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The Chemical and Microbiological Synthesis of 14-Hydroxy-gibberellins

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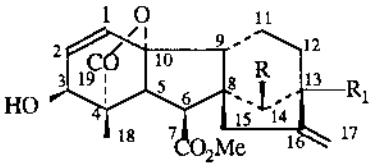
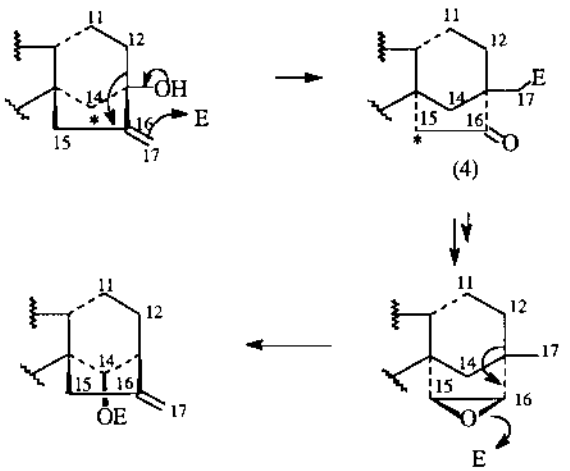
Abstract: Gibberellic acid has been transformed into 14β-hydroxy-gibberellin A_7 methyl ester *via* the rearrangement of an epoxy-beyergibberellin intermediate using a titanium-amide complex. The diterpene *ent*-beyer-15-en-19-ol has been transformed by chemical methods into *ent*-14α,19-dihydroxy-kaur-16-ene. The biotransformation of this compound with the fungus *Gibberella fujikuroi* gave 14β-hydroxy-gibberellin A_7, *ent*-7α,14α,19-trihydroxy-kaur-16-ene and *ent*-6α,7α,14α-trihydroxy-kaur-16-en-19-oic acid. A minor gibberellin 14β-hydroxy-GA_{14} was identified by GC-MS.

The gibberellins are a group of widely distributed plant hormones which participate in the regulation of the growth and development of higher plants.[1] These compounds have also been isolated from fungi such as *Gibberella fujikuroi*, *Sphaceloma manihoticola* and *Phaeosphaeria* sp.[2-4] Whilst they possess a common carbon skeleton, they differ mainly from one another in their hydroxylation pattern. An extensive range of gibberellins with different hydroxylation patterns have been prepared from the more readily available congeners.[2b,5] Since the diterpene *ent*-kaurene is a precursor of gibberellins, the microbiological transformation of selected kaurene derivatives by the fungus *G. fujikuroi* can be a useful complementary approach to the preparation of these hormones particulary in the cases in which a chemical route is very difficult or impracticable.

Although gibberellins with hydroxyl groups at almost every center are known, no 14-hydroxy gibberellins have hitherto been isolated. In a preliminary communication[6] we have describe the first results of our studies directed toward the synthesis of 14-hydroxy-gibberellins by chemical transformations of the gibberellin A_3 methyl ester (**1**). In this paper, we give the full account of this study as well as the results of the microbiological preparation of this group of gibberellins hydroxylated at C-14.



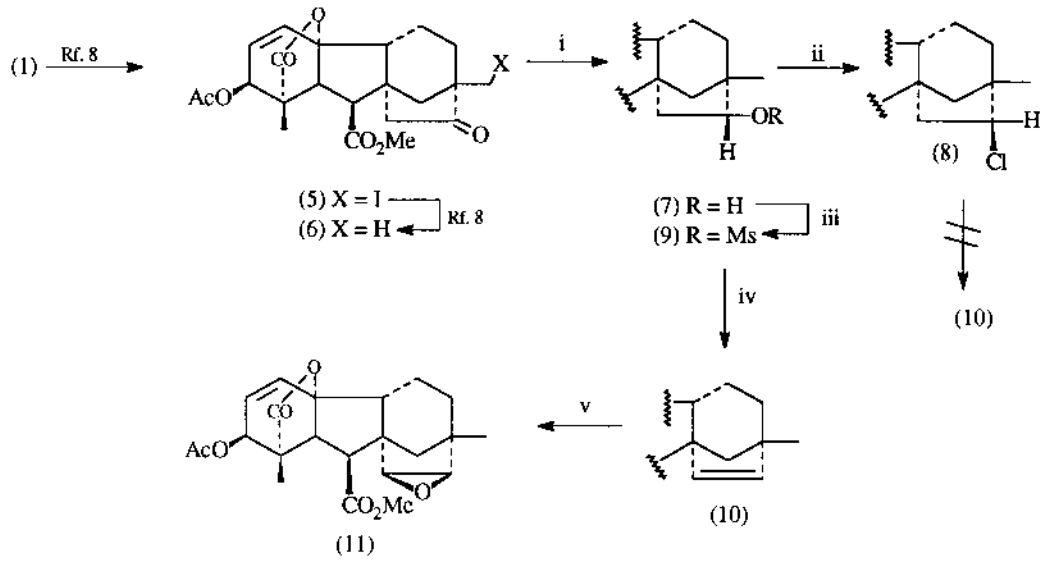
(1) R = H; R_1 = OH
(2) R = OH; R_1 = H
(3) R = R_1 = OH



Scheme 1

Three general strategies have been used to functionalize the C-ring of gibberellins: a) extension of the A-ring functionalization; b) cleavage of the C-13,C-16 bond and reconstruction of the D-ring at a later stage or c) by means of transannular processes from substituents attached to the ring D.[7] Although these strategies appear to be suitable for the introduction of functionality at a position such as C-11 or C-12, their application at C-14 for functionalization seemed to us very difficult. On the other hand, the necessary activation of C-14 to be functionalized can be achieved by a Wagner-Meerwein type rearrangement of the rings C and D to produce the ketone **4**.[8] The correct stereochemistry by a second rearrangement of these rings can be regenerated (See scheme 1). This double rearrangement of rings C and D has been used by Mori[9] in the synthesis of some gibberellins. Several ways to introduce a hydroxyl function at the activated C-15 position in ketone **4** are chemically possible, but the relatively low reactivity of this ketone as well as the low acidity of the α-hydrogens precludes several of them. We chose the formation of an epoxide as a feasible way to introduce the required hydroxyl group because: a) The known *exo*-selectivity in the epoxidation of a related system[10] defines the final stereochemistry at C-14 and b) the antiperiplanar position of the C-12,C-13 bond and the oxirane ring is ideal to achieve the required second rearrangement to restore the correct stereochemistry of the gibberellin skeleton. This strategy was successfully applied to the synthesis of the 14β-hydroxy-gibberellin A_7 methyl ester (**2**), which in turn was also obtained by the

microbiological transformation of *ent*-19,14α-dihydroxy-kaur-16-ene (**24**) by the fungus *G. fujikuroi*.

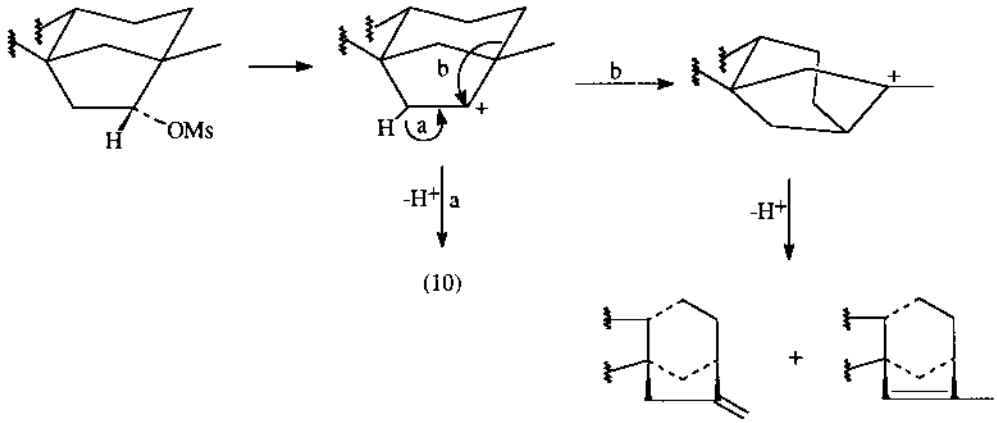


i) $NaBH_4$, MeOH, 0°C, 1h; ii) Ph_3P, CCl_4, MeCN, reflux, 2h 30 min; iii) MsCl, Py, 0°C, 45 min; iv) 2,4,6-collidine, reflux, 9h 30 min; v) MCPBA, 0°C, 65 h.

