kaur-16-ene (1)** (130 mg), m.p. 135-137° C; ^{1}H NMR (200 MHz): δ 0.78 and 1.13 (each 3H, s), 3.05 (1H, br s, H-13), 3.14 and 3.43 (each 1H, d, J = 11 Hz, H-18), 5.25 and 5.94 (each 1H, s, H-17); EIMS *m/z* (rel. int.): 302 $[M]^+$ (8), 287 (3), 284 (2), 272 (49), 271 (32), 257 (5), 253 (14), 239 (9), 229 (2), 215 (3), 201 (7), 189 (16). Further elution gave **18-hydroxy-17-methoxy-15-oxo-*ent*-(16R)-kaurane** (5 mg), ^{1}H NMR (200 MHz): δ 0.76 and 1.12 (each 3H, s), 2.34 and 3.44 (each 1H, br m, H-17), 2.49 (1H, br s, H-13), 3.12 and 3.45 (each 1H, d, J = 11 Hz, H-18), 3.30 (3H, s, -OMe), 3.44 (1H, br m, H-16); EIMS *m/z* (rel. int.): 334 $[M]^+$ (12), 319 (3), 304 (41), 303 (30), 302 (17), 289 (10), 271 (12), 253 (11), 246 (63), 215 (17), 199 (6), 181 (32). Further elution gave a mixture of the methyl ethers (150 mg), and pure **18-hydroxy-17-methoxy-15-oxo-*ent*-(16S)-kaurane** (18 mg), ^{1}H NMR (200 MHz): δ 0.76 and 1.11 (each 3H, s), 2.59 and 3.79 (each 1H, dd, J = 10 and 4 Hz, H-17), 2.62 (1H, br s, H-13), 3.12 and 3.41 (each 1H, d, J = 11 Hz, H-18), 3.37 (1H, br m, H-16), 3.37 (3H, s, -OMe); EIMS *m/z* (rel. int.): 334 $[M]^+$ (23), 319 (14), 304 (100), 303 (58), 302 (24), 289 (23), 285 (17), 271 (31), 246 (79), 215 (24), 181 (27).

The mixture of methyl ethers obtained above (150 mg) in collidine (1 ml) was refluxed for 12 h. Usual work-up and chromatography on silica gel, using petrol-EtOAc (8:2) as eluent, afforded **1** (81 mg) and part of the starting *ent*-(16R)-derivative (55 mg). The *ent*-(16S)-derivative of the mixture was totally transformed during the reaction.

Hydrogenation of 1.- Compound **1** (10 mg) dissolved in EtOH (5 ml) was hydrogenated over C/Pd (5%) (10 mg) at room temp. for 5 hr. The solution was filtered and evaporated affording (**14**), identical with the product obtained in the incubation of **1**.

Incubation experiments. The fungus *Gibberella fujikuroi* (IMI 58289) inhibited with $5x10^{-5}$ M AMO 1618, was grown in shake culture at 25° for 2 days in 65 conical flasks (250 ml) each containing sterile medium (50 ml).[19] The substrate **1** (200 mg) in EtOH (15 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The broth was filtered and extracted with EtOAc. The mycelium was treated with liquid N_2, crushed with a mortar and extracted with EtOAc. The two extracts were combined, dried and concd adjusted to pH 2 with dil HCl, and extracted with EtOAc. The extract was separated into acidic and neutral fractions with $NaHCO_3$.

Chromatography of the neutral fraction and elution with mixtures of petrol-EtOAc gave starting material (8 mg), 18-hydroxy-15-oxo-*ent*-(16S)-kaurane (**14**) (11 mg), 11β,18-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**16**) (18 mg), 18,19-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**20**) (10 mg), 7α,18-dihydroxy-15-oxo-*ent*-(16S)-kaurane (**24**) (5 mg), and a mixture of compounds, which was resolved by acetylation and chromatography eluting with petrol-EtOAc (9:1) to afford 11β,16β,18-triacetoxy-15-oxo-*ent*-(16S)-kaurane (**19**) (9 mg) and 16β,18,19-triacetoxy-15-oxo-*ent*-(16S)-kaurane (**23**) (2 mg). No metabolites were isolated from the acidic fraction.

