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The Chemical and Microbiological Preparation of 15-oxo-Gibberellin Derivatives

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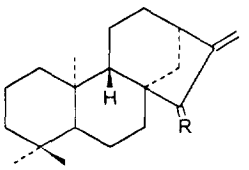
Abstract: The biotransformation of 15-oxo-*ent*-kaur-16-ene (**1**) with the fungus *Gibberella fujikuroi* gave 16α,17-dihydro-7β-hydroxy-15-oxo-kaurenolide (**5**), 16α,17-dihydro-15-oxo-GA_{12} (**7**) 16α,17-dihydro-15-oxo-GA_{25} (**8**) 16α,17-dihydro-15-oxo-GA_{24} (**9**), 7-aldehyde of 16α,17-dihydro-15-oxo-GA_{14} (**10**), *ent*-3α,7α-15-oxo-kauran-19-oic acid (**11**), and 16α,17-dihydro-15-oxo-GA_7 (**12**). The hydrogenation of the 16,17-double bond observed in this incubation is directed by the presence of the 15-oxo group, and it does not take place in the normal biosynthetic gibberellin pathway. The chemical preparation of some of these 15-oxo-gibberellins has also been carried out.

The gibberellins are a group of diterpenes that participate in the regulation of the growth and development of higher plants.[1] They possess a common carbon skeleton and differ mainly from one another in their hydroxylation pattern. Natural gibberellins with an oxo group at C-15 are unknown and we decided to prepare 15-oxo-gibberellins by chemical and microbiological methods. The latter form, using the fungus gibberellin producer *Gibberella fujikuroi* and 15-oxo-*ent*-kaur-16-ene derivatives as substrates, also permits the effects of the 15-oxo-function to be seen in the biotransformation products formed. The preparation of 15-oxo-*ent*-kaur-16-ene derivatives may also be useful because it is known that this type of products also has biological properties.[2]

We have incubated 15-oxo-*ent*-kaur-16-ene (**1**) with *G. fujikuroi*. This diterpene had been isolated from the liverwort *Jungermannia infusca*[3] and we have now prepared it by oxidation of 15α-hydroxy-*ent*-kaur-16-ene (**2**)[4] with pyridinium dichromate. In this reaction a mixture of the aldehydes **3** and **4** was also obtained. The fermentation of **1** was carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene and consequently its endogenous metabolites.[5,6] The transformation was carried out for a period of 6 days, and the combined broth and mycelium extract separated into neutral and acid fractions.

Only the lactone 16α,17-dihydro-7β-hydroxy-15-oxo-kaurenolide (**5**) was obtained from the neutral fraction. Its ^{1}H NMR spectrum was in accordance with this structure showing the signals of the H-5, H-6 and H-7 protons at δ 1.88 (d), 5.05 (t) and 4.21 (d), with a coupling constant of J = 6 Hz in all cases. The C-17 methyl resonated at δ 1.14 as a doublet (J = 7 Hz). Acetylation of this compound formed the acetate **6**, which showed the H-5, H-6 and H-7 hydrogens at δ 1.95 (d), 5.15 (t) and 5.69 (d). The β-stereochemistry assigned to C-17 was given on the basis that the biological and synthetic hydrogenation of this type of compounds occurs by the α-face of the molecule.[7] A similar α-attack must occur in the epoxidation of **3** to give **4**.[8]



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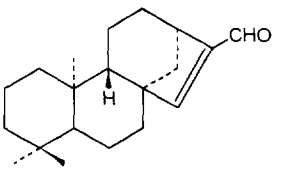
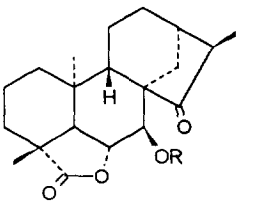
2 R = β-OH, H



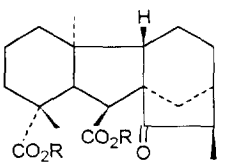
3

4 15α,16α-epoxy



5 R = H

6 R = Ac



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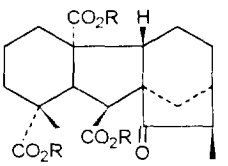
13 R = Me



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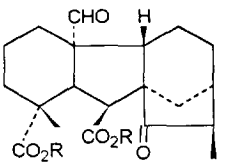
14 R = Me



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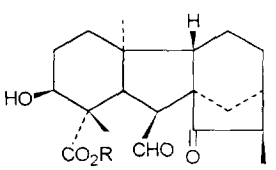
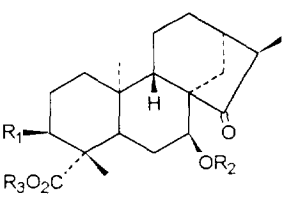
15 R = Me



10 R = H

16 R = Me



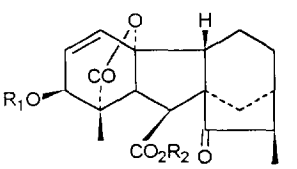
11 R_1 = OH R_2 = R_3 = H

17 R_1 = OH R_2 = H R_3 = Me

18 R_1 = OAc R_2 = H R_3 = Me

19 R_1 = OAc R_2 = Ac R_3 = Me

20 R_1 = R_2 = R_3 = H



12 R_1 = R_2 = H

21 R_1 = H R_2 = Me

22 R_1 = Ac R_2 = Me

The acidic fraction was methylated and chromatographed. In this way the substances **7-12** were isolated and identified as their methyl esters: 16α,17-dihydro-15-oxo-GA_{12} methyl ester (**13**), 16α,17-dihydro-15-oxo-GA_{25} trimethyl ester (**14**), 16α,17-dihydro-15-oxo-GA_{24} dimethyl ester (**15**), the 7-aldehyde of 16α,17-

dihydro-15-oxo-GA_{14} methyl ester (**16**), *ent*-3α,7α-dihydroxy-15-oxo-kauran-19-oic methyl ester (**17**) and 16α,17-dihydro-15-oxo-GA_7 methyl ester (**21**).

The high resolution MS of the least polar compound was in accordance with the formula $C_{22}H_{32}O_5$ and was identified as 16α,17-dihydro-15-oxo-GA_{12} methyl ester (**13**) on the basis of the ^1H NMR spectrum, which showed two singlets of methyl groups, the typical H-5,H-6 doublets (δ 2.05 and 3.36, J = 12 Hz) and a doublet at δ 1.03 (J = 7 Hz) assignable to the C-17 methyl. The assignment of its ^{13}C NMR spectrum (table 2) was carried out by a 2D NMR HMQC experiment, and confirmed the structure **13**.