Scheme 2

The transformation of methyl gibberellate (**1**) into the target epoxide **11** is outlined in scheme 2. The ketone 6 was prepared from methyl gibberellate as previously described.[8] We noted that both a longer reaction time and a larger excess of iodine increased the yield of the iodo-ketone **5** to 80%. The radical deiodination with tri-n-butyltin hydride afforded the ketone **6**, which was easily reduced with sodium borohydride to give the 16-*endo* alcohol **7** as the only diastereoisomer. The value of the vicinal coupling constant between H-16 and H-15α (J = 4.2 Hz) was consistent with the stereochemistry assigned at this centre.[10] The direct transformation of this compound into the alkene **10** by treatment with CCl_4-Ph_3P in acetonitrile was unsuccessful, giving the *exo*-chloro derivative **8** as the only transformation product. The elimination of the chlorine proved to be fruitless: Either no reaction or an intractable mixture of polar compounds was obtained, depending on the reaction conditions. The better leaving group ability of the mesylate function compared to the chlorine was exploited to transform the alcohol into the alkene **10**. Thus, the alcohol was transformed into the mesylate **9** by treatment with mesyl chloride in cold pyridine. Different bases and conditions were examined to effect the desired elimination, achieving the best results with neat 2,4,6-collidine under reflux. Under these conditions, the formation of **10** (34%) competes with a rearrangement of rings C and D leading also to a mixture of the 3β-acetates of Δ^{15}- and Δ^{16}-gibberellin A_7 methyl esters. These results can be explained by admitting an E_1 mechanism in which the carbonium ion formed at C-16 is neutralized by proton loss from C-15, in the first case, or by a C-12 to C-13 bond migration in the second although the formation of a non-classical carbonium ion cannot be ruled out (See

scheme 3).

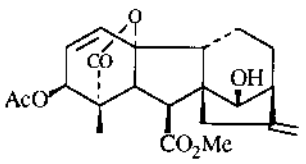


Scheme 3

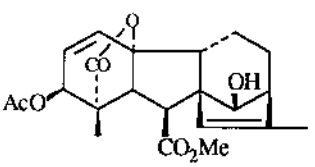
The epoxidation of **10** (MCPBA, $CHCl_3$, 0°C) yielded the expected 15β,16β-epoxide **11** as the only isomer. The β-stereochemistry of the epoxide was assigned on the basis of other related examples given in the literature.[10] The chemoselectivity observed in this reaction is a direct consequence of the low reactivity of the A-ring double bond.[3]

With the required epoxide to hand, we undertook the study of the second rearrangement to restore the gibberellin skeleton. The first attempts to rearrange the epoxide **11** using Lewis acid catalysis ($BF_3.Et_2O$) were frustrating. Instead of the target compound **12**, the Δ^{15} isomer **13** was always the major reaction product, contaminated with variable amounts of the 2,19-lactone isomer **14**. Two serious obstacles became evident from the first results of this Lewis acid-catalyzed rearrangement. Firstly, the susceptibility of the ring A of gibberellin A_3 and its derivatives to acidic conditions[3] and secondly, the formation of the undesirable Δ^{15} isomer as the major transformation compound in the rearrangement. In order to see if this rearrangement could be controlled in some way, the epoxides **18** and **20** were used as models (scheme 4). They were easily obtained from the natural occurring *ent*-18-hydroxy- and *ent*-19-hydroxy-beyerenes, (**15**) and (**16**),[11] by acetylation and epoxidation in the normal way. The $BF_3.Et_2O$-catalyzed rearrangement of both epoxides gave predominantly the respective 14β-hydroxy-Δ^{15} isomers **21** and **25**. In some cases, a variable amount of the diol **30** was also obtained. It was established that this diol could be easily transformed into an equimolecular mixture of Δ^{15}- and Δ^{16}-*ent*-14β,19-diacetoxy-kaurenes by simple treatment with acetic anhydride and p-toluenesulfonic acid (cat.), from 0°C to room temperature. No further studies to improve the Δ^{16}/Δ^{15} ratio were carried out.

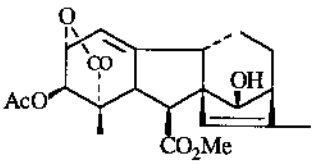
A Dreiding stereomodel of the epoxy-beyergibberellin derivative **11** showed that the conformation of the oxirane ring and the methyl group seemed suitable for a regioselective base-controlled Lewis acid rearrangement such as those used in the synthesis of allylic alcohols from epoxides.[12,13] When the epoxy-beyerane **20** was treated with the Corey reagent (bromomagnesium isopropylcyclohexylamide)[12] no reaction took place below 100°C. After 5h at this temperature, the C-18 hydrolysis product of the starting



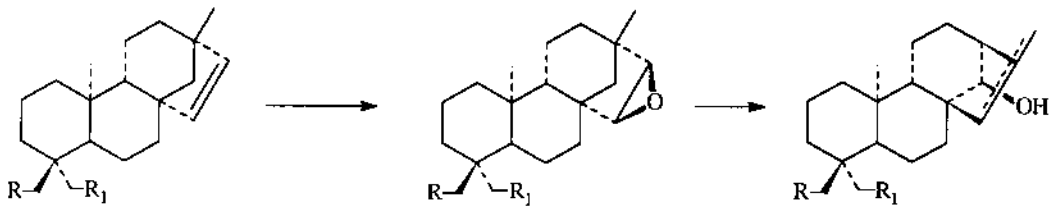
(12)



(13)



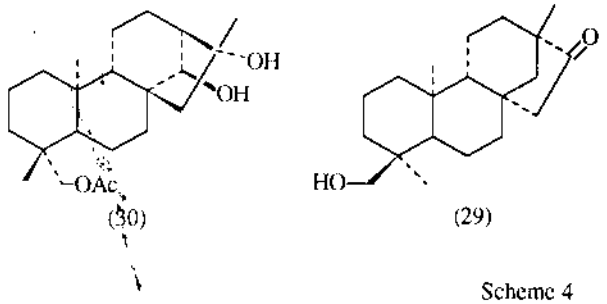
(14)



(15) R = H; R_1 = OH
(16) R = OH; R_1 = H

(17) R = H; R_1 = OH
(18) R = H; R_1 = OAc
(19) R = OH; R_1 = H
(20) R = OAc; R_1 = H

(21) R = H; R_1 = OAc; Δ^{15}
(22) R = H; R_1 = OAc; Δ^{16}
(23) R = H; R_1 = OH; Δ^{15}
(24) R = H; R_1 = OH; Δ^{16}
(25) R = OAc; R_1 = H; Δ^{15}
(26) R = OAc; R_1 = H; Δ^{16}
(27) R = OH; R_1 = H; Δ^{15}
(28) R = OH; R_1 = H; Δ^{16}



(30) (29)

Scheme 4

epoxide was recovered in a significant amount (30%) together with the 16-ketone **29** (30%) and the isomeric mixture of the rearranged compounds **27** (24%) and **28** (6%). When the reaction was repeated with the epoxide **19** in the presence of HMPA (2 eq.) the same temperature was required and the result was very similar.

