Pergamon

PII: S0031-9422(96)00269-5

Phytochemistry, Vol. 43, No. 2, pp. 393–395, 1996

0031-9422/96 $15.00 + 0.00

BIOTRANSFORMATION OF ISOSTEVIOL BY *FUSARIUM VERTICILLOIDES*

BRÁS H. DE OLIVEIRA and ROGÉRIO A. STRAPASSON

Universidade Federal do Paraná, Departamento de Química, C.P. 19081, 81531-990, Curitiba, PR, Brazil

(*Received in revised form* 21 *March* 1996)

Key Word Index—*Fusarium verticilloides*; biotransformation; isosteviol; diterpenoid.

Abstract—The biotransformation of isosteviol (*ent*-16-ketobeyeran-19-oic acid) by *Fusarium verticilloides* (Sacc.) Nirenberg I33 produced *ent*-7β-hydroxy-16-ketobeyeran-19-oic acid and *ent*-12α-hydroxy-16-ketobeyeran-19-oic acid. The metabolites were isolated and characterized by spectroscopic methods. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Isosteviol (**1**) is a tetracyclic diterpenoid obtained by acid hydrolysis of the sweet glycoside stevioside [1].

Although a number of reports on the biotransformation of diterpenoids [2] have appeared in the literature, not many concern the biotransformations of beyerenes by microorganisms. Among the few studies carried out, *ent*-3-ketobeyer-15-en-17-oic acid (**2**) was shown to be reduced to its 3-hydroxyl derivative by *Aspergillus ochraceous* and hydroxylated at C-6 by *Calonectria decora* [3]; *ent*-7α,18-diacetoxy-14β-hydroxybeyer-15-ene (**3**) was found to be transformed to the corresponding epoxide, the 3α-hydroxyl derivative and the metabolite with both new functions by *Rhizopus nigricans* [4]. More recently [5], isostevic acid (*ent*-beyeran-19-oic acid) (**4**), *ent*-16β-hydroxybeyeran-19-oic acid (**5**) and *ent*-16β-hydroxy-16α-methylbeyeran-19-oic acid (**6**) were shown to be transformed by *Gibberella fujikuroi*, producing beyergibberellins and hydroxylated beyeranes.

As far as **1** is concerned, however, the only reported biotransformation was carried out with a mutant of *G. fujikuroi* [6] and the metabolites were analysed by GC–mass spectrometry. Among the products obtained, were beyergibberellins.

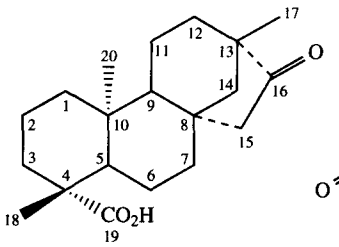
In view of the lack of studies on the biotransformation of **1**, we decided to investigate the ability of *Fusarium verticilloides* to transform this diterpenoid, and determine the positions in the beyerane skeleton where the transformations took place.

RESULTS AND DISCUSSION

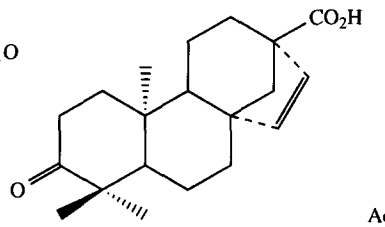
Initial experiments to evaluate the ability of *F. verticilloides* to take up **1** were conducted in conical flasks containing two-day-old cultures of the fungus. After addition of **1**, the reaction was monitored by TLC. After four days, new metabolites were detected in the broth, compared to a control.

