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PREPARATION OF *ENT*-3β-HYDROXYKAUR-6,16-DIENE AND ITS MICROBIOLOGICAL TRANSFORMATION BY *GIBBERELLA FUJIKUROI*

CARMEN E. DIAZ, BRAULIO M FRAGA and MELCHOR G. HERNANDEZ

Instituto de Productos Naturales Orgánicos, C S I.C, La Laguna, 38206-Tenerife, Canary Islands, Spain

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Key Word Index—*Gibberella fujikuroi*, gibberellins, kaurenolides, *ent*-3β-hydroxykaur-6,16-diene, linearol

Abstract—The microbiological transformation of *ent*-3β-hydroxykaur-6,16-diene into *ent*-6α,7α-epoxy-3β-hydroxykaur-16-ene has been carried out The substrate incubated was synthesized from the diterpene linearol.

INTRODUCTION

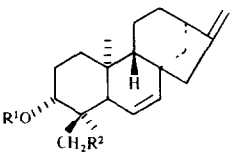
In the past few years we have studied the microbiological transformation of *ent*-kaurene diterpenes by *Gibberella fujikuroi* [1–6]. The purpose of these works was to obtain gibberellin analogues and to define the substrate requirements of the enzymes that participate in various biosynthetic steps in the gibberellin and kaurenolide pathway in this fungus. Continuing these studies we present here the results obtained in the chemical preparation and microbiological incubation of *ent*-3β-hydroxykaur-6,16-diene (**1**) with *G fujikuroi*

RESULTS AND DISCUSSION

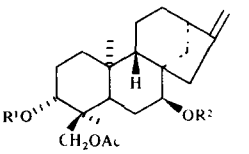
The substrate **1** was prepared in the following way Partial acetylation of linearol (**2**) [7] gave the triacetate **3** and the diacetates **4** and **5**. The 3α,18-diacetate **4** was treated with tosyl chloride in pyridine to afford the corresponding tosylate **6** Treatment of this derivative under reflux with collidine formed compound **7**, which was then hydrolysed to give *ent*-3β,18-dihydroxykaur-6,16-diene (**8**) [5]. Partial acetylation of this diene afforded the two monoacetates, **9** and **10**, and the diacetate **7**. Additional quantities of compound **9** were obtained by transacetylation of **10** Treatment of the 3α-monoacetate **9** with triphenylphosphine–carbon tetrachloride gave the chloride derivative **11**, reduction of which with tri-*n*-butyl tin hydride and subsequent hydrolysis of the acetate **12** formed, yielded *ent*-3β-hydroxykaur-6,16-diene (**1**).

When compound **1** was incubated with the fungus *G fujikuroi*, in the presence of AMO-1618, which blocks the formation of endogenous *ent*-kaur-16-ene [8, 9], *ent*-3β-hydroxy-6α,7α-epoxykaur-16-ene (**13**) was obtained by epoxidation of the 6,7 double bond However, we were unable to detect any kaurenolide or gibberellin The structure of the epoxide **1** followed from its ^{1}H NMR spectrum, in which the geminal hydrogens to the oxirane ring appear at δ 2.91 (*d*, *J* = 4 Hz) and 3.08 (*dd*, *J* = 2 5 and 4 Hz) The ^{13}C NMR spectrum of **13** also confirmed its structure; the spectrum is summarized in Table 1 together with that of the parent compound **14**. The H-5, H-6 coupling of 2 5 Hz is in accordance with a β-epoxide [5, 10] The result of the incubation confirms that a 3α-hydroxyl group in the kaur-16-ene derivatives inhibits their hydroxylation at C-19 by *G. fujikuroi* [3]

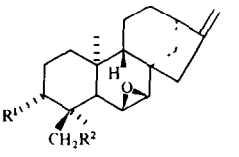
It has been suggested that the microbiological epoxidation of an alkene is sometimes equivalent to the hydroxylation of the corresponding alkane [11, 12] Thus, in a previous work we have shown that *ent*-3β,18-dihydroxykaur-6,16-diene (**8**) is transformed into the epoxide **14** [4, 5], and *ent*-3β,18-dihydroxykaur-16-ene (**15**) is converted into the corresponding triol **16** [3]. On the other hand, in a previous work we have also shown that *ent*-3β-hydroxykaur-16-ene (**17**) is not transformed