We then replaced magnesium by the harder titanium (IV) as the Lewis acid maintaining the N-isopropylcyclo-hexylamide as the base counterpart. Thus, three different chloro-titanium isopropylcyclohexylamide pairs **31**, **32** and **33** were prepared by reaction of titanium tetrachloride (1 eq), (0.5 eq) and (0.3 eq) with lithium isopropylcyclohexylamide (1 eq) and directly assayed with the beyergibberellin epoxide **11**. While the pairs **31** and **32** were sufficiently reactive to promote a clean and smooth rearrangement, the pair **33** was totally inactive even at room temperature. It was observed that the

epoxide reactivity decreased when the number of amide units increased as a result of the lowering hardness of the Lewis acid and of the steric impediment introduced. The best result was obtained with the pair **32** giving the target 3β-acetate of 14β-hydroxy-gibberellin A_7 methyl ester (**12**) (50%) and the endocyclic isomer **13**, in a ratio of 1.4:1. With the pair **31**, the ratio was worse, (1:1), and the yield of **12** dropped to 40%. The isomeric mixtures could be easily resolved by repeated flash chromatography on silica gel or by simple chromatography on silica gel impregnated with silver nitrate (20% w/w). Carefully controlled hydrolysis of the 3β-acetate derivative **12** gave the target 14β-hydroxy-gibberellin A7 methyl ester (**2**).

Once the chemical access to this family of 14-hydroxylated C19 gibberellins was successfully achieved, we turned our attention to their possible microbiological preparation. The *ent*-19,14β-dihydroxy-

Table 1. ^{13}C NMR Data[c] for Compounds **2**, **24**, **25**, **30**, **35** and **38**

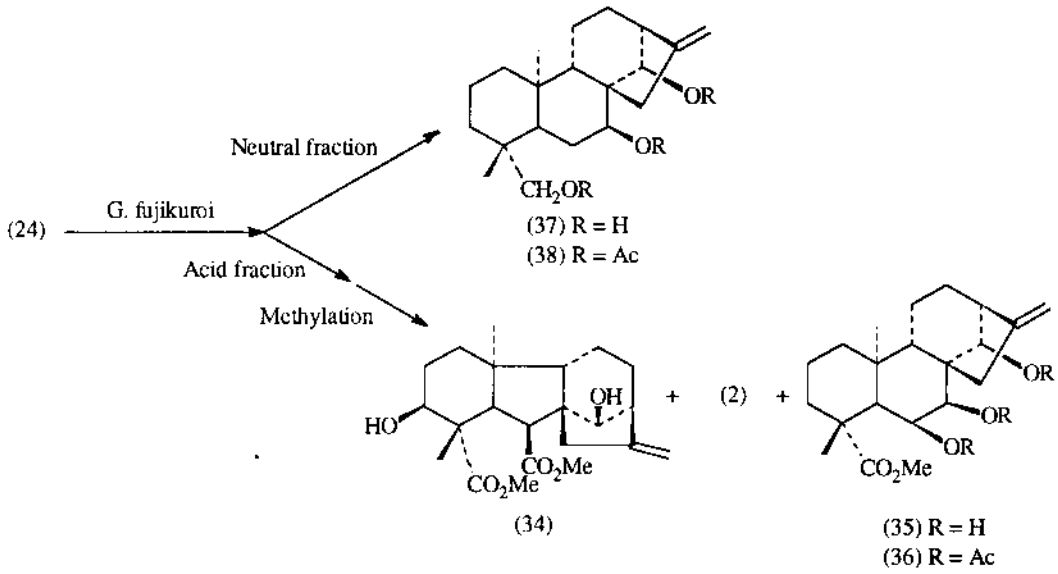
C	2	24	25	30	35	38
1	132.64	40.57	40.52	40.35	40.48[a]	40.15
2	132.51	18.41	18.30	18.30	19.20	18.29
3	70.00	35.66	36.50	36.36	39.19[a]	36.64
4	53.49	39.34[a]	37.07	37.03	43.97	36.31
5	53.39	56.95	56.61	56.80	51.93[b]	47.67
6	46.74	20.09	19.15	20.09	71.23	24.88
7	173.36	32.81[b]	25.57	27.32	77.36	72.96
8	56.79	49.37	55.17	51.07	53.21	50.90
9	48.98	58.97	53.45	53.73	51.77[b]	54.15
10	90.75	38.65a	39.56	39.28	41.15	38.98
11	15.24	17.66	18.30	17.54	17.46	16.90
12	31.43	33.02[b]	31.10	33.75	32.57	32.69
13	54.13	51.93	51.65	59.63	51.14	49.21
14	70.00	76.11	79.08	78.53	74.12	78.69
15	40.04	44.71	131.73	56.09	41.15	42.03
16	153.93	152.80	139.63	79.96	152.18	151.36
17	109.57	106.57	15.43	18.35	107.65	105.78
18	14.28	27.10	27.53	27.50	32.88	27.57
19	178.26	65.56	67.14	67.15	179.13	67.43
20	--	18.49	20.87	20.91	16.78	20.81

[a,b] These values may be interchanged
[c] Taken at 20.15 MHz, except that of **35** at 50.32

kaur-16-ene **24** was selected as the substrate to explore the microbiological approach because: a) its easy preparation (see above) and b) since *ent*-19-hydroxy-kaur-16-ene (**41**) is an intermediate in the gibberellin biosynthesis and the 14β-hydroxy group appeared to be far enough from C-6 and C-7, the centres involved in the B-ring contraction, the chance of success in its biotransformation by the fungus *G. fujikuroi* into

14β-hydroxy-gibberellins promised to be very high.

Compound **24** was incubated with *G. fujikuroi* for six days (scheme 5) in the presence of AMO 1618, which inhibits the formation of endogenous *ent*-kaur-16-ene derivatives and thus facilitates the purification of metabolites of exogenous kaurenoids.[14] The acidic products were methylated and chromatographed. The major gibberellin metabolite was assigned the structure 14β-hydroxy-gibberellin A_7



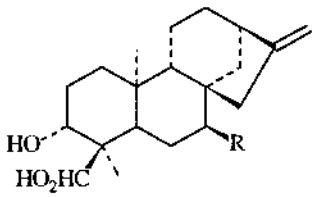
Scheme 5

methyl ester (**2**) (6%). Its spectroscopic and physical properties were identical with those of the synthetic material. The other gibberellin obtained in this feeding was the 14β-hydroxy-gibberellin A_{14} dimethyl ester (**34**), which was identified by GC-MS, as a contaminent of one of the chromatographic fractions that contained 14β-hydroxy-gibberellin A_7 methyl ester. No 14β-hydroxy-gibberellic acid methyl ester (**3**) was detected, and hence it would appear that the 14β-hydroxy group of the substrate inhibited hydroxylation at C-13, characteristic of gibberellic acid.

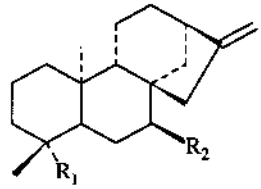
The triol ester **35** was also obtained from the methylated acid fraction. Its ^1H and ^{13}C NMR spectra are in accordance with this structure. The geminal hydrogens to the two vicinal hydroxyl groups at C-6 (α,axial) and C-7 (α,equatorial) showed the expected multiplicity: a broad doublet at δ 4.33 (J = 10 Hz) and a broad singlet at δ 4.30, respectively.