16α,17-Dihydro-15-oxo-GA_{25} trimethyl ester (**14**), molecular formula $C_{23}H_{32}O_7$, showed a similar pattern of signals with H-5 and H-6 at δ 2.30 and 3.85, the C-17 methyl at δ 1.00 (d, J = 7 Hz) and three methoxyl groups at δ 3.60, 3.61 and 3.62. Likewise, the biogenetic precursor of this gibberellin, 16α,17-15-oxo-dihydro-GA_{24} (**9**), was also obtained in this feeding and characterized by the ^1H NMR spectrum of its dimethyl ester **15**, which showed a singlet typical of the aldehyde group at δ 9.66, the H-5 and H-6 doublets at δ 2.38 and 3.93, respectively, and the C-17 methyl doublet at δ 1.03.

Another gibberellin obtained in the incubation of **1** was the 7-aldehyde of 16α,17-dihydro-15-oxo-GA_{14} (**10**). The ^1H NMR spectrum of its dimethyl ester **16** showed characteristic signals of three methyl groups, one secondary at δ 1.04 (d), a methoxyl group at δ 3.67, the H-5 and H-6 at δ 2.67 and 2.99, the first as a doublet (J = 12 Hz) and the second as a double doublet (J = 12 and 4 Hz), the H-3 as a broad singlet and the aldehydic hydrogen at δ 10.13 (d, J = 4 Hz). Double resonance experiments were in accordance with these assignments. Thus, irradiation of the doublet at δ 10.13 collapsed the double doublet at δ 2.99 to a doublet. Alternatively, irradiation of this latter signal transformed the doublet at δ 10.13 into a singlet, and the doublet at δ 2.67 into another singlet. This compound by acetylation formed a monoacetate, the H-3 now appearing at δ 5.44. Its MS showed a peak at 376 *m/z* formed by loss of 28 mass units from the molecular ion, which is characteristic of gibberellin methyl esters with an aldehydic function.[9]

The sole C_{19} gibberellin obtained in this biotransformation was 16α,17-dihydro-15-oxo-GA_7 (**12**). The high resolution mass spectrum of its methyl ester indicated its formula, $C_{20}H_{24}O_6$. Its ^1H NMR spectrum showed one angular methyl (δ 1.32, s), a secondary methyl (δ 1.08, d, J = 7 Hz), the H-5 and H-6 at δ 3.24 and 2.68 as two doublets (J = 10 Hz), the hydrogen geminal to the hydroxyl group at C-3 (δ 4.16) and the H-1 and H-2 at δ 6.28 and 5.94, the first as a doublet (J = 10 Hz) and the second as a double doublet (J = 10 and 3.6 Hz).

An *ent*-kaurane derivative was also obtained in this feeding, and characterized as *ent*-3α,7α-dihydroxy-15-oxo-kauran-19-oic acid (**11**). The MS of its methyl ester **17** showed the fragment of higher mass at *m/z* 346 ($M^+ - H_2O$). Its ^1H NMR spectrum showed besides the signals of the three methyls and one methoxyl group, those of two geminal hydrogens to two hydroxyl groups at δ 3.86 and 4.10, each resonating as a broad singlet. The first resonance is characteristic in chemical shift and form of the geminal proton to a 7β-alcohol.[10] The second resonance can be assigned to a geminal hydrogen to a 1β- or 3β-hydroxyl group. The ^{13}C NMR spectrum (table 2) was in accordance with the 3β position for this alcoholic group, and also for the 7β-hydroxyl. This product by acetylation in the usual manner gave the monoacetate **18** and the diacetate **19**. Biosynthetically, compound **11** can be formed by 3β hydroxylation of **20**, which is also precursor of the other compounds obtained in this biotransformation.

To confirm the structure and especially the stereochemistry at C-16 of C-17 of this class of products compound **17** was subjected to X-ray analysis. Figure 1 shows a perspective view of the molecule. This

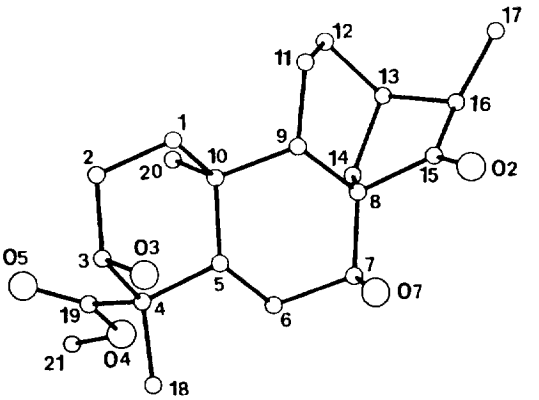


Fig. 1. A PLUTO[24] drawing of **1** with the atomic number

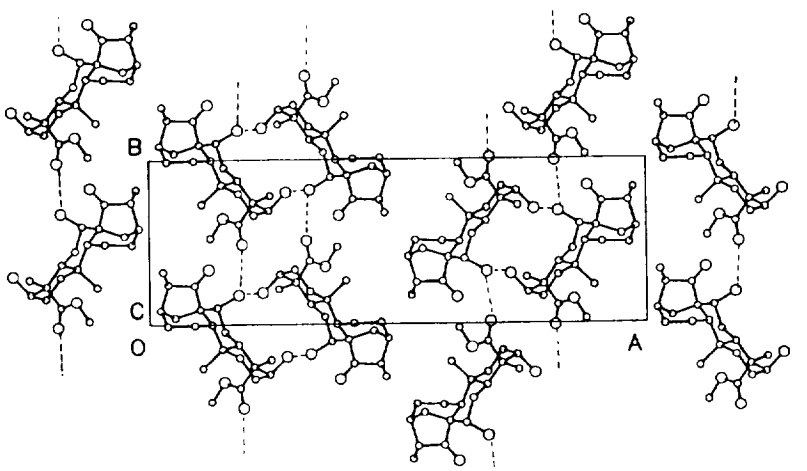


Fig. 2. A PLUTO[24] plot of the crystal packing. H-bonds are indicated by broken lines

Table 1

D--H.....A	D----H	D.....A	H.....A	D--H.....A	Symmetry
O-3-H....O-7	0.92(4)	2.788(3)	1.89(3)	163.0(3)	1/2+x, -y, 1/2+z
O-7-H....O-5	0.94(3)	2.849(6)	2.21(3)	131.9(3)	x, y-1, z

consists of three six-membered rings and one five-membered ring, labelled A, B, C and D, respectively. An ethylene group bridges C-8 and C-13 forming a five-membered ring which has a conformation between a half-chair and an envelope. The three six-membered rings adopt a chair conformation with endocyclic torsion angles between 49° and 64° in rings A and B, but ring C shows greater distortions from the ideal geometry, showing dihedral angles from 37° to 71°. The fusion ring A/B (C-20,C-10 α-oriented, H-5,C-5 β-oriented) is *trans* and the fusion ring B/C is *cis* (H-9,C-9 β-positioned, C-15,C-8 β-positioned). The hydroxyl groups at C-3 and C-7 have a β configuration. The several chiral centres in the molecule have the following configuration: C-3, C-4, C-5 and C-7 are (S), C-8 and C-10 are (R) configured.