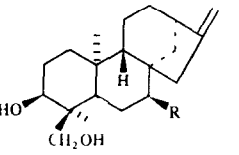
1 R^1 = R^2 = H
7 R^1 = Ac, R^2 = OAc
8 R^1 = H, R^2 = OH
9 R^1 = Ac, R^2 = OH
10 R^1 = H, R^2 = OAc
11 R^1 = Ac, R^2 = Cl
12 R^1 = Ac R^2 = H



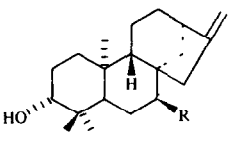
2 R^1 = R^2 = H
3 R^1 = R^2 = Ac
4 R^1 = Ac, R^2 = H
5 R^1 = H, R^2 = Ac
6 R^1 = Ac, R^2 = Ts



13 R^1 = OH R^2 = H
14 R^1 = R^2 = OH



15 R = H
16 R = OH



17 R = H
18 R = OH

Table 1 ^{13}C NMR spectral data of compounds **13** and **14** (50 MHz)

C	13	14	C	13	14
1	39 50	39 50	11	16 51	16 51
2	27 40	26 73	12	33 24	33 12
3	78 95	76 41	13	42 30	42 24
4	38 71	41 88	14	36 34	35 87
5	55 89	51 08	15	45 93	45 85
6	53 70	53 23	16	154 69	154 56
7	60 81	60 75	17	104 78	104 88
8	42 95	42 83	18	28 00	71 13
9	50 94	50 69	19	16 28	11 73
10	37 91	37 64	20	19 59	19 88

by the fungus into the 7β-hydroxy analogue **18** [3] In contrast we now report that the corresponding dehydro-derivative, *ent*-3β-hydroxykaur-6,16-diene (**1**), is transformed into the 6β,7β-epoxide **13** Therefore, it appears unlikely that the same enzymes are involved in 7β-hydroxylation and 6β,7β-epoxidation of *ent*-3β-hydroxykaur-16-ene analogues However, there is still insufficient evidence to exclude this possibility in the gibberellin and kaurenolide biosynthetic pathways

EXPERIMENTAL

Mps uncorr, IR $CHCl_3$, NMR $CDCl_3$, MS 70 eV (probe) CC was performed on silica gel 0 063–0 2 mm Substances were crystallized from petrol–EtOAc except where otherwise indicated

Partial acetylation of linearol (ent-18-*acetoxy*-3β,7α-*dihydroxykaur*-16-*ene*) Compound **2** [7] (1 1 g) in pyridine (15 ml) was treated with Ac_2O at 0° for 4 hr Usual work-up and chromatography of the residue, eluting with petrol–EtOAc (4 : 1), afforded the triacetate of foliol (**3**) (60 mg), the diacetates **4** and **5**, 800 and 150 mg, respectively, and starting material (130 mg) *ent*-3β,18-Diacetoxy-7α-hydroxykaur-16-ene (**4**) Mp 210–213°, ^1H NMR (90 MHz) δ 0.81 and 1 07 (each 3H, s), 2 01 and 2 03 (each 3H, s), 3 58 (1H, *br* s, H-7), 3 52 and 4 05 (each 1H, *d*, *J* = 12 Hz, H-18), 4 82 (3H, *br*, H-3 and H-17), EIMS *m/z* (rel int) 404 $[M]^+$ (1), 386 (1), 344 (1), 326 (20), 298 (1), 284 (6), 266 (34), 253 (21), 251 (22) *ent*-7α,18-Diacetoxy-3β-hydroxykaur-16-ene (**5**) Mp 169–171°, ^1H NMR (90 MHz) δ 0 76 and 1 09 (each 3H, s), 2 07 and 2 09 (each 3H, s), 3 68 (1H, *br* s, H-3), 3 58 and 4 38 (each 1H, *d*, *J* = 12 Hz, H-18), 4 85 (3H, *br*, H-7 and H-17), EIMS *m/z* (rel int) 344 $[M-EtOAc]^+$ (1), 326 (12), 248 (8), 266 (16), 253 (14), 251 (7), 225 (6), 199 (7), 197 (5), 185 (12), 171 (6), 157 (6), 149 (17)