Preparative experiments were carried out with the same culture medium under the same conditions. The crude extract obtained was chromatographed and two new metabolites were isolated and characterized. The first one showed an IR spectrum typical of a carboxylic acid i.e. carboxyl absorption bands at 1671 and 2500–3200 cm^{-1}. There was also an absorption at 1717 cm^{-1}, which indicated that the keto group at C-16 had not been affected. The mass spectrum showed the molecular ion with a *m/z* 334 (53%), compatible with the molecular formula $C_{20}H_{30}O_4$. Analysis of the ^{13}C NMR spectrum (Table 1), compared to that of **1**, and a DEPT experiment showed eight CH_2 and six CH/CH_3 for **2** whilst for isosteviol there are nine and five, respectively. Therefore, one methylene carbon of **1** was converted into a methine. The only differences in the normal spectrum were the resonances of C-5, C-6 and C-7. The resonance of C-6 suffered a downfield shift from δ 21.48 to δ 29.32. The resonance of C-5 was located at δ 45.25, which had undergone a γ effect. Therefore, the resonance at δ 75.22 was attributed to C-7, where the hydroxyl was located. The ^1H NMR spectrum showed the three methyl resonances of H-20, H-17 and H-18 at δ 0.80, 0.95 and 1.22, respectively. The –C<u>H</u>OH proton was located at δ 3.29 as a double doublet (J = 10 and 3.3 Hz). Thus, the –C<u>H</u>OH must be in the axial position coupled with the axial H-6 (J = 10 Hz) and with the equatorial H-6 (J = 3.3 Hz). Therefore, the hydroxyl was located in the α (equatorial) position leading to the structure **7**.

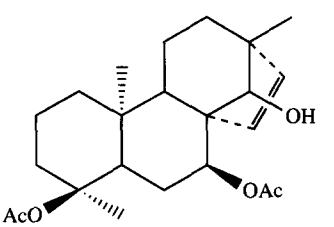
The second metabolite (**8**) showed an IR spectrum also typical of a carboxylic acid with its carboxyl absorption bands at 1695 and 2500–3200 cm^{-1}. It also showed an absorption at 1743 cm^{-1}, which indicated that the keto group at C-16 had not been affected. The mass spectrum showed a molecular ion with a *m/z* 334



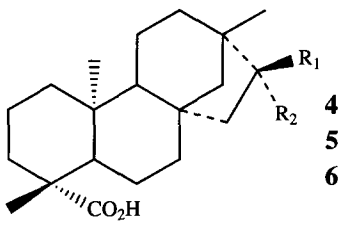
1



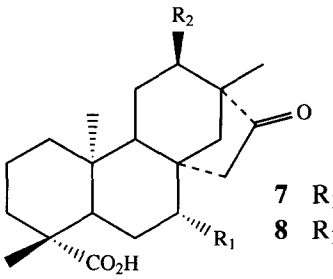
2



3



4 $R_1 = R_2 = H$
5 $R_1 = H$; $R_2 = OH$
6 $R_1 = CH_3$; $R_2 = OH$



7 $R_1 = OH$; $R_2 = H$
8 $R_1 = H$; $R_2 = OH$

(94%), compatible with the molecular formula $C_{20}H_{30}O_4$. Its ^{13}C NMR spectrum was compared with that of **1**. Again, a DEPT experiment showed 8-CH_2 and 6-CH/CH_3. Therefore, one methylene carbon of **1** was converted into methine. The differences in the normal spectrum were on the resonances of C-9, C-11, C-12, C-13, C-14 and C-17. The resonances of C-11 and C-13 suffered a downfield shift from δ 21.44 to 29.97 and from δ 41.05 to 55.67, respectively. The resonances of C-9, C-14 and C-17 suffered an upfield shift from δ 55.88 to 50.70, from δ 55.18 to 48.14 and from δ 20.20 to 17.51, respectively. Consequently, the hydroxyl group was located at position C-12, for which the resonance at δ 72.05 was attributed. The ^1H NMR spectrum showed the three methyl resonances of H-20, H-17 and H-18 at δ 0.71, 0.89 and 1.11, respectively. The –CHOH proton was located at δ 3.29 as a broad singlet. Therefore, the –CHOH must be in the equatorial (α) position and the hydroxyl was located in the axial (β) position, giving the structure **8**.

Table 1. ^{13}C NMR chemical shifts of isosteviol (**1**) and its metabolites

C	1	7	8
1	40.72	40.71	40.73
2	20.13	20.04	20.01
3	38.48	38.27	39.06
4	44.64	44.39	44.65
5	58.16	55.00	58.19
6	22.94	32.01	22.89
7	42.47	75.83	42.04
8	48.16	46.80	41.21
9	55.88	54.70	50.70
10	39.25	39.31	38.84
11	21.44	21.25	29.97
12	39.08	38.86	72.05
13	41.05	39.33	55.67
14	55.18	50.04	48.14
15	49.85	41.40	48.25
16	225.08	224.97	223.13
17	20.20	20.24	17.51
18	29.54	29.42	29.48
19	181.50	181.21	181.59
20	13.97	13.91	13.47

The hydroxylation of kaurenes and beyerenes at C-7 is a common occurrence with *F. moniliforme* and *G. fujikuroi*. Although 7α-hydroxylation has already been observed in kaurenes [3], this is the first time it has been obtained for a beyerane compound. The 12β-hydroxylation, on the other hand, is novel and it gives access to ring C functionalization. These results increase the range of applicability of fungi of the genus *Fusarium* in the biotransformation of tetracyclic diterpenoids.