The *ent*-7α,14α,19-trihydroxy-kaur-16-ene (**37**) (scheme 5) was obtained as the only metabolite from the neutral fraction. This product was characterized as its triacetate **38** by acetylation of the fraction containing it. The position of the extra hydroxyl group was assigned to C-7 (β, axial) on the basis of its ^1H NMR spectrum which showed the broad singlet (δ 5.10) characteristic of a geminal hydrogen at a C-7(β) acetoxyl group. A consideration of its ^{13}C NMR spectrum (table 1) led to other positions such as C-1 (β) and C-3 (β) being excluded.

In the biosynthetic pathway of gibberellins the intermediate *ent*-19-hydroxy-kaur-16-ene (**41**) is oxidized to *ent*-kaur-16-en-19-oic acid (**42**) and then hydroxylated at C-7 to form **43**. An alternative route in which the 7β-hydroxylation occurs first, and subsequently the oxidation at C-19, does not take place.[2a] However, we have now found that compound **24** is hydroxylated at C-7 by *G. fujikuroi* to give **37**, without the existence of an acid group at C-19 in the molecule of **24**. This was also the case in the microbiological transformation with this fungus of *ent*-3β,18-dihydroxy-kaur-16-ene (**39**) which gave the triol **40**.[15] These



(39) R = H
(40) R = OH



(41) $R_1 = CH_2OH$; $R_2 = H$
(42) $R_1 = CO_2H$; $R_2 = H$
(43) $R_1 = CO_2H$; $R_2 = OH$

results suggest that a minimum polarity in the *ent*-kaurene substrate such as a carboxyl group at C-19 or two hydroxyl groups (e.g. at C-14 and C-19, or C-3 and C-18) is necessary for the 7β-hydroxylation to take place, and that under these latter abnormal conditions a metabolic grid relationship may exist amongst the biosynthetic transformation in *G. fujikuroi*.

EXPERIMENTAL

M.P.s were determined with a Kofler hot-plate apparatus and are uncorrected. ^1H and ^{13}C NMR spectra, at 200 and 50.32 MHz respectively, were determined on a Bruker WP 200 SY for solutions in $CDCl_3$. The 60 MHz ^1H and the 20.15 ^{13}C NMR spectra were run on a Perkin Elmer R-12 and on a Bruker AC80, respectively. The solvent for the samples was $CDCl_3$. MS were taken on a Hewlett-Packard 5930A and HRMS on a VG-Micromass ZAB-2F. Silica gel Merck (0.05-0.2 mm) was used for column chromatography.

Iodination of methyl gibberellate.- Methyl gibberellate (**1**) (3.5 g; 9.8 mmol) in a mixture of dichloromethane (60 ml), tetrahydrofuran (60 ml) and aq. sat. sodium hydrogen carbonate (120 ml) was vigorously stirred with iodine (11 g; 43 mmol) at room temperature for 4 h. More iodine (11 g; 43 mmol) was added and the reaction mixture stirred for a further 24 h. Work-up and acetylation as previously described[18] gave the *ent-3α-acetoxy-10β-hydroxy-17-iodo-16-oxo-20-nor-8:13-isogibberell-1-en-7,19-dioic acid 19:10β lactone 7-methyl ester* (**5**) (4.1 g) (yield: 80%)

Reduction of the 16-oxo derivative **6**.- The 16-oxo compound **6** (2.845 g; 7.08 meq) in chloroform (7 ml) and methanol (100 ml) was added dropwise for 1h to a stirred and cooled (0°C, crushed ice- water bath) solution of sodium borohydride (393 mg; 42.5 meq) in methanol (50 ml). The mixture was stirred for 45 min and more sodium borohydride (130 mg; 14.2 meq) was added. After 30 min, a few drops of acetone and 2N hydrochloric acid were carefully and consecutively added to destroy the reagent excess and the resulted mixture was concentrated. 2N Hydrochloric acid was added and the mixture extracted with ethyl acetate. The organic fractions were combined and washed with aq. sat. sodium hydrogen carbonate and brine, dried over sodium sulfate, filtered and concentrated to dryness to give a gummy residue which was flash chromatographed. Elution with ethyl acetate - petroleum ether: 2/8 (v/v) gave *starting material* (273 mg) and the reduction compound *ent-3α-acetoxy-10β,16β-dihydroxy-13-methyl-17,20-bisnor-8:13-isogibberell-1-en-7,19-dioic acid 19:10 lactone 7-methyl ester* (**7**) (2.46 g) (yield: 93%) as a gum. Found

(M^+ -31), at 373.1617. $C_{21}H_{25}O_6$ requires (M-31), 373.1648. IR υ_{max} (nujol): 3.800 (s), 3.400 (br), 1770, 1735 and 895 cm^{-1}. ^1H NMR (360 MHz) δ: 0.99 (3H, s, H-17), 1.18 (3H, s, H-18), 2.10 (3H, s, Ac), 2.60 (1H, d, J = 7.5 Hz, H-6), 3.24 (1H, d, J = 7.5 Hz, H-5), 3.74 (3H, s, OMe), 3.88 (1H, d.d, J = 4.3 and 10.8 Hz, H-16), 5.36 (1H, d.d, J = 0.7 and 3.6 Hz, H-3), 5.84 (1H, d.d, J = 3.6 and 10 Hz, H-2), 6.46 (1H, d.d, J = 0.7 and 10 Hz, H-1). EIMS m/z (rel. int.): 373 (M^+- 31) (4), 344 (3), 329 (3), 312 (13) 300 (21), 268 (24),255 (19), 240 (98), 43 (100). The recovered starting material (255 mg) was newly reduced to give the *alcohol* **7** (250 mg).

Attempts to obtain the 15,16-alkene derivative **10**.- a) Alcohol **7** (50 mg; 0.124 mmol) in dry acetonitrile (0.7 ml) was refluxed with triphenylphosphine (48.8 mg; 0.186 mmol) and dry carbon tetrachloride (0.015 ml; 0.155 mmol) under a nitrogen atmosphere for 2h 30 min.

b) Alcohol **7** (40 mg; 0.1 mmol) in dry acetonitrile (0.7 ml) was refluxed with triphenylphosphine (39.3 mg; 0.15 mmol), dry carbon tetrachloride (0.015 ml; 0.155 mmol) and dry pyridine (0.015 ml; 0.185 mmol) under a nitrogen atmosphere for 2h and 30 min.

Both experiments were followed by TLC, showing to give the same transformation compound. The solvents were evaporated off, ethyl acetate added and the resulted mixtures were filtered, combined and concentrated to dryness to give a gum residue which was flash chromatographed. Elution with ethyl acetate- petroleum ether (1/9 (v/v) gave the ent-*3α-acetoxy-16α-chloro-10β-hydroxy-13-methyl-17,20-bisnor-8:13-isogibberell-1-en-7,19-dioic acid 19:10β-lactone 7-methyl ester* (**8**) (80 mg) as a crystalline solid. Found: C, 62.02; H, 6.45. $C_{22}H_{27}O_6Cl$ requires C, 62.54; H, 6.45%. IR υ_{max} (nujol): 1.768, 1735, 890 and 764 cm-1. 1H NMR (360 MHz) δ: 1.14 (3H, s, H-17), 1.19 (3H, s, H-18), 1.97 (1H, d.d, J = 3.7 and 15.3 Hz, H-15β), 2.10 (3H, s, Ac), 2.63 (1H, d, J = 7.6 Hz, H-6), 3.17 (1H, quartet of doublets, J = 2.2, 7.9 and 15.3 Hz, H-15α), 3.25 (1H, d, J = 7.6 Hz, H-5), 3.78 (3H, s, OMe), 3.86 (1H, m, H-16), 5.36 (1H, d.d, J = 0.7 and 3.7 Hz, H-3), 5.86 (1H, d.d, J = 3.7 and 9.3 Hz, H-2), 6.43 (1H, d.d, J = 0.7 and 9.3 Hz, H-1). EIMS m/z (rel. int.): 391 (M+-31) (3), 365 (2), 334 (4), 320 (5), 318 (5), 283 (5), 275 (8), 258 (100)

c) The chlorine derivative **8** (40 mg; 0.95 mmol) in dry toluene (1 ml) was refluxed with DBU (0.2 ml; 1.33 mmol) for 4h under a nitrogen atmosphere. At this point, a TLC did not show any reaction. Dry pyridine (0.5 ml) was added and the solution refluxed for a further 15h. The solvents were distilled off to give the starting material and an intractable mixture of polar compounds.