18-Hydroxy-15-oxo-*ent*-(16S)-kaurane (14).- $[M]^+$ at *m/z* 304.2430. $C_{20}H_{32}O_2$ requires 304.2402; ^{1}H NMR (400 MHz): δ 0.76 and 1.10 (each 3H, s), 1.10 (3H, d, J = 7 Hz, H-17), 3.13 and 3.43 (each 1H, d, J = 11 Hz, H-18); EIMS *m/z* (rel. int.): 304 $[M]^+$ (3), 289 (1), 274 (15), 273 (11), 246 (12), 215 (6), 203 (3),

191 (4). **Acetate (15)** $[M]^+$ at *m/z* 346.2498. $C_{22}H_{34}O_3$ requires 346.2508; m.p. 146-148° C; ^1H NMR (200 MHz): δ 0.82 and 1.11 (each 3H, s), 1.10 (3H, d, J = 7 Hz, H-17), 2.09 (3H, s), 3.63 and 3.85 (each 1H, d, J = 11 Hz, H-18); EIMS *m/z* (rel. int.): 346 $[M]^+$ (6), 331 (1), 328 (1), 288 (36), 286 (19), 273 (16), 272 (4), 255 (8), 243 (18), 228 (20), 213 (17), 191 (5).

11β,18-Dihydroxy-15-oxo-*ent*-(16S)-kaurane (16).- $[M]^+$ at *m/z* 320.2348. $C_{20}H_{32}O_3$ requires 320.2351; ^1H NMR (400 MHz): δ 0.75 and 1.02 (each 3H, s), 1.25 (3H, d, J = 6.8 Hz, H-17), 3.11 and 3.42 (each 1H, d, J = 11 Hz, H-18), 3.94 (1H, d, J = 5.7 Hz, H-11), EIMS *m/z* (rel. int.): 320 $[M]^+$ (2), 305 (10), 302 (3), 289 (17), 275 (5), 271 (6), 246 (4), 213 (11), 199 (3), 193 (3): **Diacetate (17)** $[M]^+$ at *m/z* 404.2554. $C_{24}H_{36}O_5$ requires 404.2562; ^1H NMR (400 MHz): δ 0.81 and 1.07 (each 3H, s), 1.18 (3H, d, J = 7 Hz, H-17), 1.96 and 2.08 (each 3H, s), 3.62 and 3.85 (each 1H, d, J = 11 Hz, H-18), 5.07 (1H, d, J = 5.3 Hz, H-11); EIMS *m/z* (rel. int.): 404 $[M]^+$ (10), 386 (3), 362 (3), 344 (58), 331 (10), 316 (5), 301 (8), 284 (61), 271 (57), 269 (55), 266 (10), 256 (22), 248 (24), 228 (29), 226 (33), 213 (25), 211 (30), 201 (17), 199 (14).

18,19-Dihydroxy-15-oxo-*ent*-(16S)-kaurane (20).-^1H NMR (200 MHz): δ 1.06 (3H, s), 1.10 (3H, d, J = 7 Hz, H-17), 3.41 and 3.89 (each 1H, d, J = 11 Hz, H-18), 3.76 and 3.92 (each 1H, d, J = 11 Hz, H-19); EIMS *m/z* (rel. int.): 320 $[M]^+$ (1), 305 (2), 288 (2), 274 (2), 273 (3), 262 (2), 256 (2), 243 (3), 241 (8), 215 (6), 203 (15), 199 (9). **Diacetate (21)**. $[M]^+$ at *m/z* 404.2554. $C_{24}H_{36}O_5$ requires 404.2562; ^1H NMR (400 MHz): δ 1.10 (3H, d, J = 7 Hz, H-17), 1.13 (3H, s), 2.05 and 2.09 (each 3H, s), 3.95 and 4.03 (each 1H, d, J = 11 Hz, H-18), 4.02 and 4.29 (each 1H, d, J = 11 Hz, H-19); EIMS *m/z* (rel. int.): 404 $[M]^+$ (17), 386 (2), 374 (1), 346 (35), 344 (9), 329 (5), 316 (3), 304 (4), 284 (73), 272 (8), 269 (21), 226 (63), 214 (13), 211 (29), 199 (12).

