fused silica capillary column (25 m × 0.32 mm CP sil 5; Chrompack, Middelburg, The Netherlands) was used; temp. program 60–300° at 6°/min; carrier gas helium; 70 eV and a cycle time during acquisition of mass spectra: 1 sec and by direct inlet procedure.

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THE MICROBIOLOGICAL TRANSFORMATION OF CANDIDIOL, *ENT*-15β,18-DIHYDROXY-KAUR-16-ENE, BY *GIBBERELLA FUJIKUROI*

BRAULIO M. FRAGA, PEDRO GONZÁLEZ*, MELCHOR G. HERNANDEZ, FERNANDO G. TELLADO and AUREA PERALES†

Instituto de Productos Naturales Orgánicos, C.S.I.C., La Laguna, Tenerife, Canary Islands, Spain; *Departamento de Química Orgánica, Universidad de La Laguna, Tenerife, Spain; †Departamento de Rayos X, Instituto Rocasolano, C.S.I.C., Serrano 119, 28006-Madrid, Spain

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Key Word Index—*Gibberella fujikuroi*; microbiological transformation; diterpenes; candidiol; *ent*-kaur-16-ene derivatives.

Abstract—Incubation of candidiol, *ent*-15β,18-dihydroxy-kaur-16-ene, with *Gibberella fujikuroi* affords *ent*-11α,15β,18-trihydroxy-kaur-16-ene. Its structure was determined by X-ray analysis.

The fungus *Gibberella fujikuroi* produces a series of metabolites such as the gibberellins and the kaurenolide lactones, which derive biogenetically from the diterpene *ent*-kaur-16-ene [1]. This fungus is able to handle some natural and synthetic diterpenoids. Thus the microbiological transformation of *ent*-kaur-16-ene derivatives hydroxylated at different positions has been carried out [2]. In contrast to these investigations the metabolism of 15α-hydroxylated *ent*-kaurenes has been little studied. *ent*-15β-Hydroxy-kaur-16-en-19-oic acid has been incubated with *G. fujikuroi* and the products obtained tentatively identified as 7β,15α-dihydroxykaurenolide, GA_{14} 7,15-lactone and a hydroxy GA_{14} 7,15-lactone [3]. We now describe the results of incubation with this fungus of candidiol, *ent*-15β,18-dihydroxy-kaur-16-ene (**1**), a diterpene isolated from *Sideritis candicans* [4], and also synthesized from *ent*-18-hydroxy-kaur-15-ene [5].

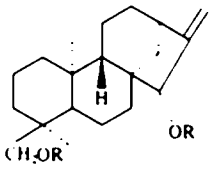
The fermentation was carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene without perturbing the post-kaurene metabolism [6, 7], thus facilitating the analysis of the products formed. Candidiol (**1**) was incubated with *G. fujikuroi*, the fermentation harvested after 6 days, and the combined broth and mycelium extracts separated into neutral and acidic fractions.

Chromatography of the neutral fraction afforded a triol, $C_{20}H_{32}O_3$ (**3**). Its ^{1}H NMR spectrum showed resonances for two methyl groups and one hydroxymethylene group, the two protons of an exocyclic double bond and the geminal hydrogens to two secondary hydroxyl groups. This spectrum was very similar to that of candidiol (**1**) [4, 5], the difference being the appearance of a geminal hydrogen to a new alcoholic group. This compound formed a triacetate (**4**). Its ^{1}H NMR spectrum in $CDCl_3$ showed the geminal proton to one of the acetates overlapped with one of the olefinic hydrogens, but in C_6D_6 a doublet centred at δ 5.13 was observed. The form of this signal indicated that C-14 was a possible place for this new function, but the ^{13}C NMR spectrum was more in accordance with an alcoholic group at C-11 (Table 1). To solve this ambiguity we submitted the alcohol to an X-ray analysis. In this way the structure of *ent*-11α,15β,18-trihydroxy-kaur-16-ene was assigned to this new metabolite.

