

## THE MICROBIOLOGICAL TRANSFORMATION OF SOME *ENT-13-EPI-MANOYL OXIDE* DITERPENES BY *GIBBERELLA FUJIKUROI*

BRAULIO M. FRAGA, PEDRO GONZÁLEZ,\* RICARDO GUILLERMO, MELCHOR G. HERNÁNDEZ and JUANA ROVIROSA

Instituto de Productos Naturales Orgánicos, C.S.I.C., La Laguna, 38206-Tenerife, Canary Islands, Spain; \*Departamento de Química Orgánica, Universidad de La Laguna, Spain

(Received 11 November 1988)

**Key Word Index:** *Gibberella fujikuroi*; microbiological transformation; diterpenes; *ent-13-epi-manoyl oxide* derivatives; varol; varodiol.

**Abstract**—Incubation of *ent-19-hydroxy-13-epi-manoyl oxide* with *Gibberella fujikuroi* afforded *ent-12 $\alpha$ ,19-dihydroxy-13-epi-manoyl oxide* and *ent-7 $\beta$ ,12 $\alpha$ ,19-trihydroxy-13-epi-manoyl oxide*, the first of which was transformed by chemical methods into varol (*ent-12 $\alpha$ -hydroxy-13-epi-manoyl oxide*). The incubation of *ent-3 $\beta$ -hydroxy-13-epi-manoyl oxide* (ribenol) with the same fungus gave the 12 $\beta$ -hydroxy, the 11 $\beta$ -hydroxy and the 11 $\beta$ ,12 $\beta$ -dihydroxy derivatives of ribenol, the first of which was identical with varodiol.

### INTRODUCTION

The gibberellins and the kaurenolides, isolated from the fungus *Gibberella fujikuroi*, are diterpenoid metabolites derived from *ent-kaur-16-ene* (1) [1]. During the past year we have been interested in the study of the microbiological transformations of natural and synthetic diterpenoids by this fungus. These studies have been directed to preparing modified gibberellins and to obtaining information on the substrate requirements of the enzymes involved in several steps of the biosynthesis of the gibberellins and kaurenolides. The results obtained in the incubations of *ent-kaurene* derivatives and other tetracyclic diterpenes have been reported [2-4].

### RESULTS AND DISCUSSION

The fungus *G. fujikuroi* produces *ent-13-epi-manoyl oxide* (5), a labdane diterpene which seems to be a final metabolite of a biosynthetic branch [5]. Its 19-hydroxy derivative (6) has been obtained from *Baccharis tola*, a plant endemic to Chile [6, 7]. This compound possesses a hydroxyl group in the same position as *ent-19-hydroxykaur-16-ene* (2), an intermediate in the biosynthesis of gibberellins. Thus, we thought it would be interesting to know if the alcohol 6 could be transformed by this fungus.

The results obtained in the feeding indicated that the alcohol 6 was not oxidized to the corresponding aldehyde, and then to the acid, as occurs with the *ent-kaurene* analogue 2, which is transformed into 3, and then to 4, in the biosynthetic pathway of gibberellins [1]. However, a series of interesting hydroxylations on the molecule of 6, did occur as described below.

The microbiological transformations were carried out in the presence of the *ent-kaur-16-ene* biosynthesis inhibitor, AMO 1618, to suppress the formation of the normal metabolites, thus facilitating the analysis of the products formed [8, 9]. The fermentations were harvested after eight days, and the broth and mycelium extracts

were mixed and separated into neutral and acidic fractions. The incubations of *ent-19-hydroxy-13-epi-manoyl oxide* (6) gave the 12 $\beta$ -hydroxy derivative 7 and the 7 $\alpha$ , 12 $\beta$ -dihydroxy derivative 15. These metabolites were identified as follows.

