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THE MICROBIOLOGICAL TRANSFORMATION OF SOME TRACHYLOBANE DITERPENOIDS BY *GIBBERELLA FUJIKUROI*

CARMEN E. DIAZ, BRAULIO M. FRAGA, ANTONIO G. GONZALEZ, PEDRO GONZALEZ, JAMES R. HANSON* and MELCHOR G. HERNANDEZ

Instituto de Productos Naturales Organicos, C.S.I.C. and Departmento de Química Orgánica, Universidad La Laguna, Tenerife, Canary Islands, Spain; *School of Molecular Sciences, University of Sussex, Brighton, BN1 9QJ, U.K.

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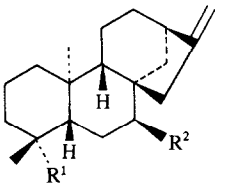
Key Word Index—*Gibberella fujikuroi*; gibberellins; *ent*-trachylobane; trachylobagibberellins.

Abstract—The microbiological transformation of *ent*-trachylobane, *ent*-7α-hydroxytrachylobane and *ent*-19-hydroxytrachylobane into trachylobagibberellins A_7, A_9, A_{13}, A_{25}, A_{40} and A_{47} by *Gibberella fujikuroi* is described. Whereas 7β-hydroxy- and 7β,18-dihydroxytrachylobanolides were obtained from *ent*-trachylobane and *ent*-trachyloban-19-ol, the presence of a 7β-hydroxyl group directed metabolism exclusively into the gibberellin pathway. An 18-hydroxyl group as in *ent*-7α,18-dihydroxytrachylobane inhibited oxidation at C-6 affording *ent*-7α,18,19-trihydroxytrachylobane as the major metabolite.

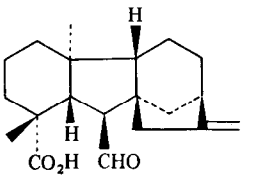
INTRODUCTION

The diterpenoid hydrocarbon *ent*-kaurene (**1**) is the parent hydrocarbon of the gibberellins which are produced by *Gibberella fujikuroi* [1]. The biosynthetic pathway [2] involves oxidation of C-19 followed by hydroxylation at C-7 to afford compound **2** and contraction of ring B. The product, gibberellin A_{12} 7-aldehyde (**3**), may either be hydroxylated at C-3 or further oxidized to gibberellin A_{12}. Subsequent oxidative removal of C-20 leads to the C_{19}-gibberellins of either the 3-hydroxy- or 3-desoxy series. Hydroxylation at C-13 is a late stage in this pathway. Despite the occurrence of several structurally similar families of polycyclic diterpenoids related to *ent*-kaur-16-ene, the naturally-occurring gibberellin plant hormones which have been isolated so far [3] belong to the *ent*-kaurene series. A number of pentacyclic diterpenoids are known which possess the *ent*-trachylobane skeleton [4–7]. Apart from hydroxylation at C-13, nothing mechanistic is known about the biosynthesis of gibberellins which would preclude their transformation along the pathway. Indeed, some of the known compounds possess hydroxylation patterns reminiscent of gibberellin intermediates. Hence the transformation of some *ent*-trachylobane diterpenoids (**4**–**7**) has been examined in the context of our studies on the substrate specificity of the gibberellin biosynthetic pathway [2, 8–14]. The conversion of *ent*-trachylobanic acid (**8**) into pentacyclic analogues of the gibberellins by a mutant of the fungus, *G. fujikuroi*, which is blocked for gibberellin biosynthesis between *ent*-kaur-16-en-19-al and *ent*-kaur-16-en-19-oic acid, has been reported [15, 16] and reveals the lack of structure specificity in the formation of the gibberellins once the 19-carboxylic acid is formed. In this work, the trachyloba-(12,16-cyclo)-gibberellins A_4, A_9, A_{12}, A_{13}, A_{14}, A_{25}, A_{40}, A_{47} and 7β-hydroxytrachylobanolide were detected by GC/MS and the A_9 and A_{12} derivatives were isolated from larger scale fermentations. However, the enzyme systems responsible for the immediate oxidation of *ent*-kaur-16-ene show some features of structure specificity. Thus *ent*-kaur-15-ene accumulates in the gibberellin-deficient d-5 mutant of maize [17] whilst *ent*-16,17-epoxykaurane displaces *ent*-kaur-16-ene and inhibits its oxidative metabolism by *Gibberella fujikuroi* [18]. Consequently, we have examined the metabolism by *G. fujikuroi*, of the hydrocarbon, *ent*-trachylobane (**4**), *ent*-trachyloban-19-ol (**5**), *ent*-trachyloban-7α-ol (trachinol) (**6**) and *ent*-trachyloban-7α,18-diol (trachinodiol) (**7**) [19].