The hydroxyl groups are involved in the crystalline state. There are two intermolecular H-bonds, the geometry of which is described in table 1. Figure 2 shows a PLUTO drawing of the molecular packing in the unit cell. It can be observed that the two intermolecular H-bonds produce bendings between the molecules.

No gibberellins with an alcoholic group at C-13 were obtained in the biotransformation of **1** indicating an inhibition of the hydroxylation at this carbon, which may be due to the presence of the 15-oxo group or, more probably, to the hydrogenated 16,17-double bond. This hypothesis is confirmed by a previous result obtained in the biotransformation of 16,17-dihydro-GA_4 by *G. fujikuroi*, which led to a 1α-hydroxy derivative, and not to a 13-hydroxy one.[11]

On the other hand, in previous works, we have shown that in the incubation with *G. fujikuroi* of *ent*-kaur-16-ene derivatives, the presence of a 15α-hydroxyl, a 16α-hydroxyl, a 15α,16α-epoxy or a 16α,17-epoxy group in the molecule inhibits oxidation at C-19, which is characteristic of the biosynthesis of gibberellins and kaurenolides.[12,13] However, the results of this biotransformation now show that a 15-oxo group does not inhibit oxidation at C-19.

The compounds obtained in this biotransformation of **1** also indicate that the presence of the 15-oxo group favoured the stereospecific reduction of the 16,17-double bond. This fact is very interesting, because in the biosynthetic pathway of gibberellins and kaurenolides there is no analogous reduction step. This reaction can occur in two ways: a) The direct stereospecific hydrogenation of the 16,17-double bond or b) The reduction of the 15-oxo group to the 15β-alcohol and the rearrangement of this product to form the corresponding 15-oxo-16α,17-dihydro derivative. We think that the biotransformation occurs by the first route, direct hydrogenation, because all the metabolites isolated are 15-oxo-16α,17-dihydro derivatives. If the second route were operative also 15β-hydroxy-derivatives should be obtained in the incubation, because it has been shown that metabolites of this type are efficiently transformed by the fungus,[14] without any rearrangement to a 15-oxo-16α,17-dihydro derivative. In any case, this reduction occurs in one of the initial steps of the biotransformation, and may be due to an enzyme involved in another metabolic route.

Once the microbiological access to this family of 15-oxo-16α,17-dihydrogibberellin derivatives was achieved, we turned our attention to their chemical preparation. Allylic oxidation of GA_{25} trimethyl ester (**23**) with selenium dioxide gave the 15α-hydroxyl derivative **24**, which was converted by Swern oxidation[15,16] into 15-oxo-GA_{25} trimethyl ester (**25**). Hydrogenation of the double bond in the presence of C/Pd led to compound **14**, which was identical with that obtained in the biotransformation of **1** (see above). A similar procedure was used for the preparation of **13**, which proved to be identical with the methyl ester of **7**. This product **7** had also been formed in the microbiological transformation of **1**. The starting material, in the partial synthesis of **13**, was GA_{12} dimethyl ester (**26**), and the intermediate compounds obtained were the alcohol **27** and the ketone **28**.

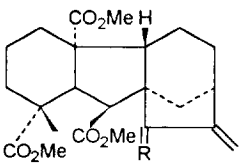
Table 2. ^{13}C NMR data of compounds **1**, **6**, **13-15**, **17-19**, **21** and **25**

C	1	6	13	14	15	17	18	19	21	25
1	39.5	36.5	39.0	35.4	32.3	33.1	33.6	33.5	132.0	35.5
2	18.3	17.3	19.4[a]	21.4	19.1	25.9	23.5	23.4	132.7	21.3
3	41.7	28.2	37.9	38.0	37.9	72.7	73.2	73.2	70.2	38.0
4	33,1	41.3	44.8	45.6	45.7	47.1	46.4	46.2	53.9	45.6
5	55.2	52.1[a]	54.9	55.1	54.5	38.7	40.2	41.7	51.6	54.9
6	18.4	80.3	50.4	50.6	49.7	27.3	27.2	26.2	49.4	49.6
7	33.4	72.1	173.2	172.5	172.4	70.8	72.1	72.2	171.8	172.9
8	52.4	53.8	57.7	58.1	57.8	53.1	53.0	51.6	60.0	58.2
9	52.5	52.6[a]	54.6	53.4	53.7	46.4	46.3	47.4	48.3	52.6
10	39.9	35.2	45.1	58.4	61.4	39.4	39.3	39.1	90.9	58.4
11	18.0	16.7	16.7	18.8[a]	17.8	18.0	18.0	17.8	15.7	19.3
12	32.3	19.8	19.6[a]	18.9[a]	20.6	25.0	24.9	24.7	18.2	31.0
13	38,0	31.9	33.2	32.8	32.7	34.8	34.8	34.6	32.6	35.7
14	36.6	30.1	34.8	32.5	34.4	35.7	35.7	36.3	33.0	29.1
15	210.6	n.o.	221.5	221.4	220.9	228.9	229.0	n.o.	220.3	206.7
16	149.5	49.0	48.9	49.1	48.9	48.5	48.6	48.9	48.5	151.7
17	114.2	11.1	10.2	10.1	10.2	9.7	9.8	9.9	10.4	115.2
18	33.4	25.7	30.2	29.3	29.1	23.9	23.5	23.5	14.8	29.5
19	21.4	181.4	178.1	176.3	177.1	177.8	176.6	176.2	178.4	176.4
20	17.4	19.8	14.7	174.3	205.2	15.0	15.0	14.9	--	174.4

Table 3. ^{13}C NMR data of compounds **27**, **28**, **30-32** and **34-38**

C	27	28	30	31	32	34	35	36	37	38
1	39.2	39.1	27.9	27.3	27.0	134.5	133.3	134.5	132.4	132.1
2	19.5	19.5	25.6	25.4	27.8	129.0	129.5	128.7	133.0	132.8
3	38.0	37.9	71.6	71.3	70.3	70.4	70.3	70.3	70.0	70.1
4	44.4[a]	44.8	53.2	53.7	55.0	51.9	52.6	50.8	53.3	53.9
5	59.2	54.8	52.9	51.1	50.2	53.5	52.2	54.6	53.6	52.0
6	48.9	49.4	49.3	49.1	49.1	48.6	48.3	50.8	50.6	48.3
7	178.4	173.1	174.6	171.8	172.0	173.0	171.6	174.9	175.0	171.7
8	56.3	58.0	57.5	59.4	59.5	58.2	60.1	55.7	55.7	60.3
9	53.5	53.9	50.6	50.4	50.4	48.4	48.2	41.2	41.6	48.4
10	44.5[a]	45.4	93.3	93.4	93.9	90.4	90.5	91.3	91.3	90.7
11	16.6	17.2	16.2	16.6	16.6	15.9	16.4	15.3	15.5	16.5
12	32.7	33.9	31.3	30.9	30.9	31.2	30.7	31.3	31.2	30.7
13	37.9	36.2	36.8	35.7	35.8	36.6	35.6	36.7	36.7	35.6
14	38.7	29.8	33.6	28.9	29.0	33.1	28.9	31.9	32.1	28.9
15	80.0	206.3	78.5	205.3	n.o	78.3	204.9	77.9	77.7	205.0
16	158.8	151.1	160.8	151.2	151.3	160.6	151.0	156.8	157.0	151.0
17	110.9	115.1	112.2	117.1	117.0	112.4	117.5	109.4	109.7	117.4
18	27.7	30.1	14.3	14.9	15.1	14.1	14.8	14.2	14.4	14.9
19	177.5	178.0	175.6	176.8	174.0	176.1	177.2	177.3	178.6	178.4
20	14.8	15.1	--	--	--	--	--	--	--	--