Preparation of the mesylate **9**.- Alcohol **7** (1.8 g; 4.45 mmol) in dry pyridine (12 ml) was stirred with methanesulphonyl chloride (freshly distilled) (1 ml; 12.8 mmol) at 0°C for 3h. The reaction mixture was transferred to a decantation funnel with the aid of ethyl acetate and washed with cold 2N hydrochloric acid, aq. sat. sodium hydrogen carbonate and brine, dried over sodium sulfate, filtered and concentrated to dryness to give a gummy residue which was flash chromatographed. Elution with ethyl acetate - petroleum ether: 40/60 (v/v) gave the *mesylate* **9** (2.09 g) (yield: 97%). Found: (M^+-31), at 451.1420. $C_{22}H_{27}O_8S$ requires (M-31), 451.1424. IR υ_{max} ($CHCl_3$): 1770, 1740, 1730 and 1168 cm^{-1}. ^1H NMR (200 MHz) δ: 1.09 (3H, s, H-17), 1.19 (3H, s, H-18), 2.10 (3H, s, Ac), 2.60 (1H, d, J = 7.6 Hz, H-6), 3.00 (3H, s, Ms), 3.25 (1H, d, J = 7.6 Hz, H-5), 3.75 (3H, s, OMe), 4.69 (1H, d.d, J = 4.2 and 11 Hz, H-16), 5.37 (1H, d, J = 3.7 Hz, H-3), 5.87 (1H, d.d, J = 3.7 and 9.3 Hz, H-2), 6.45 (1H, d, J = 9.3 Hz, H-1). EIMS m/z (rel. int.): 451 (M^+-31) (3), 423 (1), 422 (1), 378 (5), 318 (38), 222 (100).

Mesylate elimination.- Mesylate **9** (1.9 g; 3.94 mmol) was refluxed in dry collidine (17 ml) for 9h 30 min. The collidine was distilled off under reduced pressure and the residue flash chromatographed (ethyl acetate - petroleum ether: 1/9 (v/v)) to give a mixture of three compounds which was resolved by chromatography on silica gel impregnated with silver nitrate (10%). Elution with ethyl acetate - petroleum ether: 15/85 (v/v) gave the isomeric mixture of Δ^{15}- and Δ^{16}-ent-*3α-acetoxy-10β-hydroxy-20-norgibberell-1-en-7,19-dioic acid 19:10β-lactone 7-methyl ester*^{16,17} (260 mg). Further elution gave the

ent-*3α-acetoxy-10β-hydroxy-13-methyl-17,20-bisnor-8:13-isogibberell-1,15-dien-7,19-dioic acid 19:10β-lactone 7-methyl ester* (**10**) (520 mg) as a gum (yield: 34%). Found: M^+, at 386.1736. $C_{22}H_{26}O_6$ requires M, 386.1729. IR υ_{max} ($CHCl_3$): 3040, 1765, 1730, 1725 and 880 cm^{-1}. ^1H NMR (200 MHz) δ: 1.09 (3H, s, H-17), 1.23 (3H, s, H-18), 2.10 (3H, s, Ac), 2.79 (1H, d, J = 7.6 Hz, H-6), 3.29 (1H, d, J = 7.6 Hz, H-5), 3.73 (3H, s, OMe), 5.38 (1H, d, J = 3.7 Hz, H-3), 5.44 and 5.52 (1H each, d, J = 5.5 Hz, H-15, H-16), 5.85 (1H, d.d, J = 3.7 and 9.3 Hz, H-2), 6.44 (1H, d, J = 9.3 Hz, H-1). EIMS m/z (rel. int.): 386 (M^+) (3), 355 (6), 326 (2), 282 (4), 222 (100).

Preparation of the 15β,16β-epoxy derivative **11**.- Diene **10** (496 mg; 1.28 mmol) in chloroform (30 ml) was treated with m-chloroperbenzoic acid (80% purity, 305 mg; 1.4 mmol) at 0°C for 65h. The reaction mixture was transferred to a decantation funnel with the aid of more chloroform and washed with aq. sat. sodium hydrogen carbonate and brine, dried over sodium sulfate, filtered and concentrated to dryness to give the crude *epoxide* **11** in practically quantitative yield. The epoxide crystallized from ethyl acetate - petroleum ether as needles, m.p.: 168 - 169°C. Found: C, 65.38; H, 6.75. $C_{22}H_{26}O_7$ requires C, 65.66; H, 6.51%. IR υ_{max} ($CHCl_3$): 3020, 1765, 1730, 1725 and 850 cm^{-1}. ^1H NMR (200 MHz) δ: 0.85 (1H, d, J = 10 Hz, H-14α), 1.10 (3H, s, H-17), 1.27 (3H, s, H-18), 1.44 (1H, d, J = 10 Hz, H-14β), 2.11 (3H, s, Ac), 3.01 and 3.46 (1H each, d, J = 3.1 Hz, H-15, H-16), 3.10 (1H, d, J = 7 Hz, H-6), 3.36 (1H, d, J = 7 Hz, H-5), 3.74 (3H, s, OMe), 5.40 (1H, d, J = 3.6 Hz, H-3), 5.88 (1H, d.d, J = 3.6 and 9.3 Hz, H-2), 6.44 (1H, d, J = 9.3 Hz, H-1). EIMS m/z (rel. int.): 402 (M^+) (0.03), 371 (4.7), 315 (7.1), 282 (11.5), 209 (42.2), 155 (30.9), 91 (24.9), 43 (100).

Epoxidation of **15**.- *ent*-19-Hydroxy-beyer-15-ene (**15**) (900 mg; 2.95 mmol) was stirred with m-chloroperbenzoic acid (80% purity, 750 mg; 3.48 mmol) at room temperature and in the dark for 7h. Work-up gave the ent-*19-hydroxy-15α,16α-epoxy-beyerane* (**17**)[18] which was acetylated to give the ent-*19-acetoxy-15α,16α-epoxy-beyerane* (**18**) (840 mg). Found M^+, at 346.2504. $C_{22}H_{34}O_3$ requires M, 346.2508. ^1H NMR (60 MHz) δ: 0.93, 0.95 and 1.00 (3H each, s, 3xMe), 2.04 (3H, s, Ac), 3.02 and 3.40 (1H each, d, J = 3 Hz, H-15, H-16), 3.91 and 4.31 (1H each, d, J = 11 Hz, H-18). EIMS m/z (rel. int.): 346 (M^+) (9), 328 (6), 287 (12), 273 (8), 268 (7), 255 (17), 245 (9), 227 (6), 213 (10), 203 (10), 197 (3), 185 (11), 175 (15), 173 (19), 161 (21), 55 (100).