7α,18-Dihydroxy-15-oxo-*ent*-(16S)-kaurane (24).-^1H NMR (400 MHz): δ 0.76 and 1.11 (each 3H, s), 1.10 (3H, d, J = 7 Hz, H-17), 3.10 and 3.43 (each 1H, d, J = 11 Hz, H-18), 3.98 (1H, dd, J = 11 and 4 Hz, H-7); EIMS *m/z* (rel. int.): 320 $[M]^+$ (39), 302 (8), 290 (15), 289 (5), 288 (5), 271 (32), 262 (38), 253 (10), 244 (6), 231 (3), 213 (12), 199 (8). **Diacetate (25)**; $[M - C_2H_2O]^+$ at *m/z* 362.2447. $C_{22}H_{34}O_4$ requires 362.2457; ^1H NMR (400 MHz): δ 0.81 and 1.14 (each 3H, s), 1.11 (3H, d, J = 7 Hz, H-17), 1.93 and 2.13 (each 3H, s), 3.58 and 3.85 (each 1H, d, J = 11 Hz, H-18), 4.97 (1H, dd, J = 11 and 4 Hz, H-7); EIMS *m/z* (rel. int.): 404 $[M]^+$ (1), 362 (9), 346 (2), 344 (8), 304 (33), 302 (2), 284 (41), 273 (4), 271 (20), 257 (14), 241 (16), 227 (20), 213 (18), 199 (9).

11β,16α,18-Triacetoxy-15-oxo-*ent*-(16S)-kaurane (19). $[M - HOAc]^+$ at *m/z* 402.2394. $C_{24}H_{34}O_5$ requires 402.2406; ^1H NMR (200 MHz): δ 0.83, 1.07 and 1.66 (each 3H, s), 1.96, 2.01 and 2.11 (each 3H, s), 3.65 and 3.87 (each 1H, d, J = 11 Hz, H-18), 5.08 (1H, d, J = 5 Hz, H-11); EIMS *m/z* (rel. int.): 462 $[M]^+$ (11), 402 (17), 360 (83), 342 (85), 271 (50), 269 (13), 257 (47), 227 (51), 199 (10).

16α,18,19-Triacetoxy-15-oxo-*ent*-(16S)-kaurane (23). $[M - HOAc]^+$ at *m/z* 402.2408. $C_{24}H_{34}O_5$ requires 402.2406; ^1H NMR (400 MHz): δ 1.12 and 1.53 (each 3H, s), 1.99, 2.05 and 2.10 (each 3H, s, -OAc), 3.96 and 4.04 (each 1H, d, J = 11 Hz, H-19), 4.03 and 4,28 (each 1H, d, J = 11 Hz, H-18); EIMS *m/z* (rel. int.): 462 $[M]^+$ (2), 402 (27), 346 (5), 342 (17), 300 (9), 287 (20), 282 (30), 271 (34), 254 (20), 239 (27), 227 (32), 211 (13), 199 (14).

Part 2

3α-Hydroxy-*ent*-kaur-15-ene (27).- The acetate **26** (650 mg), which had been prepared from linearol,[14] was treated with 5% methanolic KOH (40 ml) at room temp. overnight. Usual work up gave the alcohol **27** (530 mg), ^1H NMR (400 MHz): δ 0.77, 0.98. 1.03 and 1.70 (each 3H, s), 2.32 (1H, br s, H-13), 3.19 (1H, dd, J = 10.7 and 5.6 Hz, H-3), 5.05 (1H, br s, H-15).