1 $R^1 = Me$, $R^2 = H$

2 $R^1 = CO_2H$, $R^2 = OH$



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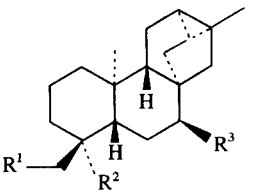


4 $R^1 = R^3 = H$, $R^2 = Me$

5 $R^1 = R^3 = H$, $R^2 = CH_2OH$

6 $R^1 = H$, $R^2 = Me$, $R^3 = OH$

7 $R^1 = R^3 = OH$, $R^2 = Me$

8 $R^1 = R^3 = H$, $R^2 = CO_2H$

17 $R^1 = R^3 = OH$, $R^2 = CH_2OH$

18 $R^1 = R^3 = OAc$, $R^2 = CH_2OAc$

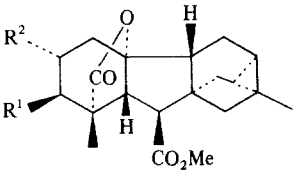
RESULTS

The fermentations were carried out in the presence of the *ent*-kaur-16-ene biosynthesis inhibitor AMO 1618 to suppress the formation of the normal metabolites and facilitate the analysis of the products [20, 21]. The substrates were incubated with *G. fujikuroi* for 6 days, the fermentations were harvested, and the broth extracts separated into acidic and neutral fractions. The acidic fractions were methylated with diazomethane and purified as their methyl esters.

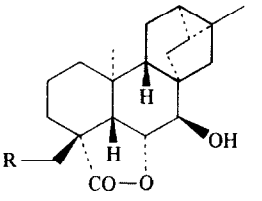
The *ent*-trachylobane transformation was relatively inefficient and the only acidic metabolite to be isolated in sufficient quantity for identification was trachylobagibberellin A_{40} (**9**). The ^{1}H NMR spectrum showed a CH(OH) signal as a triplet, δ4.26 (J = 4 Hz). The 5-H and 6-H signals were co-incident (δ2.49) whilst the cyclopropane signals (H-12 and H-13, δ0.6 and 0.9) showed little perturbation [23] which might be assigned to adjacent hydroxylation, thus suggesting that the hydroxyl group was located on the α-face of ring A. Structure **9** was confirmed by an X-ray analysis of the methyl ester [14]. The neutral fraction furnished 7β-hydroxytrachylobanolide (**10**) ($[M]^+$ at m/z 316.201) and 7β,18-dihydroxytrachylobanolide (**11**) ($[M]^+$ at m/z 332.196). The IR spectra of these compounds contained hydroxyl and γ-lactone absorption (3600 and 1760 cm^{-1}) whilst the ^{1}H NMR spectra contained signals (δ4.09, 1H, *d*, J = 5 Hz, H-7; 4.60, 1H, *dd*, J = 5 and 7 Hz, H-6) characteristic of the vicinal oxygenation of ring B found in the kaurenolides [23]. Whereas the ^{1}H NMR spectrum of 7β-hydroxytrachylobanolide showed three methyl group resonances (δ0.84, 1.18 and 1.22, all *s*), that of 7β,18-dihydroxytrachylobanolide possessed only two (δ0.92 and 1.17) and a CH_2OH signal (δ3.66).