Preparation of ent-*19-acetoxy-14α-hydroxy-kaur-16-ene* (**22**).- Epoxide **18** (880 mg; 2.5 mmol) in dry benzene (15 ml) was treated with freshly distilled boron trifluoride etherate (5 drops) at 0°C for 3h. Aq. sat. potassium carbonate was added and the mixture was extracted with ethyl acetate. The organic phases were combined and washed with 2N hydrochloric acid and brine, dried over sodium sulfate, filtered and concentrated in vacuo to give a solid residue which was chromatographed on silica gel. Elution with ethyl acetate - petroleum ether: 7/93 (v/v) gave a mixture of isomers and ent-*19-acetoxy-14α,16β-dihydroxy-kaurane* (**30**) (105 mg), m.p.: 191 - 193°C (ethyl acetate- petroleum ether). Found (M^+-18), at 346.2510. $C_{22}H_{34}O_4$ requires (M-18), 346.2508. ^1H NMR (200 MHz) δ: 0.95, 099 and 1.36 (3H each, s, 3xMe), 2.04 (3H, s, Ac), 3.85 and 4.22 (1H each, d, J = 11 Hz, H-19), 4.20 (1H, br. s, H-14). EIMS m/z : 346 (M^+-18), 288 (20), 273 (2), 255 (2), 229 (7), 228 (5), 215 (2), 43 (100). The isomeric mixture was resolved by chromatography over silica gel impregnated with silver nitrate (10%). Elution with ethyl acetate - petroleum ether 2/8 (v/v) gave the ent-*19-acetoxy-14α-hydroxy-kaur-16-ene* (**22**) (185 mg), mp 147-149°C (ethyl acetate -petroleum ether). Found: M^+, at 346.2510. $C_{22}H_{34}O_3$ requires M, 346.2508. ^1H NMR (60 MHz) δ: 0.96 and 1.01 (each 3H, s, 2xMe), 2.04 (3H, s, Ac), 3.87 and 4.27 (each 1H, d, J 11 Hz, H-19), 4.13 (1H, s, H-14), 4.94 (2H, br s, H-17). EIMS m/z (rel. int.): 346 (M^+)(14), 331 (2), 328 (7), 273 (6), 255 (10), 189 (2), 185 (3), 173 (6). *Diacetate*, Found: M^+, at 388.2621. $C_{24}H_{36}O_4$ requires M, 388.2614. ^1H NMR (60 MHz) δ: 0.94 and 1.10 (each 3H, s, 2xMe), 2.04 (6H, s, 2xAc), 3.88 and 4.27 (each 1H, d, J 11 Hz, H-19), 4.89 (2H, br s, H-17), 5.34 (1H, br s, H-14). EIMS m/z (rel. int.): 388 (M^+) (2), 373 (2), 346 (4), 328 (60), 268 (13), 255 (24), 253 (9) 238 (6), 225 (4), 213 (4), 199 (6). Further elution gave ent-*19-acetoxy-14α-hydroxy-kaur-15-ene* (**21**) (360 mg), mp 153-154°C,

Found: M^+, at 346.2504. $C_{22}H_{34}O_3$ requires M, 346.2508. ^1H NMR (200 MHz) δ: 0.94 and 1.03 (each 3H, s, 2xMe), 1.71 (3H, d, J 2 Hz, H-17), 2.03 (3H, s, Ac), 3.84 and 4.23 (each 1H, d, J 11 Hz, H-19), 4.10 (1H, br s, H-14), 4.96 (1H, br s, H-15). EIMS m/z (rel. int.): 346 (M^+)(20), 328 (8), 300 (4), 252 (10), 192 (18). *Diacetate,* Found: M^+, 388.2651. $C_{24}H_{36}O_4$ requires M, 388.2613. ^1H NMR (60 MHz) δ: 0.93 and 1.13 (each, 3H, s, 2xMe), 1.73 (3H, d, J 1.5 Hz, H-17), 2.04 (3H, s, Ac), 3.88 and 4.26 (each 1H, d, J 11 Hz, H-19), 5.03 (1H, br s, H-15), 5.45 (1H, s, H-14). EIMS m/z 388 (M^+) (3), 3.46 (4), 328 (12), 300 (23), 268 (15), 255 (20), 240 (10), 227 (5), 225 (4), 220 (6), 199 (3), 175 (6), 164 (26).

Epoxidation of **16**.- *ent*-18-Hydroxy-beyer-15-ene (**16**) (330 mg; 1.08 mmol) in chloroform (20 ml) was stirred with m-chloroperbenzoic acid (80% purity, 324 mg; 1.5 mmol) at room temperature and in the dark for 6h 30 min. Work-up and flash chromatography on silica gel [ethyl acetate - petroleum ether: 15/85 (v/v)] gave the ent-*18-hydroxy-15α,16α-epoxy-beyerane* (**19**) (330 mg). m.p.: 114 - 115°C (ethyl acetate - petroleum ether). Found: M^+, at 304.2417. $C_{20}H_{32}O_2$ requires M, 304.2402. ^1H NMR (200 MHz) δ: 0.79, 0.97 and 1.02 (3H each, s, 3xMe), 3.03 and 3.43 (1H each, d, J = 3 Hz, H-15, H-16), 3.12 and 3.44 (1H each, d, J = 10.8 Hz, H-18), 4.12 (1H, br. s, H-14). EIMS m/z (rel. int.): 304 (M^+) (4.9), 273 (40.8), 255 (50.6), 245 (16.1), 203 (17.2), 191 (30,2), 185 (12.6), 173 (42), 41 (100). Treatment of this compound with acetic anhydride and pyridine gave the ent-*18-acetoxy-15α,16α-epoxy-beyerane* (**20**). m.p.: 95 - 95.5°C (petroleum ether, needles). Found: M^+, at 346.2500. $C_{22}H_{34}O_3$ requires M, 346.2508. ^1H NMR (200 MHz) δ: 0.87, 0.97 and 1.03 (3H each, s, 3xMe), 2.07 (3H, s, Ac), 3.03 and 3.42 (1H each, d, J = 3 Hz, H-15 and H-16), 3.67 and 3.88 (1H each, d, J = 11 Hz, H-18). EIMS m/z (rel. int.): 346 (M^+) (5.1), 286 (5.1), 273 (25.3), 255 (44.2), 245 (21.9), 203 (15.6), 191 (19.7), 185 (12.1), 173 (34.7), 43 (100).

Rearrangement experiments with the epoxides **19** *and* **20** *as models.*- a) 18-Acetoxy-epoxide **20** (320 mg; 0.92 mmol) in dry benzene (5 ml) was treated with freshly distilled boron trifluoride etherate (3 drops) as before (see above in the preparation of **22**). Work-up and chromatography on silica gel impregnated with silver nitrate (10%) [ethyl acetate - petroleum ether 60/40 (v/v)] gave the ent-*18-acetoxy-14α-hydroxy-kaur-16-ene* (**26**) (60 mg), m.p.: 173 - 174°C (ethyl acetate, needles). Found M^+, at 346.2517. $C_{22}H_{34}O_3$ requires M, 346.2508. ^1H NMR (200 MHz) δ: 0.81 and 1.0 (3H each, s, 2xMe), 2.04 (3H, s, Ac), 3.62 and 3.83 (1H each, d, J = 11 Hz, H-18), 4.12 (1H, br. s, H-14). EIMS m/z (rel. int.): 346 (M^+) (13.7), 328 (3.5), 273 (10), 255 (14.2), 185 (7.8), 173 (14.2), 109 (100). Further elution gave the ent-*18-acetoxy-14α-hydroxy-kaur-15-ene* (**27**) (240 mg), m.p.: 189 - 190°C (ethyl acetate, needles). Found M^+, at 346.2523. $C_{22}H_{34}O_3$ requires (M-18), 346.2508. ^1H NMR (200 MHz) δ: 0.80 and 1.03 (3H each, s, 2xMe), 1.73 (3H, d, J = 1.4 Hz, H17), 2.04 (3H, s, Ac), 3.62 and 3.83 (1H each, d, J = 11 Hz, H-18), 4.16 (1H, br. s, H-14) and 5.01 (1H, t, J = 1.4 Hz., H-15). EIMS m/z (rel. int.): 346 (M^+) (11.5), 328 (7.8), 252 (17.8), 240 (9), 203 (8.4), 189 (8.5), 177 (110.7), 94 (100).