3α-Hydroxy-15α,16α-epoxy-*ent*-kaurane (29). Compound **27** (20 mg) in $CHCl_3$ (5 ml) was treated with m-chloroperbenzoic acid (10 mg) at room temp. for 20 h. The solution was diluted with $CHCl_3$ and thoroughly washed with an aqueous solution of. $NaHCO_3$. The solvent was evaporated and the residue chromatographed. Elution with petrol-EtOAc (7:3) afforded **29** (18 mg), ^1H NMR (200 MHz): δ 0.77, 0.99 and 1.00 (each 3H, s), 1.45 (3H, s, H-17), 2.11 (1H, br s, H-13), 2.66 (1H, s, H-15), 3.21 (1H, dd, J = 10.1 and 6.2 Hz, H-3).

Oxidation of 29 with PDC. Compound **29** (18 mg) in CH_2Cl_2 was treated with pyridinium dichromate (3.1 mg) at room temp. for 48 hr. The solution was diluted with CH_2Cl_2, filtered and evaporated, affording 3-oxo-15α,16α-epoxy-*ent*-kaurane (**30**) (14 mg).

Oxidation of 27 with Jones reagent. Compound **27** (43 mg) in Me_2CO (5 ml) was treated dropwise with a light excess of Jones reagent and left at room temp. for 15 min. Then, MeOH was added to destroy the excess reagent. The mixture was poured into H_2O and worked up. The solvent was evaporated and the residue chromatographed on silica gel. Elution with petrol-EtOAc (8:2) gave **3-oxo-*ent*-kaur-15-ene (28)** (18 mg), $[M]^+$ at *m/z* 286.2292. $C_{20}H_{30}O$ requires 286.2296; ^1H NMR (200 MHz): δ 1.03, 1.08 and 1.12 (each 3H, s), 1.71 (3H, d, J = 1.2 Hz, H-17), 2.35 (1H, br s, H-13), 2.45 (2H, m, H-2), 5.08 (1H, d, J = 1.2 Hz, H-15); EIMS *m/z* (rel. int.): 286 $[M]^+$ (21), 271 (8), 243 (24), 201 (16), 187 (8). Further elution gave **3-oxo-15α,16α-epoxy-*ent*-kaurane (30)** (10 mg); ^1H NMR (400 MHz): δ 1.02, 1.08 and 1.09 (each 3H, s) and 1.43 (3H, s, H-17), 2.13 (1H, br s, H-13), 2.48 (2H, m, H-2), 2.67 (1H, s, H-15),; EIMS *m/z* (rel. int.): 302 $[M]^+$ (9), 287 (24), 269 (8), 259 (30), 241 (32), 217 (29), 199 (8). Further elution afforded **3-oxo-15α-hydroxy-*ent*-kaur-16-ene (31)** (1 mg), ^1H NMR (200 MHz): δ 1.04, 1.08 and 1.10 (each 3H, s), 2.47 (2H, m, H-2), 3.82 (1H, s, H-15), 5.10 and 5.23 (each 1H, s, H-17).

Oxidation of 27 with PDC. Compound **27** (200 mg) in CH_2Cl_2 (12 ml) was treated with pyridinium dichromate (360 mg) at room temp. for 24 h. The soln was diluted with Et_2O, filtered and evaporated. The residue was chromatographed, eluting with petrol-EtOAc (7:3), to give 3-oxo-*ent*-kaur-15-ene (**28**) (180 mg) and 3-oxo-15α,16α-epoxy-*ent*-(16S)-kaurane (**30**) (12 mg).

Epoxidation of (28). Compound (**28**) (195 mg) was dissolved in $CHCl_3$ (25 ml) and treated with m-chloroperbenzoic acid (120 mg) at room temp. for 6 h, as described above for **27**. In this way product **30** (190 mg) was obtained.