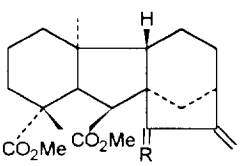
[a] These values can be interchanged



23 R = H_2

24 R = α-OH, H

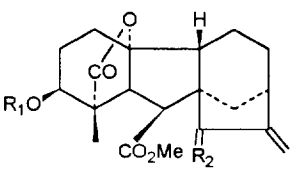
25 R = O



26 R = H_2

27 R = α-OH, H

28 R = O

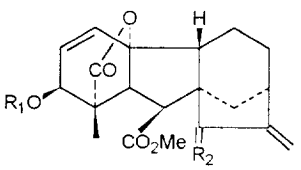


29 R_1 = Ac R_2 = H_2

30 R_1 = Ac R_2 = α-OH,H

31 R_1 = Ac R_2 = O

32 R_1 = H R_2 = O



33 R_1 = Ac R_2 = H_2

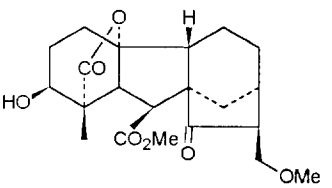
34 R_1 = Ac R_2 = α-OH,H

35 R_1 = Ac R_2 = O

36 R_1 = Ac R_2 = β-OH,H

37 R_1 = H R_2 = β-OH,H

38 R_1 = H R_2 = O



39

40 $\Delta^{1(2)}$

A commercial mixture of GA_4/GA_7 was used in the partial synthesis of 15-oxo-16α,17-dihydro-GA_7 methyl ester (**21**). Selenium dioxide oxidation of the acetate methyl esters of this mixture (**29** and **33**) afforded the corresponding 15α-alcohols **30** and **34**, which were separated by column chromatography. Compound **34** was oxidized by the Swern procedure to give the corresponding 15-oxo derivative **35**. This was treated with zinc in acetic acid to afford the compound **36**, which was impurified with its rearranged product **22**. This type of acid rearrangement of a 15β-hydroxy derivative, such as **36**, into a 15-oxo-16α,17-dihydro one, such as **22**, has previously been reported in *ent*-kaur-16-ene and gibberellin derivatives.[13,16-18] The mixture of **36** and **22** was treated with HCl/MeOH, but the amount of **22** was not increased. In this reaction the acetoxyl groups were hydrolysed giving the methyl ester of 15β-hydroxy-GA_7 (GA_{68}) (**37**) and compound **21**, which were separated by column chromatography. This latter compound was identical with that obtained by methylation of **12**, which had been formed in the incubation of **1** (see above). On the other hand, GA_{68} had been isolated from seeds of apple and pear, and synthesized from GA_3.[19]

Finally, 15-oxo-GA_4 methyl ester (**32**) and 15-oxo-GA_7 methyl ester (**38**) were prepared. Swern oxidation of **30** gave the 15-oxo derivative **31**, which was hydrolysed to afford **32** and the Michael addition product **39**. Analogously, the hydrolysis of **35** led to **38** and **40**.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-plate apparatus and are uncorrected. IR spectra were run on a Perkin Elmer 257. The NMR spectra were run on a Bruker WP200 SY and a Bruker AMX 400, for solutions in $CDCl_3$. MS were taken 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.

Incubation experiments. *Gibberella fujikuroi* (ACC 917), inhibited with 5 x 10^{-5} M AMO 1618, was grown in shake culture at 25° for 2 days in 75 conical flasks (250 ml) each containing sterile medium (50 ml).[20] 15-oxo-*ent*-kaur-16-ene (**1**) (310 mg) in EtOH (13 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The broth was filtered, adjusted to pH 2 with dil. HCl, and extracted with EtOAc. The extract was separated into acidic and neutral fractions with $NaHCO_3$. The acidic fraction was methylated with CH_2N_2. In the neutral fractions starting material (10 mg) and 16α,17-dihydro-7β-hydroxy-15-oxo-kauranolide (**5**) (7 mg) were obtained. The acid fraction contained 16α,17-dihydro-15-oxo-GA_{12} methyl ester (**13**) (4 mg), 16α,17-dihydro-15-oxo-GA_{25} trimethyl ester (**14**) (2 mg), 16α,17-dihydro-15-oxo-GA_{24} dimethyl ester (**15**) (10 mg), the 7-aldehyde of 16α,17-dihydro-15-oxo-GA_{14} methyl ester (**16**) (3 mg), *ent*-3α,7α-dihydroxy-15-oxo-kauran-19-oic methyl ester (**17**) (33 mg) and 16α,17-dihydro-15-oxo-GA_7 methyl ester (**21**) (2 mg).

16α,17-Dihydro-7β-hydroxy-15-oxo-kaurenolide (**5**). ^{1}H NMR (200 MHz) δ: 0.86 and 1.26 (each 3H, s), 1.14 (3H, d, J = 7 Hz, H-17), 1.88 (1H, d, J = 6 Hz, H-5), 4.21 (1H, d, J = 6 Hz, H-7), 5.05 (1H, t, J = 6 Hz, H-6). **Acetate** (**6**): ^{1}H NMR (200 MHz) δ: 0.95 and 1.31 (each 3H,s), 1.14 (3H, d, J = 7 Hz, H-17), 1.95 (1H, d, J = 6 Hz, H-5), 2.07 (3H, s), 5.15 (1H, t, J = 6 Hz, H-6), 5.69 (1H, d, J = 6 Hz, H-7); MS *m/z* (rel. int.): 332 [M - C_2H_2O]$^+$ (10), 314 (29), 300 (24), 286 (76), 271 (21), 258 (42), 243 (26), 229 (18), 213 (19), 137 (39), 109 (3).