Tosylation of **4**. *ent*-3β,18-Diacetoxy-7α-hydroxykaur-16-ene (**4**) (800 mg) in dry pyridine (10 ml) was treated with tosyl chloride (2 5 g) at room temp for 5 days Usual work-up afforded **6**, mp 164–165°, ^1H NMR (60 MHz) δ 0 80 and 1 07 (each 3H, *s*), 2 00 and 2 14 (each 3H, *s*), 2 45 (each 3H, s), 3 48 and 3 88 (each 1H, *d*, *J* = 12 Hz, H-18), 4 75 (1H, *br* s, H-7), 4 80 (3H, *br*, H-3 and H-17), 7 35 and 7 80 (each 2H, *d*, *J* = 9 Hz), EIMS *m/z* (rel int) 386 $[M-TsOH]^+$ (2), 326 (6), 266 (72), 251 (37), 238 (15), 223 (14), 209 (7), 195 (7), 172 (34), 169 (9), 157 (12), 155 (8), 143 (11), 131 (9)

ent-3β,18-*Diacetoxykaur*-6,16-*diene* (**7**) The tosylate **6**, obtained above, in collidine (10 ml) was refluxed for 30 min Usual work-up gave **7** (710 mg), ^1H NMR (60 MHz) δ 0 88 and 1 01 (each 3H, *s*), 2 04 (6H, *s*), 3 85 (2H, s, H-18), 4 88 (3H, *br*, H-3 and H-17), 5 55 (2H, s, H-6 and H-7), EIMS *m/z* (rel int) 326 $[M-HOAc]^+$ (1), 311 (1), 284 (2), 266 (64), 251 (100), 223 (16), 195 (11)

Hydrolysis of **7** The diacetate **7** (700 mg) in MeOH was treated with MeOH–KOH (5%) (15 ml) at room temp for 24 hr Usual work-up gave **8**, mp 140–142°, $[M]^+$ at *m/z* 302 2253 $C_{20}H_{30}O_2$ requires 302 2245, ^1H NMR (90 MHz) δ 0 90 and 0 99 (each 3H, s), 3 40 and 3 72 (each 1H, *d*, *J* = 11 Hz, H-18), 3 60 (1H, *br*, H-3), 4 80 (2H, *br* s, H-17), 5 45 (1H, s, H-6 and H-7), EIMS *m/z* (rel int) 302 $[M]^+$ (5), 284 (26), 269 (100), 266 (10), 251 (16), 241 (10), 223 (25), 211 (38), 209 (8), 199 (15)

Partial acetylation of **8** Compound **8** (2 g) in pyridine (15 ml) was treated with Ac_2O at 0° for 2 5 hr Usual work-up and chromatography of the residue, eluting with petrol–EtOAc, afforded **7** (650 mg) Further elution gave ent-3β-*acetoxy*-18-*hydroxykaur*-6,16-*diene* (**9**) (200 mg), mp 108–110°, $[M-HOAc-Me]^+$] at 269 1853 $C_{19}H_{25}O$ requires 269 1905, ^1H NMR (90 MHz) δ 0 73 and 1 03 (each 3H s), 2 02 (3H, *s*), 3 01 and 3 48 (each 1H, *d*, *J* = 12 Hz, H-18), 4 90 (2H, *br*, H-17), 4 98 (1H, *t*, H-3), 5 57 (2H, *s*, H-6 and H-7), EIMS *m/z* (rel int) 284 $[M-HOAc]^+$ (13), 269 (64), 266 (40), 251 (72), 223 (17), 211 (24), 199 (30), 195 (11), 183 (19) Further elution gave *ent*-3β-hydroxy-18-acetoxykaur-6,16-diene (**10**) (1 2 g), mp 131–133°, $[M-HOAc-Me]^+$ at 269 1904 $C_{19}H_{25}O$ requires 269 1905, ^1H NMR (90 MHz) δ 0 93 and 1 00 (each 3H, s), 2 11 (3H, *s*), 3 48 (1H, *br*, H-3), 3 81 and 4 29 (each 1H, *d*, *J* = 12 Hz, H-18), 4 48 (2H, *br*, H-17), 5 55 (2H, s, H-6 and H-7), EIMS *m/z* (rel int) 284 $[M-HOAc]^+$ (8), 269 (30), 266 (21), 251 (11), 223 (10), 211 (13), 199 (16), 195 (9), 183 (12)