Dehydrogenation of 30. To a solution of the ketone **30** (225 mg) in dry dichlorobenzene (10 ml) was added benzeneselenic anhydride (245 mg). The mixture was heated at 95°C with stirring for 2 h. The solvent was evaporated off and the residue chromatographed on silica gel. Elution with petrol-EtOAc (8:2) gave **3-oxo-15α,16α-epoxy-*ent*-kaur-1-ene (32)** (180 mg), m.p. 166-168° C; $[M]^+$ at *m/z* 300.2082. $C_{20}H_{28}O_2$ requires 300.2089; IR ($CHCl_3$) υ_{max}: 1731, 1662, 1601, 1085, cm^{-1}; UV (EtOH) λ_{max}: 226.5 nm; ^1H NMR (400 MHz): δ 1.03, 1.08, 1.18 and 1.39 (each 3H, s), 2.10 (1H, br s, H-13), 2.65 (1H, s, H-15), 5.79 (1H, d, J = 10.2 Hz, H-2), 7.04 (1H, d, J = 10.2 Hz, H-1); EIMS *m/z* (rel. int.): 300 $[M]^+$ (41), 285 (14), 267 (5), 257 (12), 241 (21), 229 (10), 199 (4).

Incubation experiments. The fungus *G. fujikuroi*, inhibited with $5x10^{-5}$ M AMO 1618, was grown in shake culture at 25° for 2 days in 58 conical flasks (250 ml), each containing sterile medium (50 ml).[19] The substrate **32** in EtOH (10 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The broth was filtered and extracted with EtOAc. The mycelium was treated with liquid N_2, crushed with a mortar and extracted with EtOAc. The two extracts were combined, dried and concentrated.

Chromatography of the residue on silica gel eluting with petrol-EtOAc (9:1) gave starting material (15 mg) and four product mixtures, which were separately resolved by acetylation and chromatography in the following way. The chromatography of the first mixture, eluting with petrol-EtOAc (8:2), gave **44** (3 mg), **52** (24 mg) and **35** (11 mg). The chromatography of the second mixtureon silica gel, by elution with petrol-EtOAc (7:3), led to the substances **46** (2 mg), **42** (3 mg), **54** (3 mg), and **50** (7 mg). The chromatography of the third mixture, eluting with petrol-EtOAc (7:3), yielded **56** (24 mg) and **58** (6 mg). Finally, the chromatography of the fourth mixture, eluting with petrol-EtOAc (6:4) afforded **60** (3 mg), **48** (10 mg), **62** (3 mg), **64** (2.5 mg), and **66** (2 mg)

3-Oxo-7β,15α-diacetoxy-*ent*-kaur-1,16-diene (44). $[M]^+$ at *m/z* 400.2251. $C_{24}H_{32}O_5$ requires 400.2249; ^1H NMR (200 MHz): δ 0.75, 0.85 and 0.88 (each 3H, s), 1.98 and 1.99 (each 3H, s), 2.91 (1H, br s, H-13), 5.06 (1H, br s, H-7), 5.12 and 5.26 (each 1H, s, H-17), 5.45 (1H, s, H-15), 5.90 and 7.13 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 400 $[M]^+$ (9), 358 (4), 340 (71), 325 (22), 298 (58), 280 (39), 265 (29), 252 (14), 237 (20), 229 (11), 199 (11).

3-Oxo-15α-acetoxy-11β,16β-epoxy-*ent*-kaur-1-ene (52). M.P. 133-135° C; $[M]^+$ at *m/z* 358.2139. $C_{22}H_{30}O_4$ requires 358.2144; ^1H NMR (200 MHz): δ 1.11 (6H, s), 1.24 and 1.28 (each 3H, s), 2.07 (3H, s), 2.28 (1H, br s, H-13), 4.51 (1H, br s, H-11), 4.82 (1H, br s, H-15), 5.88 and 7.06 (each 1H, d, J = 10.2 Hz, H-1 and H-2); EIMS *m/z* (rel. int.): 358 $[M]^+$ (71), 343 (15), 316 (76), 298 (44), 283 (22), 270 (24), 265 (9), 255 (25), 242 (13), 204 (29), 203 (24), 199 (10).