16α,17-Dihydro-15-oxo-GA_{12} dimethyl ester (**13**). Found: [M]$^+$ at *m z* 376.2230. $C_{22}H_{32}O_5$ requires 376.2249. ^{1}H NMR (200 MHz) δ: 0.70 (3H, s, H-20), 1.28 (3H, s, H-18), 1.03 (3H, d, J = 7 Hz, H-17), 2.05 (1H, d, J = 12 Hz, H-5), 2.42 (1H, br s, H-13), 3.36 (1H, d, J = 12 Hz, H-6), 3.58 and 3.65 (each 3H, s, -OMe); MS *m z* (rel. int.): 376 [M]$^+$ (4), 348 (7), 344 (8), 316 (100), 301 (7), 288 (9), 285 (2), 273 (10), 258 (13), 256 (5), 229 (27), 199 (12).

16α,17-Dihydro-15-oxo-GA_{25} trimethyl ester (**14**). Found: [M]$^+$ at *m z* 420.2146. $C_{23}H_{32}O_7$ requires 420.2148. ^{1}H NMR (200 MHz) δ: 1.00 (3H, d, J = 7 Hz, H-17), 1.31 (3H, s, H-18), 2.30 (1H, d, J = 12 Hz, H-5), 3.60, 3.61 and 3.62 (each 3H, s, -OMe), 3.84 (1H, d, J = 12 Hz, H-6). MS *m/z* (rel. int.) 420 [M]$^+$ (1), 392 (11), , 360 (6), 332 (7), 328 (11), 316 (13), 300 (100), 272 (54), 241 (27), 213 (40), 204 (19), 183 (21).

16α,17-Dihydro-15-oxo-GA_{24} dimethyl ester (**15**). Unstable compound, ^{1}H NMR (200 MHz) δ: 1.03 (3H, d, J = 7 Hz, H-17), 1.32 (3H, s, H-18), 2.38 (1H, d, J = 12 Hz, H-5), 3.61 (6H, s, 2-OMe), 3.93 (1H, d, J = 12 Hz, H-6), 9.66 (1H, s, H-20).

7-Aldehyde of 16α,17-dihydro-15-oxo-GA_{14} methyl ester (**16**). ^{1}H NMR (200 MHz) δ: 0.69 (3H, s, H-20), 1.32 (3H, s, H-18), 1.04 (3H, d, J = 7 Hz, H-17), 2.67 (1H, d, J = 12 Hz, H-5), 2.99 (1H, dd, J = 4 and 12 Hz, H-6), 3.67 (3H, s, -OMe), 4.19 (1H, br s, H-3), 10.13 (1H, d, J = 4 Hz, H-7). **Acetate**: Unstable compound, ^{1}H NMR (200 MHz) δ: 0.70 (3H, s, H-20), 1.22 (3H, s, H-18), 1.06 (3H, d, J = 7 Hz, H-17), 2.14 (3H, s), 2.61 (1H, d, J = 12 Hz, H-5), 3.02 (1H, dd, J = 4 and 12 Hz, H-6), 3.68 (3H, s, -OMe), 5.44 (1H, br s, H-3), and 10.14 (1H, d, J = 4 Hz, H-7); MS *m z* (rel. int.): 376 [M - CO]$^+$ (4), 358 (5), 346 (3), 344 (23), 343 (84), 316 (30), 298 (24), 284 (41), 273 (21), 256 (36), 239 (34), 277 (19), 199 (23).

***ent*-3α,7α-Dihydroxy-15-oxo-kauran-19-oic methyl ester (17)**. Found: $[M]^+$ at *m/z* 364.2237. $C_{21}H_{32}O_5$ requires 364.2249. ^1H NMR (200 MHz) δ: 0.90 (3H, s, H-20), 1.25 (3H, s, H-18), 1.09 (3H, d, J = 7 Hz, H-17), 3.65 (3H, s, -OMe), 3.86 (1H, br s, H-7) and 4.10 (1H, br s, H-3); MS (rel. int.) 346 $[M - H_2O]^+$ (43), 328 (9), 288 (12), 286 (11), 270 (18), 269 (20), 268 (11), 211 (15), 187 (28). **3β-Acetate (18)**: ^1H NMR (200 MHz) δ: 0.93 (3H, s, H-20), 1.18 (3H, s, H-18), 1.11 (3H, d, J = 7 Hz, H-17), 2.09 (3H, s, -OAc), 3.69 (3H, s, -OMe), 3.89 (1H, br s, H-7), and 5.36 (1H, br s, H-3); MS *m/z* (rel. int.): 388 $[M - H_2O]^+$ (3), 375 (1), 346 (3), 330 (4), 328 (13), 296 (68), 286 (7), 271 (15), 270 (15), 268 (9), 253 (12), 241 (11), 211 (14). **Diacetate (19)** ^1H NMR (200 MHz) δ: 0.95 (3H, s, H-20), 1.13 (3H, s, H-18), 1.05 (3H, d, J = 7 Hz, H-17), 2.08 and 2.17 (each 3H, s, -OAc), 3.69 (3H, s, -OMe), 5.09 (1H, br s, H-7) and 5.36 (1H, br s, H-3); MS *m/z* (rel. int.): 388 $[M - HOAc]^+$ (1), 346 (2), 328 (4), 296 (5), 286 (2), 271 (3), 270 (3), 268 (2), 211 (3), 189 (100).

16α,17-Dihydro-15-oxo-GA_7 methyl ester (21). Found: $[M]^+$ at *m/z* 360.1566. $C_{20}H_{24}O_6$ requires 360.1573. ^1H NMR (200 MHz) δ: 1.08 (3H, d, J = 7 Hz, H-17), 1.32 (3H, s, H-18), 2.68 (1H, d, J = 10 Hz, H-6), 3.24 (1H, d, J = 10 Hz, H-5), 3.64 (3H, s, -OMe), 4.16 (1H, d, J = 3.6 Hz, H-3), 5.94 (1H, dd, J = 3.6 and 10 Hz, H-2), and 6.28 (1H, d, J = 10 Hz, H-1), MS *m/z* (rel. int.): 360 $[M]^+$ (20), 342 (4), 332 (6), 328 (23), 314 (10), 302 (14), 300 (29), 297 (12), 292 (6), 271 (38), 255 (18), 238 (60), 227 (31), 209 (39), 199 (34).

Crystal data of 17.- $C_{21}H_{32}O_5$, Mw = 364.481; Dc = 1.2665 mg cm^{-1}; μ = 6.807 cm^{-1}; orthorhombic, space group $P2_12_12_1$, a = 27.678(1) Å, b = 9.2285(4) Å, c = 7.4836(2) Å; Z = 4, F(000) = 792.0, CuKα radiation (λ = 1.5418 Å).