The less polar compound isolated has the empirical formula C<sub>20</sub>H<sub>34</sub>O<sub>3</sub>. Its <sup>1</sup>H NMR spectrum, when compared with that of the starting material 6, showed the resonance of a new geminal proton to a hydroxyl group at  $\delta$ 4.08, as a broad singlet. The form of this signal is characteristic of an equatorial hydrogen at C-1, C-3, C-7 or C-12. In this spectrum the resonances of the hydrogens of the double bond also appear modified in comparison with those of 6. Thus, considering that the conformation of ring C in these compounds is of the boat type, we assigned the location of the new alcoholic group to C-12( $\beta$ ). This was confirmed by the <sup>13</sup>C NMR spectrum, whose assignments are given in Table 1. The structure of this metabolite is therefore *ent-12 $\alpha$ ,19-dihydroxy-13-epi-manoyl oxide* (7).

The second product isolated from the fermentation showed a <sup>1</sup>H NMR spectrum similar to that of the less polar 7. The chemical shift and the form of resonance of the geminal hydrogen to the alcohol at C-12, were identical with those of 7, but the geminal hydrogen to a new alcohol appeared at  $\delta$ 3.55, as a doublet ( $J = 11$  and 4.5 Hz), indicating the presence of another equatorial hydroxyl. Thus, this function can be at C-1, C-3 or C-7. The study of its <sup>13</sup>C NMR spectrum (Table 1) led us to choose the C-7 position. Therefore the structure of *ent-7 $\beta$ ,12 $\alpha$ ,19-trihydroxy-13-epi-manoyl oxide* (15) was assigned to this compound.

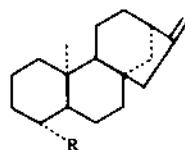
The formation of the diol 7 in the feeding prompted us to transform it by chemical procedures into varol (14), a labdane diterpene isolated from *Sideritis varoi* [10, 11]. Acetylation of 7 in the usual way gave the corresponding diacetate 8. Partial hydrolysis of 8 afforded a mixture of the monoacetates 9 and 10, inseparable by silica gel chromatography. Treatment of this mixture with triphenylphosphine-carbon tetrachloride gave the chloro-deriva-

tives 11 and 12. The 19-chloride 12 was reduced with tri-*n*-butyltin hydride to give compound 13. Hydrolysis of this with a saturated solution of sodium carbonate in methanol gave 14, identical with varol [10]. The enantiomer of 14 has been isolated from Turkish tobacco [12].

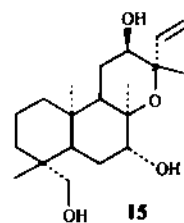
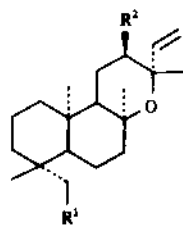
Continuing with this work we decided to incubate with the fungus the diterpene ribenol (16), a compound isolated from *Sideritis canariensis* [13] and *S. varoi* [10, 11], because it also possesses an *ent*-13-*epi*-manoyl oxide skeleton and a hydroxyl group at C-3( $\alpha$ ), a spatial position similar to that of a C-19 hydroxyl. Thus, we thought that it could also be transformed into a 12 $\beta$ -hydroxy derivative such as 17, a compound also isolated from *S. varoi* and named varodiol.

In fact, in the fermentation of ribenol (16) with *G. fujikuroi* we obtained varodiol (*ent*-3 $\beta$ ,12 $\alpha$ -dihydroxy-13-*epi*-manoyl oxide) (17) in addition to a further two compounds, 18 and 19. Varodiol (17) was identified by its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, and by comparison with those obtained from an authentic sample [10]. The structures of compounds 18 and 19 were determined on the basis of the following data.

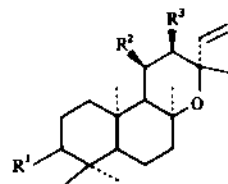
The  $^1\text{H}$  NMR spectrum of substance 18 showed the geminal proton to C-3, and a further one at  $\delta$ 4.13 as a broad multiplet ( $W_{1/2} = 26$  Hz) indicative of an equatorial alcohol at C-6 or C-11. The last position was assigned considering its  $^{13}\text{C}$  NMR spectrum, and comparing it with that of ribenol (16) (Table 1). Therefore the structure of this compound is *ent*-3 $\beta$ ,11 $\alpha$ -dihydroxy-13-*epi*-manoyl oxide (18). The most polar compound (19) showed in its  $^1\text{H}$  NMR spectrum the geminal hydrogen to the hydroxyl group at C-3, and further geminal protons to another two functions of this type at  $\delta$ 4.00 (*d*,  $J = 4.5$  Hz) and 4.13 (*dd*,  $J = 9.4$  and 4.5 Hz), which are coupled. The  $^{13}\text{C}$  NMR spectrum of this compound compared to that of 17 indicated that the vicinal hydroxyl groups are on ring C at C-11 and C-12. In accordance