3-Oxo-7β-acetoxy-15α,16α-epoxy-*ent*-kaur-1-ene (35). M.p. 141-143° C; $[M]^+$ at *m/z* 358.2152. $C_{22}H_{30}O_4$ requires 358.2144; ^1H NMR (200 MHz): δ 1.05, 1.06 and 1.25 (each 3H, s), 1.46 (3H, s, H-17), 2.07 (3H, s), 2.23 (1H, br s, H-13), 2.99 (1H, s, H-15), 4.89 (1H, br s, H-7), 5.88 and 7.09 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 358 $[M]^+$ (23), 343 (9), 316 (21), 298 (64), 283 (58), 255 (46), 241 (10), 239 (16), 237 (16), 227 (19), 215 (9), 199 (15).

3-Oxo-7α,15α-diacetoxy-*ent*-kaur-1,16-diene (46). $[M]^+$ at *m/z* 400.2240. $C_{24}H_{32}O_5$ requires 400.2249; ^1H NMR (200 MHz): δ 0.19, 0.20 and 1.27 (each 3H, s), 2.31 and 2.34 (each 3H, s), 5.07 (1H, dd, J = 10.6 and 4.5 Hz, H-7), 5.15 and 5.20 (each 1H, s, H-17), 5.30 (1H, s, H-15), 5.91 and 7.00 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 400 $[M]^+$ (5), 358 (10), 340 (31), 325 (16), 298 (63), 280 (40), 265 (38), 255 (16), 237 (27), 223 (13), 199 (12).

3-Oxo-15α,16β-diacetoxy-*ent*-kaur-1-ene (42). $[M]^+$ at *m/z* 402.2395. $C_{24}H_{34}O_5$ requires 402.2406; ^1H NMR (200 MHz): δ 1.08, 1.12 and 1.26 (each 3H, s), 1.49 (3H, s, H-17), 2.00 and 2.11 (each 3H, s), 2.35 (1H, br s, H-13), 4.72 (1H, s, H-15), 5.84 and 6.90 (each 1H, d, J = 10.2 Hz, H-2 and H-1), EIMS *m/z* (rel. int.): 402 $[M]^+$ (3), 360 (4), 342 (36), 327 (6), 314 (6), 300 (100), 285 (12), 282 (36), 267 (23), 254 (16), 239 (16), 213 (12), 199 (8).

3-Oxo-6α,15α-diacetoxy-11β,16β-epoxy-*ent*-kaur-1-ene (54). $[M]^+$ at *m/z* 416.2196. $C_{24}H_{32}O_6$ requires 416.2198; ^1H NMR (200 MHz): δ 1.18, 1.20 and 1.37 (each 3H, s), 1.50 (3H, s, H-17), 2.07 and 2.08 (each 3H, s), , 4.58 (1H, br s, H-11), 4.86 (1H, s, H-15), 5.37 (1H, br s, H-6), 5.89 and 7.04 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 416 $[M]^+$ (35), 374 (49), 356 (50), 314 (54), 296 (42), 281 (26), 268 (19), 253 (29), 239 (16), 213 (13), 199 (14).

3-Oxo-11β,15α-diacetoxy-*ent*-kaur-1,16-diene (50). M.p. 186-188° C; $[M]^+$ at *m/z* 400.2257. $C_{24}H_{32}O_5$ requires 400.2249; ^1H NMR (200 MHz): δ 1.12 (6H, s), 1.20 (3H, s), 2.01 and 2.10 (each 3H, s), 2.85 (1H, br s, H-13), 5.09 and 5.17 (each 1H, s, H-17), 5.24 (1H, br s, H-11), 5.64 (1H, s, H-15), 5.91 and 7.35 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 400 $[M]^+$ (5), 358 (7), 340 (29), 325 (8), 298 (72), 283 (21), 280 (30), 265 (32), 254 (18), 237 (13), 211 (15), 199 (8).

3-Oxo-7α,15α-diacetoxy-11β,16β-epoxy-*ent*-kaur-1-ene (56). M.p. 237-239° C; $[M]^+$ at *m/z* 416.2208. $C_{24}H_{32}O_6$ requires 416.2198; ^1H NMR (200 MHz): δ 1.10, 1.12, 1.27 and 1.28 (each 3H, s), 2.00 and 2.05 (each 3H, s), 4.50 (1H, br s, H-11), 4.99 (1H, s, H-15), 5.05 (1H, dd, J = 5 and 10.8 Hz, H-7), 5.91 and 7.04 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 416 $[M]^+$ (10), 401 (1), 374 (15), 356 (17), 314 (49), 296 (23), 271 (10), 253 (10), 240 (16), 202 (15), 199 (10).