A colourless prism-like crystal with approximate dimensions of 0.45x0.30x0.40 mm was used for data collection. The lattice parameters are based on 46 centred reflections, with θ values between 10° and 40°. The data were collected on a Philips PW 1100 diffractometer with graphite monochromated CuKα radiation. Intensities of two reflections measured every 90 reflections showed no crystal decay; ω/2θ scan technique, scan speed 1 min./refl., detector apertures 1 x 1, θ max. 65°. The data were corrected for Lorentz and polarization effects but not for absorption. The structure was solved by direct methods (SIR88)[21], and refined by full-matrix least-squares calculations of F magnitudes with anisotropic thermal parameters for non-H atoms. The H-atoms were located in successive Fourier synthesis. All the H-atoms were included in the structure factors calculations and refined. An empirical weighting scheme was used not to give dependence in <ωΔ2F> vs. <Fo> and <sin θ/λ>. The maximum height in the final difference Fourier synthesis is less than 0.16 $eÅ^{-3}$. The values of R and Rω are 3.1 and 3.4, respectively.

Atomic scattering factors were taken from reference.[22] All calculations were performed on a VAX 6410 and with X-Ray 76 programs.[23] Lists of atomic coordinates, thermal parameters, structure factors, bond lengths, bond angles and torsion angles are deposited as supplementary material.

Oxidation of 2. 15α-Hydroxy-*ent*-kaur-16-ene (**2**) (400 mg), obtained from candidiol,[4] was dissolved in dichloromethane (10 ml) and treated with pyridinium dichromate (800 mg) at room temp for 5 h. The soln. was diluted with diethyl ether, filtered and evaporated. The residue was chromatographed eluting with light petroleum-ethyl acetate (95:5) affording **ent-15-oxo-kaur-16-ene (1)** (310 mg); ^1H NMR (200 MHz) δ: 0.82, 0.88 and 1.09 (each 3H, s), 3.05 (1H, br s, H-13), 5.25 and 5.94 (each 1H, s, H-17); MS *m/z* (rel. int.): 286 [M]+ (73), 271 (37), 253 (13), 230 (6), 215 (5), 201 (14), 199 (3), 189 (12). Further elution gave an inseparable mixture of the aldehydes **3** and **4** (80 mg): ^1H NMR (200 MHz) δ: 3.04 (1H, br s, H-13), 6.59 (1H, s, H-15), 9.73 (1H, s, H-17) (70% of **3**) and 2.54 (1H, br s, H-13), 3.24 (1H, s, H-15), 9.20 (1H, s, H-17) (30% of **4**).

Oxidation of GA_{25} trimethyl ester (23) with selenium dioxide. Compound **23** (35 mg, 0.09 mmol), dissolved in 4 ml. of dioxane-water (3:1), was treated with selenium dioxide (30 mg., 0.27 mmol) and left with stirring for 10 h. at room temperature. Brine was added and the solution extracted with ethyl ether. After drying and removing the solvent, the residue was purified by chromatography. Elution with light petroleum ether-ethyl acetate (7:3) gave **15α-hydroxy-GA_{25} trimethyl ester (24)** (16 mg., 0.04 mmol, 44%). ^1H NMR (200 MHz) δ: 1.13 (3H, s, H-18), 2.08 (1H, d, J = 12 Hz, H-5), 2.58 (1H, br s, H-13), 3.62 (1H, d, J = 12 Hz, H-6), 3.62 (3H, s, -OMe), 3.70 (6H, s, -2OMe), 4.10 (1H, s, H-15), 5.07 and 5.26 (each 1H, s, H-17). MS *m/z*

(rel. int.) 420 $[M]^+$ (6), 388 (66), 370 (17), 360 (24), 356 (36), 328 (100), 310 (66), 300 (65), 284 (62), 282 (42), 269 (75), 268 (59), 241 (90), 225 (66), 219 (22), 209 (14), 197 (18).

Swern oxidation of 15α-hydroxy-GA_{25} trimethyl ester (24). Oxalyl chloride (25 μl, 0.29 mmol) was added to dry dichloromethane (2ml) in a dry flask under nitrogen and the solution was cooled to -72° C. After 10 min dimethyl sulphoxide (33μl, 0.46 mmol) was added and allowed to react for 10 min. before addition of the 15α-alcohol (16 mg, 0.04 mmol) in dry dichloromethane (1 ml). The reaction mixture was stirred at -72° C for 45 min and at -60° C for a further 15 min. Di-isopropylethylamine (200μl, 1.14 mmol) was added dropwise and the reaction mixture was then allowed to warm up to room temperature. The solvent was removed under reduced pressure and the residue purified by chromatography. Elution with light petroleum-ethyl acetate (7:3) gave **15-oxo-GA_{25} trimethyl ester (25)** (12 mg, 0.03 mmol, 75%). Found: $[M]^+$ at *m/z* 418.1999. $C_{23}H_{30}O_7$ requires 418.1992. ^1H NMR (200 MHz): 1.34 (3H, s, H-18), 2.31 (1H, d, J = 12 Hz, H-5), 2.94 (1H, br s, $W_{1/2}$= 12 Hz, H-13), 3.59 (3H, s, -OMe), 3.61 (6H, s, -2OMe), 3.89 (1H, d, J = 12 Hz, H-6), 5.24 and 5.83 (each 1H, s, H-17). MS *m/z* (rel. int.) 418 $[M]^+$ (12), 386 (100), 354 (18), 326 (46), 298 (96), 270 (49), 267 (30), 239 (47), 211 (49).

Hydrogenation of 15-oxo-GA_{25} trimethyl ester (25). Compound **25** (12 mg., 0.03 mmol) was dissolved in ethanol (4 ml) and stirred vigorously with Pd/C 5% (10 mg) under hydrogen for 8 h. The catalyst was removed by filtration to give a residue that was chromatographed. Elution with light petroleum-ethyl acetate (19:1) gave (**14**) (11 mg), identical with the compound isolated from the culture.

Oxidation of GA_{12} dimethyl ester (26) with selenium dioxide. Compound **26** (60 mg, 0.17 mmol) was treated with selenium dioxide (40 mg, 0.36 mmol) as described above for **23**, giving a residue which was purified by chromatography. Elution with light petroleum-ethyl acetate (7:3) gave **15α-hydroxy-GA_{12} dimethyl ester (27)** (32 mg, 0.09 mmol, 51%). Found: $[M]^+$ at *m/z* 376.2222. $C_{22}H_{32}O_5$ requires 376. 2250. ^1H NMR (200 MHz): 0.71 and 1.07 (each 3H, s, H-18 and H-20), 1.89 (1H, d, J = 12 Hz, H-5), 2.62 (1H, br s, H-13), 3.29 (1H, d, J = 12 Hz, H-6), 3.66 and 3.67 (each 3H, s, -OMe), 4.13 (1H, s, H-15), 5.07 and 5.26 (each 1H, s, H-17). MS *m/z* (rel. int.) 376 $[M]^+$ (0.2), 344 (100), 329 (11), 326 (15), 316 (42), 301 (33), 298 (42), 285 (34), 269 (24), 257 (40), 239 (48).