- 1 R = Me
- 2 R = CH<sub>2</sub>OH
- 3 R = CHO
- 4 R = CO<sub>2</sub>H



- 5 R<sup>1</sup> = R<sup>2</sup> = H
- 6 R<sup>1</sup> = OH, R<sup>2</sup> = H
- 7 R<sup>1</sup> = R<sup>2</sup> = OH
- 8 R<sup>1</sup> = R<sup>2</sup> = OAc
- 9 R<sup>1</sup> = OAc, R<sup>2</sup> = OH
- 10 R<sup>1</sup> = OH, R<sup>2</sup> = OAc
- 11 R<sup>1</sup> = OAc, R<sup>2</sup> = Cl
- 12 R<sup>1</sup> = Cl, R<sup>2</sup> = OAc
- 13 R<sup>1</sup> = H, R<sup>2</sup> = OAc
- 14 R<sup>1</sup> = H, R<sup>2</sup> = OH



- 16 R<sup>1</sup> = OH, R<sup>2</sup> = R<sup>3</sup> = H
- 17 R<sup>1</sup> = R<sup>3</sup> = OH, R<sup>2</sup> = H
- 18 R<sup>1</sup> = R<sup>2</sup> = OH, R<sup>3</sup> = H
- 19 R<sup>1</sup> = R<sup>2</sup> = R<sup>3</sup> = OH

Table 1.  $^{13}\text{C}$  NMR (50.3 MHz) spectral data for compounds 6, 7, 14–19

13C	6	7	14	15	16*	17	18	19
1	39.58	39.21	39.08	39.12	37.81	37.45	39.51	38.86
2	18.38	18.25	18.54	18.28	27.42	27.34	27.52	27.42
3	35.92	35.91	42.19	35.97	78.91	78.96	78.63	78.70
4	38.75	38.71	33.34	38.71	38.93	39.00	39.31	39.23
5	57.22	57.26	56.68	55.03	55.39	55.53	55.53	55.46
6	20.30	20.35	20.03	27.40	19.51	19.75	19.64	19.81
7	43.58	43.22	42.87	80.91	43.00	42.83	40.70	43.68
8	76.01	76.18	76.36	79.56	75.92	76.20	76.39	76.25
9	58.74	49.48	49.22	47.88	58.42	49.74	63.24	58.70
10	36.86	36.40	36.50	36.72	36.25	36.27	38.41	38.08
11	16.15	23.84	23.72	23.50	16.01	23.85	65.63	66.75
12	34.92	69.15	69.22	69.10	34.96	69.21	44.47	71.85
13	73.47	76.23	75.93	†	73.35	76.30	73.87	75.57
14	147.74	147.27	147.33	146.87	147.45	147.25	147.82	146.19
15	109.71	110.74	110.68	111.21	109.80	110.83	110.17	112.10
16	32.84	27.04	26.96	26.77	32.73	27.09	31.86	27.09
17	23.91	24.28	24.45	18.96	24.13	24.36	25.95	25.03
18	27.04	27.04	33.34	27.16	28.02	28.12	28.31	28.28
19	65.43	65.35	21.29	65.69	15.15	15.30	15.45	15.42
20	16.39	16.31	15.85	16.41	15.97	16.02	16.54	16.57

\*Data from ref. [11].

†Not observed.

with the coupling constants of their geminal protons both alcoholic groups have the  $\beta$ -stereochemistry. Thus the structure of this metabolite was determined as *ent*-3 $\beta$ ,11 $\alpha$ ,12 $\alpha$ -trihydroxy-13-*epi*-manoyl oxide (19). This triol can be formed in the incubation, alternatively, by hydroxylation of 17 or 18.