3-Oxo-7β,15α-diacetoxy-11β,16β-epoxy-*ent*-kaur-1-ene (58). M.p. 226-228° C;$[M]^+$ at *m/z* 416.2192. $C_{24}H_{32}O_6$ requires 416.2198; ^1H NMR (200 MHz): δ 1.05, 1.09, 1.27 and 1.28 (each 3H, s), 1.98 and 2.01 (each 3H, s), 2.32 (1H, br s, H-13), 4.54 (1H, br s, H-11), 4.98 (1H, br s, H-7), 5.07 (1H, s, H-15), 5.91 and 7.06 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 416 $[M]^+$ (24), 374 (20), 356 (60), 314 (55), 296 (70), 281 (30), 271 (13), 268 (15), 253 (29), 240 (19), 237 (14), 225 (23), 201 (25), 199 (20).

3-Oxo-13,15α-diacetoxy-11β,16β-epoxy-*ent*-kaur-1-ene (60). $[M]^+$ at *m/z* 416.2196. $C_{24}H_{32}O_6$ requires 416.2198; ^1H NMR (200 MHz): δ 1.10, 1.12, 1.26 and 1.27 (each 3H, s), 2.08 and 2.11 (each 3H, s), 4.54 (1H, br s, H-11), 4.77 (1H, s, H-15), 5.89 and 7.02 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 416 $[M]^+$ (4), 374 (42), 356 (4), 314 (6), 296 (15), 281 (8), 253 (5), 241 (5), 211 (6), 199 (5).

3-Oxo-7β,14α,16β-triacetoxy-*ent*-beyer-1-ene (48). $[M]^+$ at *m/z* 460.2465. $C_{26}H_{36}O_7$ requires 460.2465; ^1H NMR (200 MHz): δ 0.88 and 1.08 (each 3H, s), 1.13 (6H, s), 1.54 (1H, t, H-15), 2.07 (3H, s), 2.12 (6 H, s), 2.67 (1H, dd, J = 14 and 8 Hz, H-15), 4.83 (1H, m, H-16), 4.99 (1H, br s, H-7), 5.05 (1H, s, H-14), 5.86 and 6.89 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 460 $[M]^+$ (5), 445 (3), 418 (1), 400 (7), 358 (8), 340 (19), 312 (24), 298 (17), 280 (17), 263 (19), 252 (6), 199 (5).

3-Oxo-15α,17-diacetoxy-11β,16β-epoxy-*ent*-kaur-1-ene (62). $[M]^+$ at *m/z* 416.2205. $C_{24}H_{32}O_6$ requires 416.2198; ^1H NMR (200 MHz): δ 1.12 (3H, s), 1.25 (6H, s), 2.06 and 2.08 (each 3H, s), 4.03 and 4.56 (each 1H, d, J = 12 Hz, H-17), 4.60 (1H, br s, H-11), 5.03 (1H, s, H-15), 5.89 and 7.04 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 416 $[M]^+$ (10), 374 (31), 356 (13), 341 (5), 301 (2), 296 (26), 283 (9), 281 (12), 268 (8), 265 (5), 253 (11).

3-Oxo-7β,15α-diacetoxy-16β-hydroxy-*ent*-kaur-1-ene (64). $[M - H_2O]^+$ at *m/z* 400.2253. $C_{24}H_{32}O_5$ requires 400.2249; ^1H NMR (200 MHz): δ 1.07, 1.14, 1.25 and 1.28 (each 3H, s), 1,96 and 2.05 (each 3H, s), 4.53 (1H, s, H-15), 4.88 (1H, br s, H-7), 5.88 and 6.94 (each 1H, d, J = 10.2 Hz, H-2 and H-1); EIMS *m/z* (rel. int.): 418 $[M]^+$ (5), 400 (39), 376 (6), 358 (62), 343 (9), 340 (46), 316 (52), 298 (100), 283 (34), 280 (36), 270 (23), 265 (24), 255 (31), 241 (21), 237 (27), 211 (23), 199 (27).