EXPERIMENTAL

General experimental details. Mps: uncorr., IR: KBr discs; NMR: 300 MHz (^1H) and 75 MHz (^{13}C) with TMs as int. standard; EIMS: 70 eV. The fungus *F. verticilloides* (Sacc.) Nirenberg I33 was obtained from the culture collection of Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Hydrolysis of stevioside with dilute HCl. Stevioside was dissolved in H_2O, treated with conc HCl and the mixt. was heated under reflux for 2 hr. The product was recovered in $CHCl_3$ and the solvent evapd. Recrystallization from Me_2CO–petrol gave **1** as prisms, mp 227–228° (lit. [7] 231–233°).

Biotransformation with F. verticilloides. Slants of *F. verticilloides* were transferred to 1 l conical flasks containing a sterile culture medium that contained glucose (10.00 g l^{-1}), corn steep liquor (8.00 g l^{-1}) and yeast extract (2.0 g l^{-1}). The flasks were shaken at 30°

for 48 hr, and then **1** (440 mg) in DMSO was evenly distributed among 8 flasks each containing 200 ml of medium. After 4 days the mycelium was filtered off and washed with Et_2O. The broth was extracted with the same solvent, and the organic layers combined and dried with Na_2SO_4. After filtration the solvent was evapd under red. press. and the residue was subjected to CC on silica gel. Elution with EtOHAc–petrol (2:1) gave **1** (230 mg). Further elution gave a solid (24 mg) which was characterized as 7β-hydroxyisosteviol (**7**), mp 254–255°. IR ν_{max} cm^{-1}: 3540, 1718, 1678; EIMS *m/z* (rel. int.): 334 $[M]^+$ (53); ^1H NMR (CD_3OD) δ: 0.80 (3H, *s*, H-20), 0.95 (3H, *s*, H-17), 1.22 (3H, *s*, H-18), 3.29 (1H, *dd*, $J = 10$, 3.3 Hz, H-7β). Further elution gave a solid (30 mg) which was characterized as 12β-hydroxyisosteviol (**8**), mp 225–226°. IR ν_{max} cm^{-1}: 3373, 1743, 1695; EIMS *m/z* (rel. int.): 334 $[M]^+$ (94); ^1H NMR (CD_3OD) δ: 0.71 (3H, *s*, H-20), 0.89 (3H, *s*, H-17), 1.11 (3H, *s*, H-18), 3.29 (1H, *br s*, H-12α).

Acknowledgements—The financial support of CNPq (Brazil) and IFS (Sweden) is gratefully acknowledged. We also thank Fundação Oswaldo Cruz for the gift of *F. verticilloides* IOC, and NPPN-UFRJ for the mass spectra.

REFERENCES

1. Avent, A. G., Hanson, J. R. and Oliveira, B. H. de (1990) *Phytochemistry* **29**, 2712.
2. Hanson, J. R. (1992) *Nat. Prod. Rep.* 139.
3. Beilby, J. P., Ghisalberti, E. L., Jefferies, P. R., Sefton, M. A. and Sheppard, P. M. (1973) *Tetrahedron Letters* **28**, 2589.
4. Garcia-Granados, A., Martinez, A. and Onorato, M. E. (1985) *J. Nat. Prod.* **48**, 371.
5. Ali, M. S., Hanson, J. R. and Oliveira, B. H. de (1992) *Phytochemistry* **31**, 507.
6. Bearder, J. R., Frydman, V. M., Gaskin, P., McMillan, J., Wels, C. M. and Phinney, B. O. (1976) *J. Chem. Soc., Perkin Trans. I* 173.
7. Mosettig, E. and Nes, W. R. (1955) *J. Org. Chem.* **20**, 884.