These feedings indicated that although *ent*-13-*epi*-manoyl oxide (5) may be a final metabolite in *G. fujikuroi*, an increase in polarity, produced by new hydroxyl groups, may lead the fungus to transform these compounds. However, as occurs with compound 5, in these substances the oxidations at C-19 are also inhibited. Another conclusion that can be reached from the incubations with these substrates is that there is a preference for hydroxylation at C-12 ( $\beta$ ).

#### EXPERIMENTAL

Mps: uncorr. IR spectra were taken for solns in  $\text{CHCl}_3$  and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were determined for solns in  $\text{CDCl}_3$ . Silica gel Merck (0.065–0.2 mm) was used for column chromatography.

*Incubation experiments.* *Gibberella fujikuroi* (ACC 917), inhibited with  $5 \times 10^{-5}$  M AMO 1618, was grown in shake cultures at 25° for 1 day in 75 conical flasks (250 ml), each containing sterile medium (50 ml) [14]. The substrate (see below) in EtOH (16–20 ml) was distributed equally among the flasks, and the incubation was allowed to continue for a further 8 days. The broth was filtered, adjusted to pH 2 with diluted HCl and extracted with EtOAc. The mycelium was treated with liquid  $\text{N}_2$ , crushed with a mortar and extracted with EtOAc. The two extracts were combined and separated into acidic and neutral fractions with  $\text{NaHCO}_3$ .

The neutral fraction of the incubation of *ent*-19-hydroxy-13-*epi*-manoyl oxide (6) (275 mg) was chromatographed on silica gel. Elution with mixtures of petrol–EtOAc gave starting material (150 mg), *ent*-12 $\alpha$ ,19-dihydroxy-13-*epi*-manoyl oxide (7) (45 mg), and *ent*-7 $\beta$ ,12 $\alpha$ ,19-trihydroxy-13-*epi*-manoyl oxide (15) (4 mg). The acidic fraction was methylated with  $\text{CH}_2\text{N}_2$ , but in the chromatography of the residue no acidic diterpenes were detected.

The incubation of *ent*-3 $\beta$ -hydroxy-13-*epi*-manoyl oxide (16) (320 mg) gave starting material (190 mg), *ent*-3 $\beta$ ,11 $\alpha$ -dihydroxy-13-*epi*-manoyl oxide (18) (22 mg), *ent*-3 $\beta$ ,12 $\alpha$ -dihydroxy-13-*epi*-manoyl oxide (varodiol) (17) (20 mg) and *ent*-3 $\beta$ ,11 $\alpha$ ,12 $\alpha$ -trihydroxy-13-*epi*-manoyl oxide (19) (15 mg). These compounds were separated by chromatography on silica gel of the neutral fraction, eluting with petrol–EtOAc mixtures. The acidic fraction did not contain diterpenoid metabolites.

*ent*-12 $\alpha$ ,19-Dihydroxy-13-*epi*-manoyl oxide (7). Mp 175–178°. (Found:  $[\text{M}-\text{Me}]^+$  at  $m/z$  307.2239.  $\text{C}_{19}\text{H}_{31}\text{O}_3$  requires  $[\text{M}-\text{Me}]^+$  at 307.2273.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.73, 0.96, 1.19 and 1.22 (each 3H, s), 3.44 and 3.67 (each 1H, d,  $J = 11$  Hz, 19-H), 4.08 (1H, br s, 12-H), 4.95 and 4.97 (each 1H, d,  $J = 18$  and 11 Hz, H-15), 6.06 (1H, dd,  $J = 18$  and 11 Hz, H-14); MS  $m/z$  (rel. int.): 307  $[\text{M}-\text{Me}]^+$  (11), 289 (4), 279 (5), 271 (5), 221 (6), 208 (62), 178 (43), 107 (64).