b) Ethylmagnesium bromide (3M in ether, 0.6 ml, 1.8 mmol) was stirred with N-isopropylcyclohexylamine (0.3 ml, 1.8 mmol) at 0°C for 30 min under a nitrogen atmosphere. 18-Acetoxy-epoxide derivative **20** (100 mg, 0.29 mmol) in dry toluene (2 ml) was added and the mixture allowed to reach the room temperature (1h) and stirred at this temperature for a further 2h. A TLC showed no reaction. A condenser was coupled to the system and the mixture was heated to 100°C and maintained at that temperature for 5h. After cooling, 2N hydrochloric acid (0.5 ml) was carefully added to destroy the reagent excess, and the resulting mixture was dissolved in ethyl acetate and 2N hydrochloric acid. The organic layer was decanted off and the aqueous phase further extracted with ethyl acetate. The combined organic phases were washed with aq. sat. sodium hydrogen carbonate and brine, dried over sodium sulfate, filtered and concentrated to dryness to give a gummy residue which was flash chromatographed. Elution with ethyl acetate - petroleum ether: 5/95 (v/v) gave ent-*18-hydroxy-beyer-15α,16α-epoxide* (**19**) (30 mg) (see data above) and ent-*18-hydroxy-16-oxo-beyerane* (**29**) (30 mg), ^1H NMR (200 MHz) δ: 0.76, 0.90 and 0.97 (3H each, s, 3xMe), 1.75 (1H, d, J = 18.6 Hz, H-15β), 2.68 (1H, d.d, J = 3.7 and 18.6 Hz,

H-15α), 3.1 and 3.43 (1H each, d, J = 10.9 Hz, H-18). EIMS m/z (rel. int.): 304 (M^+) (14), 273 (98), 255 (12), 191 (24), 177 (43), 123 (42), 81 (77), 55 (100). Further elution gave the ent-*18-hydroxy-14α-hydroxy-kaur-16-ene* (**28**) (24 mg). ^{1}H NMR (200 MHz) δ: 0.8 and, 1.03 (3H each, s, 2xMe), 3.1 and 3.45 (1H each, d, J = 10.9 Hz, H-18), 4.15 (1H, s, H-14) and 4.93 (2H, br. s, H-17). m/z (rel. int.): 304 (M^+) (14), 286 (5), 273 (11), 255(15), 173 (), 149 (20), 109 (89), 85 (100). Final elution gave ent-*18-hydroxy-14α-hydroxy-kaur-15-ene* (**27**) (6 mg). ^{1}H NMR (200 MHz) δ: 0.74 and 1.05 (3H each, s, 2xMe), 1.72 (3H, d, J = 1.6 Hz, H-17), 3.1 and 3.44 (1H each, d, J = 10.9 Hz, H-18), 4.15 (1H, s, H-14) and 4.97 (1H, br. s, H-15). EIMS m/z (rel. int.): 304 (M^+) (6), 286 (2), 273 (3), 255(7), 210 (11), 121 (38), 94 (71), 55 (100).

Rearrangement experiments with the epoxide **11**.- a) To a cooled (0°C, ice bath) and stirred solution of lithium isopropylcyclohexylamide [N-isopropylcyclohexylamine (0.07 ml; 0.43 mmol) and n-butyllithium (2.34N in hexanes; 0.15 ml; 0.36 mmol)] in toluene (0.5 ml), was dropwise added titanium tetrachloride (freshly distilled) (0.05 ml; 0.36 mmol) under a positive pressure of nitrogen. The mixture stirred at 0°C for 30 min and then at room temperature for 1h. After cooling to 0°C, epoxide **11** (25 mg, 0.06 mmol) in toluene (0.5 ml) was added. Stirring was continued for 4h at this temperature and then the reaction mixture was allowed to reach room temperature and stirred for a further 3h. The reaction mixture was cooled to 0°C, quenched by careful addition of 2N hydrochloric acid and water and extracted with ethyl acetate. The combined organic phases were washed with aq. sat. sodium hydrogen carbonate and brine, dried over sodium sulfate, filtered and concentrated to dryness to give a gummy residue which was flash chromatographed. Elution with ethyl acetate - petroleum ether: 2/8 (v/v) gave the *3β-acetate of 14β-hydroxy GA$_7$ methyl ester* (**12**) (8 mg) as a crystalline solid. m.p.: 175 - 176°C (ethyl acetate - petroleum ether, needless). Found: M^+, at 402.1711. $C_{22}H_{26}O_7$ requires M, 402.1678. IR υ_{max} ($CHCl_3$): 3700, 1750, 1755 and 1730 cm^{-1}. ^{1}H NMR (200 MHz) δ: 1.19 (3H, s, H-19), 2.12 (3H, s, Ac), 3.025 (1H, d, J = 10.5 Hz, H-6), 3.36 (1H, d, J = 10.5 Hz, H-5), 3.77 (3H, s, OMe), 4.09 (1H, br. s, H-14), 5.02 and 5.09 (1H each, br. s, H-17), 5.35 (1H, d, J = 3.8 Hz, H-3), 5.88 (1H, d.d., J = 3.8 and 9.2 Hz, H-2) and 6.38 (1H, d, J = 9.2 Hz, H-1). EIMS m/z (rel. int.): 402 (M^+) (1), 384 (100), 371 (8), 342 (31), 324 (10), 310 (26), 297 (13), 280 (52), 221 (96). Further elution gave the *Δ^{15} isomer* **13** (8 mg) as needless solid. m.p.: 203 - 205°C (ethyl acetate - petroleum ether). Found: M^+, at 402.1674. $C_{22}H_{26}O_7$ requires M, 402.1678. IR υ_{max} ($CHCl_3$): 3750, 1770, 1730, 1725 and 890 cm^{-1}. ^{1}H NMR (200 MHz) δ: 1.20 (3H, s, H-19), 1.75 (3H, d, J = 1.5 Hz, H-17), 2.12 (3H, s Ac), 2.28 (1H, d, J = 10.7 Hz, OH), 3.12 (1H, d, J = 10.5 Hz, H-6), 3.37 (1H, d, J = 10.5 Hz, H-5), 3.74 (3H, s, OMe), 4.28 (1H, d, J = 10.7 Hz, H-14), 5.20 (1H, d, J = 1.5 Hz, H-15), 5.35 (1H, d, J = 3.8 Hz, H-3), 5.87 (1H, d.d., J = 3.8 and 9.2 Hz, H-2) and 6.31 (1H, d, J = 9.2 Hz, H-1). When a drop of D_2O was added, the signal at 2.28 ppm disappeared and that at 4.28 ppm collapsed to a singlet. EIMS m/z: 402 (M^+) (2), 384 (1), 371 (6), 342 (86), 353 (10), 342 (86), 310 (100), 297 (9), 282 (13), 265 (15), 252 (22), 237 (26), 221 (20), 209 (91).

b) To a cooled (0°C, ice bath) and stirred solution of lithium isopropylcyclohexylamide (0.7 mmol) in toluene (1 ml), was dropwise added titanium tetrachloride (freshly distilled) (0.05 ml; 0.36 mmol) under a positive pressure of nitrogen, and the resulted black-brown mixture was stirred at 0°C for 30 min. The ice-bath was removed and the mixture refluxed for 1h and chilled again at 0°C. Epoxide **11** (48 mg, 0.12 mmol) in toluene (0.5 ml) was added and the stirred reaction mixture allowed to reach the room temperature (2h) and stirred for 7h at this temperature. Quenching, work up and flash chromatography following the same procedure as in a) gave the *starting epoxide* (6 mg), the *3β-acetate of 14β-hydroxy-GA$_7$-methyl ester* (**12**) (23 mg) and the *Δ^{15}-isomer* **13** (17 mg).

c) To a cooled (0°C, ice bath) and stirred solution of lithium isopropylcyclohexylamide (1.13 mmol) in toluene (1 ml), was dropwise added titanium tetrachloride (freshly distilled) (0.05 ml; 0.36 mmol) under a positive pressure of nitrogen, and the resulted black-brown mixture was stirred at 0°C for 30 min. The ice-bath was removed and the mixture refluxed for 2h and chilled again at 0°C. Epoxide **11**

(48 mg, 0.12 mmol) in toluene (0.5 ml) was added and the stirred reaction mixture allowed to reach the room temperature (2h) and stirred for 7h at this temperature. Quenching, work up and flash chromatography following the same procedure as in a) gave the unaltered *starting epoxide*.