The crystal structure of **3** consists of discrete molecules, one of which is shown in Fig. 1. Rings A, B and C have

Table 1. ^{13}C NMR spectral data of compounds 1, 2 and 4 (50 MHz)

Carbon	1	2	4	Carbon	1	2	4
1	40.16	39.93	39.73	11	18.24	18.13	68.83
2	18.13	17.94	17.76	12	32.96	33.01	39.51
3	35.42	35.85	35.62	13	42.56	42.72	40.91
4	37.70	36.63	36.68	14	36.55	34.47	33.98
5	49.41	49.84	49.57	15	83.14	83.35	82.43
6	19.32	19.35	19.06	16	160.55	155.75	155.48
7	35.00	37.42	36.81	17	108.37	110.11	108.37
8	47.79	47.42	46.60	18	72.37	72.98	72.71
9	54.43	53.96	60.29	19	17.70	17.65	17.71
10	39.50	39.60	38.57	20	18.32	18.13	18.09



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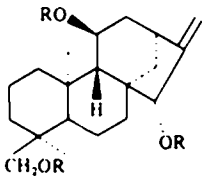
2 R = Ac



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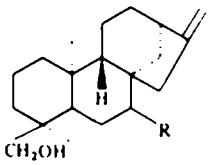
4 R = Ac



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6 R = OH

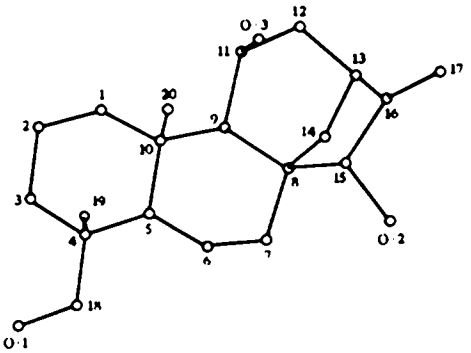

Fig. 1. Molecular structure of 11β,15α,18-trihydroxy-*ent*-kaur-16-ene (3).

chair conformations and ring D has an envelope conformation. The bond distances and angles are of normal value. The hydroxyl at C-11 has the β-configuration, while at C-15 there is the α-configuration.

It is interesting to note that in this microbiological transformation of candidiol (1) no hydroxylation occurs at C-19 in contrast to the normal gibberellin biosynthetic pathway after formation of *ent*-kaur-16-ene. We know that this inhibition is not due to the presence in candidiol (1) of the 18-hydroxyl group, because candol B (5) [8] or epicandicandiol (6) [9] is hydroxylated and oxidized at C-19 by *G. fujikuroi*. Thus this inhibition must be produced by the 15α-hydroxyl group present in candidiol (1). We have shown that an equatorial alcohol group at C-3 in an *ent*-kaur-16-ene skeleton also blocks hydroxylation at C-19 [10]. In this case it seems likely that the inhibition of reaction at this carbon was due to the proximity of the 3α-hydroxyl group, but in candidiol the 15α-hydroxyl is too far for a similar effect to be considered.

No metabolites have been isolated to date from *G. fujikuroi* with an 11β-hydroxyl group, but the structure of 7β,11α-dihydroxykaurenolide has been assigned to a compound isolated from the strain TP70 of this fungus [11].

EXPERIMENTAL

Incubation experiments. Gibberella fujikuroi (ACC 917), inhibited with 5×10^{-5} AMO 1618, was grown in shake culture at 25° for 1 day in 82 conical flasks (250 ml), each containing sterile medium (50 ml). Candidiol (1, 260 mg) in EtOH (16 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 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 separated into acidic and neutral fractions with $NaHCO_3$. The neutral fraction was chromatographed on silica gel. Elution with mixtures of petrol–EtOAc gave candidiol (1, 125 mg), an unidentified compound (6 mg) and *ent*-11α,15β,18-trihydroxy-kaur-16-ene (3, 45 mg). The acidic fraction was methylated with CH_2N_2, but in the chromatography of the residue no acidic kaurene derivatives of gibberellins were detected.