*ent*-7 $\beta$ ,12 $\alpha$ ,19-Trihydroxy-13-*epi*-manoyl oxide (15). Gum (Found:  $[\text{M}]^+$  at  $m/z$  338.2471.  $\text{C}_{20}\text{H}_{34}\text{O}_4$  requires  $[\text{M}]^+$  at 338.2457.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.75, 0.98, 1.18 and 1.20 (each 3H, s), 3.45 and 3.67 (each 1H, d,  $J = 11$  Hz, H-19), 3.55 (1H, dd,  $J = 11$  and 4.5 Hz, H-7), 4.08 (1H, br s, H-12), 4.96 and 4.99 (each 1H, d,  $J = 18$  and 11 Hz, H-15), 6.03 (1H, dd,  $J = 18$  and 11 Hz, H-14); MS  $m/z$  (rel. int.): 338  $[\text{M}]^+$  (0.2), 323 (1), 287 (1), 267 (5), 250 (3), 231 (2), 224 (3), 207 (7), 189 (6), 163 (11), 123 (29), 109 (71).

*Diacetate 8.* Acetylation of 7 with  $\text{Ac}_2\text{O}$  and pyridine in the usual way gave 8, mp 122–123°. (Found:  $[\text{M}-\text{Me}]^+$  at  $m/z$  391.2487.  $\text{C}_{23}\text{H}_{35}\text{O}_5$  requires  $[\text{M}-\text{Me}]^+$  at 391.2485.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.73, 0.96, 1.10 and 1.23 (each 3H, s), 2.04 and 2.13 (each 3H, s), 4.15 and 3.87 (each 1H, d,  $J = 11$  Hz, H-19), 5.00 and 5.15 (each 1H, d,  $J = 18$  and 11 Hz, H-15), 5.35 (1H, br s, H-12), 6.02 (1H, dd,  $J = 18$  and 11 Hz, H-14); MS  $m/z$  (rel. int.): 391  $[\text{M}-\text{Me}]^+$  (1), 346 (1), 331 (3), 276 (3), 271 (2), 250 (20), 190 (78), 177 (68), 147 (15).

*Partial hydrolysis of 8.* A soln of 8 (40 mg) in  $\text{C}_6\text{H}_6$  (minimum quantity) was treated with 2% methanolic KOH (4 ml) leaving the mixt. at room temp. for 2 hr. Usual work-up and subsequent dry CC, eluting with petrol–EtOAc (23:2), gave starting material (4 mg), an unseparable mixture of the monoacetates 9 and 10 (16 mg), and the diol 7 (14 mg).

*Chlorination of 9 and 10.* The mixture of monoacetates (16 mg) and  $\text{Ph}_3\text{P}$  (40 mg) in  $\text{CCl}_4$  (2 ml) and pyridine (0.5 ml) were heated under reflux for 12 hr. The ppt. was separated by filtration, and washed with EtOAc. The solvents were evapd and the residue chromatographed on silica, eluted with petrol–EtOAc (97:3), to afford impure 12-chloro derivative and *ent*-12 $\alpha$ -acetoxy-19-chloro-13-*epi*-manoyl oxide (12) (9 mg), mp 137–139°. (Found:  $[\text{M}-\text{AcOH}]^+$  at  $m/z$  324.2007.  $\text{C}_{20}\text{H}_{31}\text{O}^{37}\text{Cl}$  requires  $[\text{M}-\text{AcOH}]^+$  at 324.2034. Found:  $[\text{M}-\text{AcOH}]^+$  at  $m/z$  322.2063.  $\text{C}_{20}\text{H}_{31}\text{O}^{35}\text{Cl}$  requires  $[\text{M}-\text{AcOH}]^+$  at 322.2063.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.73, 1.04, 1.10 and 1.23 (each 3H, s), 2.14 (3H, s), 3.39 and 3.77 (each 1H, d,  $J = 11$  Hz, 19-H), 5.00 and 5.06 (each 1H, d,  $J = 18$  and 11.5 Hz, 15-H), 5.35 (1H, br s, 12-H), 6.02 (1H, dd,  $J = 18$  and 11.5 Hz, 14-H); MS  $m/z$  (rel. int.): 324  $[\text{M}-\text{AcOH}]^+$  (0.5), 322 (1.5), 309 (1), 307 (3), 277 (7), 228 (18), 226 (51), 211 (22), 177 (100), 135 (22), 123 (38).