Hydrolysis of **12**.- Acetate **12** (23 mg; 0.057 mmol) in methanol (3 ml) was stirred with aq. sat. potassium carbonate (0.5 ml) for 1h at room temperature. The mixture was poured on water and extracted with ethyl acetate. The combined organic fractions were washed with 2N hydrochloric acid and brine, dried over sodium sulfate, filtered and concentrated to dryness to give *14β-hydroxy-gibberellin A7 methyl ester* (**2**) (20 mg) as a gum. Found: M^+, at 360.1571. $C_{20}H_{24}O_6$ requires M, 360.1571. ^1H NMR (200 MHz) δ: 1.27 (3H, s, H-19), 3.04 (1H, d, J = 10 Hz, H-6), 3.25 (1H, d, J = 10 Hz, H-5), 3.76 (3H, s, OMe), 4.09 (1H, br. s, H-14), 4.16 (1H, br s, H-3), 5.01 and 5.08 (1H each, br s, H-17), 5.91 (1H, dd, J = 4 and 9 Hz, H-2) and 6.31 (1H, d, J = 9 Hz, H-1). EIMS m/z (rel. int.): 360 (M^+) (0.6), 342 (8), 328 (3), 297 (7), 280 (5), 267 (4), 237 (10), 221 (14), 220 (5).

Hydrolysis of **22**.- Acetate **22** (260 mg) in chloroform (minimum amount) was treated with methanolic potassium hydroxide solution (4%) (1 ml) for 6 h. The solution was poured on 2N hydrochloric acid and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The residue was chromatographed (ethyl acetate - petroleum ether), to give ent-*14α,19-dihydroxykaur-16-ene* (**24**) (250 mg), m.p. 179-181°C (ethyl acetate), Found: M^+, at 304.2410. $C_{20}H_{32}O_2$ requires M, 304.2403. ^1H NMR (200 MHz) δ: 0.94 (6H, s, 2xMe), 3.42 and 3.70 (each 1H, d, J 11 Hz, H-19), 4.08 (1H, s, H-14), 4.89 and 4.90 (each 1H, s, H-17). EIMS m/z (rel. int.): 304 (M^+) (12), 286 (7), 273 (15), 255 (21), 173 (10), 159 (10), 145 (14).

Incubation of ent-*14α,19-dihydroxy-kaur-16-ene* (**24**). The fungus *Gibberella fujikuroi* (ACC 917), inhibited with 5 x 10^{-5} M AMO 1618, was grown in shake culture at 25° for 1 day in 77 conical flasks (250 ml), each containing sterile medium (50 ml).[19] The substrate, ent-*14α,19-dihydroxy-kaur-16-ene* (**24**) (330 mg), in ethanol (15 ml) was distributed equally between the flasks and the incubation was allowed to continue for a further 6 days. The broth was filtered, adjusted to pH 2 with dilute HCl and extracted with EtOAc. The mycelium was treated with liquid nitrogen, crushed in a mortar and extracted with ethyl acetate. The two extracts were combined and separated into neutral and acidic fractions with sodium hydrogen carbonate.

The neutral fraction was chromatographed in silica gel, eluting with ethyl acetate-petroleum ether mixtures, to give untransformed product (140 mg) and ent-*7α,14α,19-trihydroxy-kaur-16-ene* (**37**) (8 mg), which was characterized as its triacetate **38** by acetylation of the fraction that contained it, m.p. 96-98°C (ethyl acetate - petroleum ether). Found: (M^+ - 60), at 386.2444. $C_{24}H_{34}O_4$ requires (M -60), 384.2457. ^1H NMR (200 MHz) δ: 0.85 and 1.11 (each 3H, s, 2xMe), 2.06 (9H, s, 3xAc), 3.87 and 4.16 (each 1H, d, J = 11 Hz, H-19), 4.88 (2H, s, H-17), 5.10 (1H, br s, H-7), 5.31 (1H, s, H-14). EIMS m/z (rel. int.): 386 (M^+-HOAc) (11), 341 (11), 326 (6), 284 (9), 266 (21), 253 (16), 251 (8).

The acidic fraction was methylated with diazomethane and chromatographed using ethyl acetate - petroleum ether: 3/7 (v/v) as eluent to give the *14β-hydroxy-GA7 methyl ester* (**2**) (14 mg) (see above) and ent-*6α,7α,14α-trihydroxy-kaur-16-en-19-oic acid methyl ester* (**35**) (10 mg). Found: (M^+ - 18), at 346.2156. $C_{21}H_{30}O_4$ requires (M - 18), 346.2144. ^1H NMR (200 MHz) δ: 0.79 and 1.22 (each 3H, s, 2xMe), 1.90 (1H, d, J 10 Hz, H-5), 3.70 (3H, s, OMe), 4.07 (1H, br s, H-14), 4.30 (1H, br s, H-7), 4.33 (1H, d, J 10 Hz, H-6), 4.96 (2H, br s, H-17). EIMS m/z (rel. int.): 346 (M^+- H_2O) (7), 328 (2), 314 (16), 296 (2), 286 (4), 269 (3), 255 (3), 225 (5), 213 (5). *Triacetate* **36**, ^1H NMR (200 MHz) δ: 0.97 and 1.29 (each 3H, s, 2xMe), 1.94 (1H, d, J = 12 Hz, H-5), 1.95, 2.12 and 2.14 (each 3H, s, 3xAc), 2.75 (1H, br s, H-13), 3.64 (3H, s, OMe), 4.89 (2H, br s, H-17), 5.38 (1H, br s, H-14), 5.41 (1H, dd J = 12 and 2.4 Hz, H-6), 5.58 (1H, d, J = 2.4 Hz, H-7). EIMS m/z (rel. int.): 490 (M^+) (2), 43.0 (52), 388 (16), 370 (49), 356 (5), 328 (63), 310 (100), 296 (22), 285 (5), 282 (5), 268 (25), 255 (15).

The dimethyl ester of *14β-hydroxy-GA14* (**34**) was identified by GC-MS in one of the fractions that contained **2**. EIMS m/z (rel. int.) 374 (M^+ -18) (13), 360 (11), 342(100), 310 (12), 299 (6), 296 (9), 282

(15), 267 (7), 264 (13), 254 (9), 237 (23), 221 (10), 211 (10), 199 (13). GC-MS conditions: Carrier gas, He at a flow of 5 ml/min He. Column of cross-linked methyl silicone (HP-1, 12 m long, 0.2 mm i.d., 0.33 μm film thickness); initial temp. 200°C, final temp. 280°C, with a program rate of 4°C/min. EIMS 70 ev.

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