Transacetylation of **10** Compound **10** (1 2 g) in $CHCl_3$ (20 ml) was treated with concd HCl (two drops) at room temp for 5 hr Neutralization with $NaHCO_3$, extraction with EtOAc and chromatography of the residue, eluting with petrol–EtOAc (5 1), gave **10** (450 mg), a mixture of **9** and **10** (300 mg) and **9** (280 mg)

ent-3β-*Acetoxy*-18-*chlorokaur*-6,16-*diene* (**11**) To the monoacetate **9** (450 mg) in dry pyridine (7 ml) and CCl_4 (15 ml) triphenylphosphine (1 g) was added and the mixt refluxed for 2 hr Extraction with EtOAc in the usual way and chromatography of the residue, eluting with petrol–EtOAc, afforded compound **11** (460 mg), mp 117–119°, $[M]^+$ at 362 1995 $C_{22}H_{31}O_2Cl$ requires 362 2013, ^1H NMR (60 MHz) δ 0 93 and 1 01 (each 3H, s), 2 02 (3H, s), 3 42 (2H, *br* s, H-18), 4 85 (2H, *br*, H-17), 5 03 (1H, *t*, H-3), 5 49 (2H, *br* s, H-6 and H-7), EIMS *m/z* (rel int) 362 $[M]^+$ (18), 347 (5), 319 (22), 302 (7), 291 (21), 287 (92), 267 (34), 253 (28), 225 (11), 211 (16), 199 (100)

Reduction of **11** Compound (**11**) (460 mg) in dry toluene (8 ml) was added dropwise to a refluxing soln of tri-*n*-butyl tin hydride (0.5 ml) and azobisisobutyronitrile (trace) in dry toluene (5 ml) The mixt was allowed to reflux for a further 18 hr when the solvent was evapd and the residue dissolved in Et_2O. An aq soln of KF was added and the ppt sepd by filtration The ether fraction was dried (Na_2SO_4) and the solvent evapd Chromatography of the residue, eluting with petrol–EtOAc (9 1) afforded *ent*-3β-acetoxykaur-6,16-diene (**12**) (320 mg), mp 124–126°, $[M]^+$ at 328 2397 $C_{22}H_{32}O_2$ requires 328 2402, ^1H NMR (60 MHz) δ 0 88 (6H, s), 0 97 (3H, s), 2 03 (3H, s), 4 52 (1H, *m*, H-3), 4,82 (2H, *br*, H-17), 5 53 (2H, *br* s, H-6 and H-7), EIMS *m/z* (rel int) 328 $[M]^+$ (20), 269 (34), 268 (22), 253 (100), 225 (46), 211 (13) 199 (100)

Hydrolysis of **12** The monoacetate **12** was treated as described above for **7** giving *ent*-3β-hydroxykaur-6,16-diene (**1**), mp 171–173° (from MeOH–EtOAc), $[M]^+$ at 286 2321 $C_{20}H_{30}O$ requires 286 2297, ^1H NMR (200 MHz) δ 0 73, 0 96 and 1 01 (each 3H, s), 3 25 (1H, *m*, H-3), 4 83 (2H, *br* s, H-17), 5 43 (1H, *dd*, *J* = 3 and 11 Hz, H-6), 5 63 (1H, *d*, *J* = 11 Hz, H-7), EIMS *m/z* (rel

int) 286 $[M]^+$ (27), 271 (8), 253 (80), 225 (24), 215 (13), 199 (68), 197 (14), 185 (25), 183 (13)

Incubation expt G fujikuroi (ACC 917), inhibited with 5×10^{-5} AMO 1618, was grown in shake cultures at 25° for 1 day in 75 conical flasks (250 ml), each containing sterile medium (50 ml) *ent*-3β-Hydroxykaur-6,16-diene (**1**) (200 mg) in EtOH (30 ml) was distributed equally among the flasks and the incubation allowed to continue for a further 5 days The broth was then filtered, adjusted to pH 2 with dil HCl 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 and sepd into acidic and neutral fractions with $NaHCO_3$ The neutral fraction was chromatographed on silica gel. Elution with petrol–EtOAc gave starting material (50 mg) and *ent*-6α,7α-epoxy-3β-hydroxykaur-16-ene (**13**) (27 mg), mp 181–183°, $[M]^+$ at 302 2245 $C_{20}H_{30}O_2$ requires 302 2244, ^{1}H NMR (200 MHz) δ 0.92, 0 98 and 1 13 (each 3H, *s*), 2.91 (1H, *d*, $J = 4$ Hz, H-7), 3.08 (1H, *dd*, $J = 2$ 5 and 4 Hz, H-6), 3 24 (1H, *t*, H-3), 4 84 (2H, *br s*, H-17), EIMS *m/z* (rel int.) 302 $[M]^+$ (3), 287 (6), 284 (5), 269 (13), 243 (7), 241 (10), 236 (9), 227 (8), 199 (8), 189 (15)

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