*Reduction of 12.* The chloro derivative 12 (8 mg) in dry toluene (3 ml) was refluxed with tri-*n*-butyltin hydride (56  $\mu\text{l}$ ) and azobisisobutyronitrile (traces) under  $\text{N}_2$  for 7 hr. The solvent was evapd and the residue dissolved in  $\text{Et}_2\text{O}$  and stirred with water satd with KF for 15 min. The solution was filtered, dried and the solvent evapd to give a gum which was chromatographed on silica. Elution with petrol–EtOAc (49:1) gave *ent*-12 $\alpha$ -acetoxy-13-*epi*-manoyl oxide (varol acetate) (13) (3 mg).  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.72, 0.79, 0.87, 1.11 and 1.25 (each 3H, s), 2.14 (3H, s), 5.00 and 5.06 (each 1H, d,  $J = 18$  and 11 Hz, 15-H), 5.35 (1H, t,  $J = 3$  Hz, 12-H), 6.04 (1H, dd,  $J = 18$  and 11 Hz, 14-H); MS  $m/z$  (rel. int.): 333  $[\text{M}-\text{Me}]^+$  (1), 273 (4), 192 (96), 177 (89), 137 (18).

*Varol (ent-12 $\alpha$ -hydroxy-13-epi-manoyl oxide) (14).* The alcohol 14 was obtained by hydrolysis of 13 (2 mg) with a saturated soln of  $\text{Na}_2\text{CO}_3$  in MeOH (2 ml) at reflux for 2 hr. (Found:  $[\text{M}-\text{Me}]^+$  at  $m/z$  291.2336.  $\text{C}_{19}\text{H}_{31}\text{O}_2$  requires  $[\text{M}-\text{Me}]^+$  at 291.2324.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.74, 0.79, 0.86, 1.20 and 1.24 (each 3H, s), 4.07 (1H, br s, 12-H), 4.96 and 4.98 (each 1H, d,  $J = 18$  and 11 Hz, 15-H), 6.08 (1H, dd,  $J = 18$  and 11 Hz, 14-H); MS  $m/z$  (rel. int.): 291  $[\text{M}-\text{Me}]^+$  (0.1), 273 (1), 192 (74), 177 (100), 137 (12).

*ent*-3 $\beta$ ,11 $\alpha$ -Dihydroxy-13-*epi*-manoyl oxide (18). Mp 80–82°. (Found:  $[\text{M}-\text{Me}]^+$  at  $m/z$  307.2308.  $\text{C}_{19}\text{H}_{31}\text{O}_3$  requires  $[\text{M}-\text{Me}]^+$  at 307.2273.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.78, 0.92, 0.99, 1.23 and 1.27 (each 3H, s), 3.24 (1H, t,  $J = 7$  Hz, 3-H), 4.13 (1H, m, 11-H), 4.93 (1H, dd,  $J = 11.5$  and 0.8 Hz), 5.08 (1H, dd,  $J = 18$  and 0.8 Hz), 5.98 (1H, dd,  $J = 18$  and 11 Hz); MS  $m/z$  (rel. int.): 307  $[\text{M}-\text{Me}]^+$  (25), 289 (12), 271 (13), 253 (6), 237 (5), 219 (4), 201 (17), 191 (5), 175 (10), 157 (11), 149 (13), 135 (49).

*Varodiol (ent-3 $\beta$ ,12 $\alpha$ -dihydroxy-13-epi-manoyl oxide) (17).* (Found:  $[\text{M}-\text{Me}]^+$  at  $m/z$  307.2269.  $\text{C}_{19}\text{H}_{31}\text{O}_3$  requires  $[\text{M}-\text{Me}]^+$  at 307.2273.  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.75 (6H, s), 0.97, 1.19 and 1.24 (each 3H, s), 3.21 (1H, dd,  $J = 10$  and 5 Hz, 3-H), 4.08 (1H, t,  $J = 3$  Hz, 12-H), 4.95 (1H, d,  $J = 18$  Hz, H-15), 4.97 (1H, d,  $J = 11.5$  Hz, 15-H), 6.07 (1H, dd,  $J = 18$  and 11 Hz, 14-H);