Swern oxidation of 15α-hydroxy-GA_{12} dimethyl ester (27). The 15α-alcohol **27** (28 mg, 0.076 mmol) was treated with oxalyl chloride (35 μl, 0.40 mmol) and dimethyl sulphoxide (55 μl, 0.76 mmol) as described above for **24**, giving a residue which was purified by chromatography. Elution with light petroleum-ethyl acetate (6:1) gave **15-oxo-GA_{12} dimethyl ester (28)** (26 mg., 0.07 mmol, 82%). Found: $[M]^+$ at *m/z* 374.2082. $C_{22}H_{30}O_5$ requires 374.2093. ^1H NMR (200 MHz) δ: 0.72 and 1.28 (each 3H, s, H-20 and H-18), 2.07 (1H, d, J = 12 Hz, H-5), 2.96 (1H, br, H-13), 3.36 (1H, d, J = 12 Hz, H-6), 3.58 and 3.64 (each 3H,s -OMe), 5.23 and 5.84 (each 1H, s, H-17). MS *m/z* (rel. int.) 374 $[M]^+$ (6), 342 (100), 314 (57), 299 (17), 286 (15), 282 (23), 271 (15), 267 (27), 255 (66), 227 (41), 199 (11).

Hydrogenation of 15-oxo-GA_{12} dimethyl ester (28). Compound **28** (26 mg) was dissolved in ethanol (4 ml) and stirred vigorously with Pd/C 5% (20 mg) under hydrogen for 18 h. Removal of the catalyst by filtration gave product **13** (21 mg), whose spectroscopical data are identical with those of **13** isolated from the culture, Found: M^+ at *m/z* 376.2249. $C_{22}H_{32}O_5$ requires 376.2249.

Oxidation of the mixture of 3β-acetoxy-GA_4/GA_7 methyl ester with selenium dioxide. The mixture of GA_4/GA_7 derivatives **29** and **33** (300 mg, 0.78 mmol) was treated with selenium dioxide (1 g, 9 mmol) as described above for **23** giving a residue, which was purified by chromatography. Elution with light petroleum-ethyl acetate (3:1) gave **3β-acetoxy-15α-hydroxy-GA_7 methyl ester (34)** (137 mg). Found: $[M]^+$ at *m/z* 402.1685. $C_{22}H_{26}O_7$ requires 402.1679. ^1H NMR (200 MHz) δ: 1.15 (3H, s, H-18), 2.13 (3H, s, -OAc), 2.70 (1H, br s, H-13), 2.71 (1H, d, J = 11 Hz, H-6), 3.33 (1H, d, J = 11 Hz, H-5), 3.69 (3H, s, -OMe), 4.09 (1H, br, H-15), 5.18 and 5.27 (each 1H, s, H-17), 5.32 (1H, d, J = 4 Hz, H-3), 5.86 (1H, dd, J = 4 and 9 Hz, H-2), and 6.38 (1H, d, J =9 Hz, H-1). MS *m/z* (rel. int.): 402 $[M]^+$ (1), 370 (1), 342 (2), 328 (6), 310 (16), 298 (26), 282 (19), 280 (33), 266 (31), 238 (81), 221 (100). Further elution gave **3β-acetoxy-15α-hydroxy-GA_4 methyl ester (30)** (60 mg.). Found $[M]^+$ at *m/z* 404.1836. $C_{22}H_{28}O_7$ requires 404.1835. ^1H NMR (200 MHz) δ: 1.06 (3H, s, H-18), 2.13 (3H, s, -OAc), 2.60 (1H, d, J = 11 Hz, H-6), 3.15 (1H, d, J = 11 Hz, H-5), 3.66 (3H, s , -OMe), 4.07 (1H, s, H-15), 4.95 (1H, br s, H-3), 5.16 and 5.25 (each 1H, s, H-17). MS *m/z* (rel. int.)

404 $[M]^+$ (0.4), 386 (1), 372 (65), 344 (29), 330 (12), 312 (37), 300 (17), 284 (48), 282 (47), 268 (55), 240 (83), 224 (72).

Swern oxidation of 3β-acetoxy-15α-hydroxy-GA_7 methyl ester (34). The 15α-alcohol **34** (31 mg, 0.08 mmol) was treated with oxalyl chloride (44 µl, 0.50 mmol) and dimethyl sulphoxide (54 µl, 0.75 mmol) as described above for **24**, giving a residue, which was purified by chromatography. Elution with light petroleum-ethyl acetate (3:7) gave **3β-acetoxy-15-oxo-GA_7 methyl ester (35)** (23 mg, 0.06 mmol, 75%). Found: $[M - AcOH]^+$ at *m/z* 340.1317. $C_{20}H_{20}O_5$ requires 340.1311. ^1H NMR (400 MHz) δ: 1.22 (3H, s, H-18), 2.14 (3H, s, -OAc), 2.68 (1H, d, J = 11 Hz, H-6), 3.07 (1H, br s, $W_{1/2}$ = 13 Hz, H-13), 3.43 (1H, d, J = 11 Hz, H-5), 3.63 (3H, s, -OMe), 5.34 (1H, d, J = 4 Hz, H-3), 5.39 and 5.95 (each 1H, s, H-17), 5.88 (1H, dd, J = 4 Hz and 9 Hz, H-2), 6.33 (1H, d, J =9 Hz, H-1). MS *m/z* (rel. int.) 400 $[M]^+$ (1), 369 (11), 340 (6), 308 (12), 296 (17), 264 (13), 253 (10), 236 (100), 209 (19).

Treatment of 3β-acetoxy-15-oxo-GA_7 (35) with Zn/AcOH. Activated zinc dust (1.5 g) was added to the ketone **35** (20 mg, 0.05 mmol) in acetic acid (1.5 ml) and the mixture stirred vigorously at room temperature for 3 h. Excess of zinc was filtered off, and the acetic acid removed under vacuum to give a residue that was purified by chromatography. Elution with light petroleum-ethyl acetate (4:6) gave **3β-acetoxy-15β-hydroxy-GA_7 methyl ester (36)** (6 mg, 0.021 mmol, 45%). Found: $[M - CH_3OH]^+$ at *m/z* 370.1405. $C_{21}H_{22}O_6$ requires 370.1416. ^1H NMR (200 MHz) δ: 1.15 (3H, s, H-18), 2.12 (3H, s, -OAc), 2.80 (1H, d, J = 11 Hz, H-6), 3.18 (1H, d, J = 11 Hz, H-5), 3.79 (3H, s, -OMe), 3.98 (1H, br s, H-15), 5.11 and 5.14 (each 1H, br s, H-17), 5.33 (1H, d, J = 4 Hz, H-3), 5.84 (1H, dd, J = 4 and 9 Hz, H-2), and 6.44 (1H, d, J = 9 Hz, H-1). MS *m/z* (rel. int.) 370 $[M- CH_3OH]^+$ (3), 342 (9), 310 (15), 298 (22), 280 (44), 266 (30), 251 (10), 238 (100), 221 (96), 209 (45). This compound was impurified with **22** (15%).