ent-Trachyloban-19-ol (**5**) was more efficiently transformed to a range of *ent*-trachylobagibberellins. Trachylobagibberellin A_{25} trimethyl ester (**12**) ($[M]^+$ at m/z 404.221) showed ^{1}H NMR signals at δ1.09 and 1.13 (each 3H, *s*) and three methyl ester signals (δ3.63, 3.67 and 3.72). The mass spectrum contained significant ions at m/z 404, 372, 344, 285 and 225, which are similar to those recorded for gibberellin A_{25} trimethylester [24]. Trachylobagibberellin A_9 methyl ester (**13**) ($[M]^+$ at m/z 330.187) showed ^{1}H NMR signals at δ1.10, 1.18 and 3.70 (each 3H, *s*) and the typical gibberellin H-5:H-6 double doublet (δ2.40 and 2.55, J = 8 Hz). The smaller value of this coupling constant in the trachylobagibberellins has been noted previously [15, 16]. Trachylobagibberellin A_7 methyl ester (**14**) ($[M]^+$ at m/z 344.165) possessed ^{1}H NMR signals at δ1.20, 1.28 and 3.73 (each 3H, *s*) and the characteristic gibberellin H-5:H-6 double doublet (δ2.56 and 3.09, J = 8 Hz). The ring A substitution pattern followed from the presence of signals at δ4.17 (1H, *d*, J = 4 Hz, H-3), 5.88 (1H, *dd*, J = 4 and 10 Hz, H-2) and 6.35 (1H, *d*, J = 10 Hz, H-1). The marked separation of the olefinic resonances is a characteristic of a Δ^1-rather than a Δ^2-double bond [25]. Trachylobagibberellin A_{40} methyl ester (**9**) was also isolated whilst two minor components were assigned the structures of trachylobagibberellin A_{13} trimethyl ester (**15**) and trachylobagibberellin A_{47} methyl ester (**16**) on the basis of their mass spectral data [26, 27]. The trachylobanolides **10** and **11** were isolated from the neutral fraction.

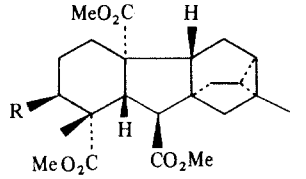
As would be expected from our previous work [11] with *ent*-7α-hydroxykaur-16-ene, *ent*-7α-hydroxytrachylobane (trachinol) (**6**) gave a comparable range of trachylobagibberellins, including trachylobagibberellins A_{25} (**12**), A_9 (**13**), A_7 (**14**), A_{40} (**9**) and A_{47} (**19**). However, the neutral fraction did not contain any trachylobanolides. Finally, incubation of *ent*-7α,18-dihydroxytrachylobane (trachinodiol) (**7**) followed the pattern of *ent*-7α,18-dihydroxykaur-16-ene (epicandicandiol) [9] in that only oxidation at C-19 took place. The major metabolite was assigned the structure *ent*-7α,18,19-trihydroxytrachylobane (**17**). On acetylation, it gave a triacetate (**18**). The ^{1}H NMR spectrum (determined in pyridine-d_5) contained two C–Me resonances (δ1.07 and 1.20) and two pairs of



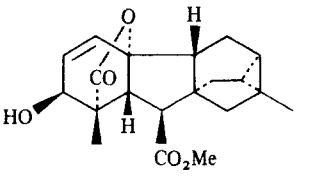
9 R^1 = H, R^2 = OH
13 R^1 = R^2 = H
16 R^1 = R^2 = OH



10 R = H
11 R = OH



12 R = H
15 R = OH



14

AB double-doublets (J = 11 Hz) at δ4.03 and 4.23, and 3.94 and 4.25. There was a broad CH(OH) signal at δ3.74. In the triacetate (**18**), these signals (determined in $CDCl_3$) appeared at δ3.79 and 3.90 (J = 11 Hz), 3.98 and 4.19 (J = 12 Hz) and 4.68.

DISCUSSION

In conclusion, we have shown that the fungal gibberellin pathway is able to handle abnormal but naturally-occurring diterpenoid hydrocarbons. There is thus the possibility that trachyloba-(12,16-cyclo)-gibberellins might be found to occur naturally. However, it is interesting to note that the dominant 2α-hydroxylation differs from the more common 3β-hydroxylation found in the natural gibberellins of *G. fujikuroi*. Indeed, the major metabolites are characteristic of the 3-desoxygibberellin pathway which proceeds via gibberellin A_{12}. The other feature which is comparable to the kaurene series is the inhibitory action of an 18-hydroxyl group on the oxidative ring contraction and the effect of a 7β-hydroxyl group in directing metabolism along the gibberellin pathway and not to the trachylobanolides. This can be rationalized in terms of the requirement for a Δ^6-ene in the formation of the lactone ring.