MS  $m/z$  (rel. int.): 307 [M]<sup>+</sup> (1), 279 (4), 208 (23), 193 (10), 190 (63), 175 (74), 147 (38), 135 (19).

ent-3 $\beta$ , 11 $\alpha$ , 12 $\alpha$ -Trihydroxy-13-epi-manoyl oxide (19). (Found: [M]<sup>+</sup> at  $m/z$  338.2380. C<sub>20</sub>H<sub>34</sub>O<sub>4</sub> requires [M]<sup>+</sup> at 338.2455). <sup>1</sup>H NMR (200 MHz):  $\delta$  0.78, 0.92, 0.99, 1.25 and 1.27 (each 3H, s), 3.24 (1H, *dd*,  $J = 10$  and 6 Hz, 3-H), 4.00 (1H, *d*,  $J = 4.5$  Hz, 12-H), 4.13 (1H, *dd*,  $J = 9.4$  and 4.5 Hz, 11-H), 5.04 (1H, *dd*,  $J = 11$  and 0.7 Hz, 15-H), 5.15 (1H, *d*,  $J = 18$  Hz, 15-H), 6.04 (1H, *dd*,  $J = 18$  and 11 Hz, 14-H); MS  $m/z$  (rel. int.): 338 [M]<sup>+</sup> (0.2), 323 (2), 307 (2), 289 (3), 268 (3), 250 (6), 191 (21), 175 (16), 135 (23).

*Acknowledgements*—This work has been supported by the CICYT, Ministry of Education and Science, Spain. We thank Dr A. Garcia-Granados (University of Granada) for copies of spectra of varol and varodiol, and Dr J. R. Hanson (University of Sussex, U.K.) for gifts of *Gibberella fujikuroi* and for his interest in this work. R. G. thanks the Ministry of Education and Science and the Spanish Research Council (CSIC) for a 'Formación de Personal Investigador' fellowship.

#### REFERENCES

1. Bearder, J. R. (1983) *The Biochemistry and Physiology of Gibberellins* (Crozier, A., ed.) Vol. 1, p. 251. Praeger, New York.
2. Diaz, C. E., Fraga, B. M., González, A. G., Hanson, J. R., Hernández, M. G. and San Martín, A. (1985) *Phytochemistry* **24**, 1489.
3. Fraga, B. M., Hernández, M. G., Rodríguez, M., Diaz, C. E., González, P. and Hanson, J. R. (1987) *Phytochemistry* **26**, 1931.
4. Fraga, B. M., Hernández, M. G., Diaz, C. E., González, P. and Guillermo, R. (1989) *Phytochemistry* (in press).
5. Cross, B. E., Galt, R. H. B. and Hanson, J. R. (1963) *J. Chem. Soc.*, 2937.
6. San Martín, A., Roviroso, J., Becker, R. and Castillo, M. (1980) *Phytochemistry* **19**, 1985.
7. San Martín, A., Roviroso, J. and Castillo, M. (1983) *Phytochemistry* **22**, 1461.
8. Dennis, D. T., Upper, C. D. and West, C. A. (1965) *Plant. Physiol.* **40**, 948.
9. Cross, B. E. and Myers, P. L. (1969) *Phytochemistry* **8**, 79.
10. Algarra, J., Garcia-Granados, A., Saez de Buruaga, A. and Saez de Buruaga, J. M. *Phytochemistry*, 1983, **22**, 1779.
11. Garcia-Granados, A., Martínez, A., Molina, A., Onorato, M. E., Rico, M., Saez de Buruaga, A. and Saez de Buruaga, J. M. (1985) *Phytochemistry* **24**, 1789.
12. Giles, J. A., Schumacher, J. N., Mims, S. S. and Bernasek, E. (1962) *Tetrahedron* **18**, 169.
13. González, A. G., Fraga, B. M., Hernández, M. G. and Luis, J. G. (1973) *Phytochemistry* **12**, 1113.
14. Hanson, J. R., Hawker, J. and White, A. F. (1972) *J. Chem. Soc., Perkin Trans. I* 1892.