Treatment of 36 and 22 with acid. Compound **36** impurified with **22** (6mg mg), dissolved in 1.5 ml of ethanol-ether (3:1), was treated dropwise with concentrated hydrochloric acid (0.2 ml), stirring for 5 h. The solvent was partially removed and brine added, extracting with ether. After drying and removing the solvent a residue was obtained that was purified by chromatography. Elution with light petroleum-ethyl acetate (4:6) gave the hydrolysis product **GA_{68} methyl ester (37)** as the main product. Found M^+ at *m/z* 360.1554. $C_{20}H_{24}O_6$ requires 360.1572. ^1H NMR (200 MHz) δ: 1.26 (3H, s, H-18), 2.61 (1H, br s, H-13), 2.83 (1H, d, J = 11 Hz, H-6), 3.07 (1H, d, J = 11 Hz, H-5), 3.80 (3H, s, -OMe), 4.02 (1H, br s, H-15), 4.12 (1H, d, J = 4 Hz, H-3), 5.14 (2H, br s, H-17), 5.89 (1H, dd, J = 4 and 9 Hz, H-2), and 6.36 (1H, d, J = 9 Hz, H-1). MS *m/z* (rel. int.) 360 M+ (2), 342 (1), 328 (86), 310 (8), 300 (8), 298 (9), 282 (7), 280 (12), 266 (8), 256 (6), 255 (12), 238 (28), 221 (28), 209 (20), 199 (11). Further elution gave **21**, whose spectroscopical data were identical to those obtained from the feeding of **1**.

Swern oxidation of 3β-acetoxy-15α-hydroxy-GA_4 methyl ester (30). Compound **30** (40 mg, 0.1 mmol) was treated with oxalyl chloride (50 µl, 0.58 mmol) and dimethyl sulphoxide (70 µl, 0.98 mmol) as described above for **24** giving a residue, which was purified by chromatography. Elution with light petroleum-ethyl acetate (3:1) gave **3β-acetoxy-15-oxo-GA_4 methyl ester (31)** (31 mg, 0.077 mmol, 77%). ^1H NMR (200 MHz) δ: 1.12 (3H, s, H-18), 2.16 (3H, s, -OAc), 2.59 (1H, d, J = 10.5 Hz, H-6), 3.06 (1H, br s, H-13), 3.32 (1H, d, J = 10.5 Hz, H-5), 3.62 (3H, s, -OMe), 4.99 (1 H, br s, H-3), 5.39 and 5.94 (each 1H, s, H-17). MS *m/z* (rel. int.) 402 $[M]^+$ (23), 384 (3), 371 (18), 360 (13), 342 (21), 314 (12), 310 (26), 298 (54), 296 (25), 282 (28), 264 (22), 238 (100), 209 (26).

Hydrolysis of 3β-acetoxy-15-oxo-GA_4 methyl ester (31). Compound **31** (9 mg, 0.022 mmol) was stirred with potassium carbonate-methanol solution (0.05%, 1 ml) for two hours at room temp. Removal of the solvent and chromatography of the residue, eluting with light petroleum-ethyl acetate (1:1), afforded **3β-hydroxy-15-oxo-GA_4 methyl ester (32)** (2 mg, 0.006 mmol). ^1H NMR (200 MHz) δ: 1.22 (3H, s, H-18), 2.61 (1H, d, J = 10.5 Hz, H-6), 3.07 (1H, br s, H-13), 3.34 (1H, d, J = 10.5 Hz, H-5), 3.63 (3H, s, -OMe), 3.67 (1H, br s, H-15), 3.87 (1H, br s, H-3), 5.38 and 5.94 (each 1H, s, H-17). MS *m/z* (rel. int.) 360 $[M]^+$ (64), 342 (15), 328 (62), 314 (52), 310 (15), 300 (96), 298 (48), 283 (25), 282 (60), 270 (27), 264 (31), 254 (38), 238 (100), 211 (36). Further elution afforded **15-oxo-16α,17-dihydro-17-methoxy-GA_4 methyl ester (39)** (5 mg, 0.013 mmol). ^1H NMR (200 MHz) δ: 1.21 (3H, s, H-18), 2.58 (1H, d, J = 10.5 Hz, H-6), 3.27 (1H, d, J = 10.5 Hz, H-5), 3.34 (3H, s, -OMe), 3.41 (2H, m, H-17), 3.59 (3H, s, -OMe), 3.86 (1H, br s, H-3). MS *m/z*

(rel. int.) 392 $[M]^+$ (6), 374 (3), 360 (43), 347 (11), 332 (100), 314 (34), 300 (64), 286 (28), 282 (16), 272 (55), 242 (45), 227 (22), 211 (16).

Hydrolysis of 3β-acetoxy-15-oxo-GA$_7$ methyl ester (35). Compound **35** (8 mg, 0.02 mmol) was hydrolyzed as above for **31** and purified by chromatography, eluting with light petroleum-ethyl acetate (1:1), giving **3β-hydroxy-15-oxo-GA$_7$ methyl ester (38)** (2 mg, 0.006 mmol). ^{1}H NMR (200 MHz) δ: 1.34 (3H, s, H-18), 2.71 (1H, d, J = 10.5 Hz, H-6), 3.08 (1H, m, H-13), 3.29 (1H, d, J =10.5 Hz, H-5), 3.65 (3H, s, -OMe), 4.17 (1H, d, J = 3.8 Hz, H-3), 5.40 and 5.96 (each 1H, s, H-17), 5.94 (1H, dd, J = 3.8 and 10 Hz, H-2), 6.28 (1H, d, J = 10 Hz, H-1). MS *m/z* (rel. int.) 358 $[M]^+$ (21), 340 (4), 326 (32), 312 (11), 298 (40), 280 (14), 265 (10), 254 (25), 236 (100). Further elution yielded **15-oxo-16α,17-dihydro-17-methoxy-GA$_7$ methyl ester (40)** (4 mg, 0.01 mmol): ^{1}H NMR (200 MHz): 1.32 (3H, s, H-18), 2.68 (1H, d, J = 10.5 Hz, H-6), 3.23 (1H, d, J = 10.5 Hz, H-5), 3.34 (3H, s, -OMe), 3.43 (2H, m, H-17), 3.62 (3H, s, -OMe), 4.16 (1H, d, J = 3.6 Hz, H-3), 5.95 (1H, dd, J = 3.6 and 9.3 Hz, H-2), 6.29 (1H, d, J = 9.3 Hz, H-1). MS *m/z* (rel. int.): 390 $[M]^+$ (8), 372 (3), 358 (43), 345 (9), 330 (100), 312 (25), 298 (74), 284 (21), 280 (14), 271 (57), 270 (56), 240 (36), 209 (28).

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