ent-11α,15β,18-*Trihydroxy-kaur*-16-*ene* (3). Mp 212–215° (from petrol–EtOAc). $[M-H_2O]^+$ 302.2239. $C_{20}H_{30}O_2$ requires 302.2246. ^1H NMR (60 MHz, pyridine-d_5): δ 0.85 and 1.02 (each 3H, *s*), 2.80 (1H, *br s*), 3.31 and 3.68 (each 1H, *d*, *J* = 11 Hz), 4.20 (1H, *br s*), 4.95 (1H, *br s*), 5.32 and 5.48 (each 1H, *s*); MS *m/z* (rel. int.): 302 $[M-H_2O]^+$ (26), 287 (10), 284 (8), 271 (69), 253 (18),

239 (8), 213 (9), 187 (13), 175 (14), 161 (15), 148 (36). *Triacetate* (4), a gum [M − AcOH]$^+$ 386.2432. $C_{24}H_{34}O_6$ requires 386.2457; ^{1}H NMR (200 MHz, $CDCl_3$): δ0.80 and 0.99 (each 3H, *s*), 1.93, 2.05 and 2.06 (each 3H, *s*), 2.74 (1H, *br s*, H-13), 3.60 and 3.83 (each 1H, *d*, *J* = 11 Hz, H-18), 5.05 (3H, complex signal, H-11 and H-17) and 5.61 (1H, *s*, H-15); ^{1}H NMR (200 MHz, pyridine-d_5): δ0.64 and 0.66 (each 3H, *s*), 1.67, 1.70 and 1.78 (each 3H, *s*), 2.49 (1H, *br s*, H-13), 3.66 and 3.87 (each 1H, *d*, *J* = 11 Hz, H-18), 5.04 and 5.25 (each 1H, *s*, H-17), 5.13 (1H, *d*, H-11), and 5.94 (1H, *s*, H-15); MS *m/z* (real. int.): 386 [M − 60]$^+$ (3), 362 (1), 344 (5), 326 (5), 311 (7), 284 (3), 253 (6), 223 (7), 149 (93).

Crystal data. Compound **3**, $C_{30}H_{32}O_3$, crystallized in the space group $P2_1$, with a = 11.1696(10), b = 12.681(2), c = 6.0806(4)Å, β = 95.669(8)°, Z = 2.

The intensities of 2950 independent Friedel pairs to θ = 65° were alternately collected on an automatic four-circle diffractometer. The size of a single crystal used was 0.2 × 0.3 × 0.35 mm, and during the experiment no decomposition was observed. The experimental details are: graphite monochromated CuKα radiation (λ = 1.5418 Å), $\omega/2\theta$ scan mode, 1.50 scan width, 0.05 sec/g scan speed, with the same measurement time for both backgrounds as for the peak. The intensities were corrected by Lorentz and polarization effects, and 2765 Friedel pairs were considered as observed when $I > 2\sigma(I)$, and were used for the structure determination and refinement. No absorption correction was applied (μ = 6.027 cm^{-1}). The atomic scattering factors and the anomalous dispersion corrections were taken from the literature [12]. The structure was solved by direct methods [13], and refined by full-matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms. All the H-atoms were found in difference Fourier maps, and were included as fixed isotropic contributors in the refinement. Convenient weighting [14] scheme was selected to obtain flat dependence of $\langle w\Delta^2F \rangle$ vs. $\langle Fo \rangle$ and vs. $\langle \sin\theta/\lambda \rangle$; afterwards several cycles of weighted anisotropic refinement, including both hkl and $\overline{hkl}$, gave the following unweighted and weighted discrepancy indices: R = 4.7%, and R_w = 5.6%.

The absolute configuration [15] was determined comparing the 28 more relevant Bijvoet pairs with Fc > 0.070 and with less experimental error that is Fo > 10σ(Fo). The averaged Bijvoet difference was 0.166 for the right enantiomer vs. 0.202 for the wrong one.

Final atom coordinates, list of temperature factors, hydrogen atom positions, and final structure factors have been deposited at the Cambridge Crystallographic Data Centre.

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