EXPERIMENTAL

Incubation experiments. Gibberella fujikuroi (ACC 917) inhibited with 5×10^{-5} M AMO 1618, was grown in shake culture at 25° for 1–2 days in 80–100 conical flasks (250 ml) each containing sterile medium (50 ml). The substrate (see below) in EtOH (16–20 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 extract was separated into acidic and neutral fractions with $NaHCO_3$. The acidic fraction was methylated with CH_2N_2. The fractions were chromatographed on silica gel in petrol–EtOAc.

ent-Trachylobane (**4**) (400 mg) gave trachylobagibberellin A_{40} (**9**) (16 mg) in the acidic fraction and 7β-hydroxytrachylobanolide (**10**) (5 mg) and 7β,18-dihydroxytrachylobanolide (**11**) (11 mg).

ent-Trachyloban-19-ol (**5**) (307 mg) gave trachylobagibberellin A_{25} (**12**) (16 mg), trachylobagibberellin A_9 (**13**) (15 mg), trachylobagibberellin A_{13} (**15**) (6 mg), trachylobagibberellin A_7 (**14**) (4 mg), trachylobagibberellin A_{40} (**9**) (18 mg) and trachylobagibberellin A_{47} (**16**) (6 mg). The neutral fraction contained 7β-hydroxytrachylobanolide (**10**) (15 mg) and 7β,18-dihydroxytrachylobanolide (**11**) (17 mg).

ent-Trachyloban-7α-ol (**6**) (300 mg) gave trachylobagibberellin A_{25} (**12**) (17 mg), trachylobagibberellin A_9 (**13**) (19 mg), trachylobagibberellin A_7 (**14**) (12 mg), trachylobagibberellin A_{40} (**9**) (17 mg) and trachylobagibberellin A_{47} (**16**) (6 mg). The neutral fraction contained the starting material (63 mg).

ent-Trachyloban-7α,18-diol (480 mg) gave *ent*-trachyloban-7,18,19-triol (**17**) (48 mg) and the starting material (180 mg) in the neutral fraction. No pure compound could be obtained from the small acid fraction.

Trachylobagibberellin A_{25} trimethyl ester. Gum ($[M]^+$ at m/z 404.221; calc. for $C_{23}H_{32}O_6$: 404.220); ^{1}H NMR (90 MHz): δ1.09, 1.13, 3.63, 3.67 and 3.72 (each 3H, *s*). EIMS m/z (rel. int.): 404 (3), 372 (15), 344 (20), 312 (34), 285 (22), 225 (23), 181 (20), 163 (100), 149 (74).

Trachylobagibberellin A_9 methyl ester (**13**). Mp 223–225° ($[M]^+$ at m/z 330.187; calc. for $C_{20}H_{26}O_4$: 330.1831); IR ν_{max} cm^{-1}: 1760, 1720; ^{1}H NMR (90 MHz): δ1.10 and 1.18 (each 3H, *s*), 2.40 and 2.55 (each 1H, *d*, J = 8 Hz), 3.70 (3H, *s*) EIMS m/z: 330, 298, 270, 225, 195.

Trachylobagibberellin A_{13} trimethyl ester (**15**). Gum ($[M]^+$ at m/z 420.223; calc. for $C_{23}H_{32}O_7$: 420.215); EIMS m/z (rel. int.): 420 (4), 402 (1), 388 (23), 360 (20), 328 (64), 310 (14), 300 (21), 284 (45), 268 (28), 251 (8), 223 (53).

Trachylobagibberellin A_7 methyl ester (**17**). Mp 197–199° ($[M]^+$ at m/z 344.165; calc. for $C_{20}H_{24}O_5$: 344.162); IR ν_{max} cm^{-1}: 3600, 1760, 1730; ^{1}H NMR (90 MHz): δ1.20 and 1.28 (each 3H, *s*), 2.56 and 3.09 (each 1H, *d*, J = 8 Hz), 3.73 (3H, *s*), 4.17 (1H, *d*, J = 4 Hz), 5.88 (1H, *dd*, J = 4 and 10 Hz), 6.35 (1H, *d*, J = 10 Hz); EIMS m/z (rel. int.): 344 (6), 326 (2), 312 (16), 298 (2), 294 (4), 284 (11), 239 (18), 223 (38), 221 (11), 164 (23).