3-Oxo-11β,15α-diacetoxy-16β-hydroxy-*ent*-kaur-1-ene (66). $[M - H_2O]^+$ at *m/z* 400.2251. $C_{24}H_{32}O_5$ requires 400.2249; ^1H NMR (200 MHz) δ: 1.05, 1.13 and 1.26 (each 3H, s), 2.11 and 2.12 (each 3H, s), 4.99 (1H, s, H-15), 5.47 (1H, t, H-11), 5.83 and 6.72 (each 1H, d, J = 10.1, H-2 and H-1); MS *m/z* (rel. int.): 400 $[M - H_2O]$ (15), 358 (12), 341 (77), 340 (22), 325 (4), 316 (4), 298 (57), 280 (78), 265 (59), 247 (3), 237 (21), 211 (20), 209 (13).

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REFERENCES AND NOTES

1. Fraga, B.M.; González, P.; Guillermo, R.; Hernández, M.G.; Perales, A. *Tetrahedron* **1995**, 51, 10053.
2. De Oliveira, A.B; Hanson, J.R.; Takahashi, J.A. *Phytochemistry* **1995**, 40, 439.
3. Fraga, B.M.; Hanson, J.R.; Hernández, M.G. *Phytochemistry* **1978**, 17, 812.
4. Matsuo, A.; Uto, S.; Nakayama, M; Hayashi S. *Chem. Lett.***1977**, 327.
5. Asakawa, Y.; Takikawa, K.; Toyota, M.; Ueda, A.; Tori, M.; Lumar, S.S. *Phytochemistry* **1987**, 26, 1019.
6. Rodríguez, B.; Valverde, S.; Rocha, J.M. *An. Quim.* **1970**, 66, 503.
7. González, A.G.; Fraga, B.M.; Hernández, M.G.; Luis, J.G; Larruga, F. *Biochem. Syst. Ecol.* **1979**, 7, 115.
8. In this reaction, the methyl ethers 18-hydroxy-17-methoxy-15-oxo-*ent*-(16R)-kaurane and 18-hydroxy-17-methoxy-15-oxo-*ent*-(16S)-kaurane were also obtained. The stereochemistry assigned at C-16 was given on the basis that, in the major compound isolated, the MeO^- group enters by the less hindered α-face.
9. Dennis, D.T.; Upper, C.D.; West, C.A. *Plant Physiol.* **1965**, 40, 948.
10. Cross, B.E.; Myers, P.L. *Phytochemistry* **1969**, 8. 79.
11. Fraga, B.M.; Hernández, M.G.; González, P. *Phytochemistry* **1992**, 31, 3845.
12. Herz, W.; Sharma, R.P. *J. Org. Chem.* **1976**, 41, 1021.
13. Boaventura, M.A.D.; Hanson; J.R.; Hitchcock, P.B.;Takahashi, J.A. *Phytochemistry* **1994**, 37, 387.
14. Fraga, B.M., Hernández, M.G., García-Tellado, F., González, P. and Perales, A. *Phytochemistry* **1993**, 34, 133.
15. Fraga, B.M.; González, A.G.; Hanson, J.R.; Hernández, M.G. *Phytochemistry* **1981**, 20, 57.
16. Barton, D.H.R.; Cussans, N.J.; Ley, S.V. *J. Chem. Soc., Chem. Commun.* **1977**, 144.
17. The facility to undergo nucleophilic attack of this group renders this type of compounds biologically actives as antifeedant, antibacterial, cytotocic and anti-HIV agents.
18. Fraga, B.M.; Hanson, J.R., Hernández, M.G.; Sarah, F.Y. *Phytochemistry* **1980**, 19, 1087.
19. Hanson, J.R.; Hawker, J.; White, A.F. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1982.

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