Trachylobagibberellin A_{40} methyl ester (**9**). Mp 227–229° ($[M]^+$ at m/z 346.175; calc. for $C_{20}H_{26}O_5$: 346.172); ^{1}H NMR (90 MHz): δ1.10 and 1.17 (each 3H, *s*), 2.49 (2H, *s*), 3.69 (3H, *s*), 4.29 (1H, *m*); EIMS m/z (rel. int.): 346 (4), 328 (2), 314 (12), 302 (4), 296 (10), 286 (8), 268 (5), 259 (5), 242 (23), 223 (7), 209 (5), 181 (5), 164 (7).

Trachylobagibberellin A_{47} methyl ester (**16**). Gum ($[M]^+$ at m/z 362.174; calc. for $C_{20}H_{26}O_6$: 362.173); ^{1}H NMR (60 MHz): δ1.19 and 1.26 (each 3H, *s*) 2.50 and 3.10 (each 1H, *d*, J = 8 Hz), 3.71 (3H, *s*), 4.05 (2H, *m*); EIMS m/z (rel. int.): 362 (13), 344 (10), 330 (93), 312 (46), 302 (62), 300 (13), 284 (49), 241 (82), 197 (21), 164 (70).

ent-6β,7α-*Dihydroxytrachyloban*-19-*oic acid* 19-6β-*lactone* (7β-*hydroxytrachylobanolide*) (**10**). Mp 252–254° ($[M]^+$ at m/z 316.201; calc. for $C_{20}H_{28}O_3$: 316.204); IR ν_{max} cm^{-1}: 3600, 1760; ^{1}H NMR (200 MHz): δ0.84, 1.18 and 1.22 (each 3H, *s*), 4.09 (1H, *d*, J = 5 Hz), 4.60 (1H, *dd*, J = 5 and 7 Hz); EIMS m/z (rel. int.): 316 (2), 298 (6), 283 (3), 255 (5), 239 (5), 223 (4), 183 (16), 165 (50), 137 (79).

ent-6β,7α,18-*Trihydroxytrachyloban*-19-*oic acid* 19-6β-*lactone* (7β,18-*dihydroxytrachylobanolide*) (**11**). Mp 230–232° ($[M]^+$ at m/z 332.196; calc. for $C_{20}H_{28}O_4$: 332.199); IR ν_{max} cm^{-1}: 3600, 2980, 1760; ^{1}H NMR (200 MHz): δ0.92 and 1.17 (each 3H, *s*), 3.66 (2H, *s*), 4.06 (1H, *d*, J = 6 Hz), 4.78 (1H, *dd*, J = 6 and 7 Hz); EIMS m/z (rel. int.): 332 (1), 314 (1), 296 (2), 284 (3), 271 (1), 169 (1), 268 (1), 255 (2), 251 (2), 239 (2), 237 (2), 223 (4), 209 (13), 203 (6), 191 (8), 181 (16).

ent-7α,18,19-*Trihydroxytrachylobane* (**17**). Mp 208–210° ($[M-H_2O]^+$ at m/z 302.221; calc. for $C_{20}H_{30}O_2$: 302.219); ^{1}H NMR (pyridine-d_5 at 200 MHz): δ1.07 and 1.20 (each 3H, *s*), 3.74 (1H, *br s*), 4.03 and 4.23 (each 1H, *d*, J = 11 Hz), 3.94 and 4.25 (each 1H, *d*, J = 11 Hz); EIMS m/z (rel. int.): 302 (52), 284 (13), 272 (10), 271 (12), 253 (32) 241 (12), 239 (13), 197 (15), 185 (22).

The triacetate, prepared with Ac_2O in pyridine, had mp 163–165° ($[M-HOAc]^+$ at m/z 386.248; calc. for $C_{24}H_{34}O_4$: 386.246); ^{1}H NMR (200 MHz): δ0.95 and 1.09 (each 3H, *s*), 2.00 (3H, *s*), 2.01 (6H, *s*), 3.79 and 3.90 (each 1H, *d*, J = 11 Hz), 3.98 and 4.19 (each 1H, *d*, J = 12 Hz), 4.68 (1H, *br s*); EIMS m/z (rel. int.): 386 (27), 326 (7), 266 (22), 251 (13).

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