BIO-TRANSFORMATION OF GIBBERELLIN 20,19-LACTONES BY RHIZOPUS STOLONIFER

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Key Word Index—Rhizopus stolonifer; microbiological transformation; gibberellin.

Abstract—In a study of the stereo-electronic requirements for microbiological hydroxylation by Rhizopus stolonifer, 20R-19-hydroxygibberellin-16-en-20-ox acid 20,19-lactone, 20S-19-dihydroxygibberellin-16-en-20-ox acid 20,19-lactone and 20R-19-hydroxygibberellin-16-en-7,20-dioic acid 20,19-lactone 7-methyl ester are shown to be hydroxylated at the 15α-position.

INTRODUCTION

Some directing effects in the microbiological hydroxylation of steroids have been rationalized [1-4] in terms of the stereochemistry of the substrates that were used. Typically, 5α-androstan-3β-ol was less reactive than 5α-androstan-3α-ol [5], but in some cases, the directing group was not significant. The stereochemistry of the active site of the hydroxylation enzyme is critical. The tetracyclic diterpenoids are rigid molecules which, whilst bearing some resemblance to the steroids, possess functionality on pendant groups attached to the carbon skeleton. Thus they allow a greater opportunity for the molecule to rotate within the active site and provide a three-dimensional mapping. There have been a number of studies using various tetracyclic diterpenoids [5] but hitherto no systematic pattern has emerged. The availability of some lactones from gibberellin A13 [2] [5, 7] enabled us to make some systematic studies with these compounds as substrates with an organism, Rhizopus stolonifer (marinus) which has been widely used in microbiological hydroxylation studies.

RESULTS AND DISCUSSION

The substrates 3, 10, 12 and 14, respectively, were obtained from the 3-lactone-7-α-methyl ether by (i) hydrogenolysis of the corresponding 7-α-methyl lactone-7-α-methyl ester by (ii) oxidation to the 7-carboxylic acid and ethyl 7-carboxyl and diethyl 7-carboxylate, and (iii) catalytic reduction of the 15α-ester.

In this series the possible directing groups are on pendant groups attached to the carbon skeleton. The results of the biotransformation by R. stolonifer are shown in Scheme 1. Some of the metabolites were difficult to separate and were purified as their acetates. There were no detectable metabolites of the 7-carboxylic acid obtained during the preparation of the 7-methyl ester (12). The location of the hydroxyl group at C-15 in the metabolites 5, 11 and 13 was established by changes in the 13C NMR spectra (Table 1). In particular the 13C NMR signals assigned to C-15 and the adjacent C-8 and C-16 had moved downfield whilst C-13 and C-14 showed shielding effects. The stereochemistry of the hydroxyl group at C-15 was confirmed by oxidation of the 7-α-methyl lactone (10) and the 7-ester (12) with hydrogen peroxide and selenium dioxide. This oxidation is known [8] to give the 15α-20-19-lactone. In both cases this afforded a 15α-methyl ester identical to the biotransformation product.

There was some additional hydroxylation of the double bond by R. stolonifer in the case of the 7-deoxy-20,19-lactone (3). The structure of these products followed from their 1H and 13C NMR spectra. The alkene 13C
resonances were replaced by two additional \( ^{13}C \) signals. Furthermore, the \(^1H\) resonances assigned to the H-17 of the alkene in the substrate were replaced by signals at \( \delta = 3.63 \) and \( 3.74 \) (\( J = 11.3 \) Hz) in 6 and \( \delta = 4.28 \) and \( 4.60 \) (\( J = 12 \) Hz) in 9.

The \(^1H\) and \(^13C\) NMR spectra showed that the major metabolite from the dihydroy-compound 14 was the 16-hydroxylated product 20. Two minor metabolites were isolated and tentatively assigned structures with 12- and 13-hydroxyl groups.

In the presence of the 16-ene hydroxylation takes place at the allylic 15\( \beta \)-position. This contrasts with a previous observation by Bearder et al. [9] that hydroxylation of gibberellin \( \Delta_6 \) methyl ester by this organism takes place at the 15\( \beta \)-position. Examination of molecular models shows that by placing the lactone carbonyl (C-20) over the C-3 carbonyl of a steroid model, C-15 and C-16 of the gibberellins lie relatively close to C-11 of the steroids, a typical site of hydroxylation by this organism. The isolation of the diol (6) and the \( 15\beta\)-epoxy-17-alkol (8) from the transformation of the 16-ene (13) and the 16-ol (12) from the transformation of the dihydro-compound (14) is interesting. Firstly, this represents a modification of the general observation [10] that an epoxide may be formed from the biotransformation of an alkene where the corresponding sp\(^3\) centre undergoes biohydroxylation. Secondly, the isolation of the \( 15\alpha\)-epoxy-17-alkol (8) which could be formed via the \( 15\beta\)-alkol (4) might suggest that the microbial oxidation of a double bond is a two-step process involving electron abstraction and then carbon-oxygen bond formation. Hydroxylation at the 16-position [11] and epoxidation of a 16-ene [12] have been observed in the biotransformation of some \( \mu \)-kaurenois and \( \mu \)-kaurenes by \textit{Rhizopus nigricans}.

**EXPERIMENTAL**

General. \(^1H\) NMR spectra were determined at 360 MHz and \(^13C\) NMR spectra at 90.56 MHz in CDCl\(_3\) except where stated. IR spectra were determined as nujol mulls. Silica for chromatography was Merck 9385. Light petrol refers to the fraction bp 60–80°.

**Fermentation details.** \textit{Rhizopus nigricans} (IMI 57761) was maintained on malt agar slopes. The incubations were carried out in shake culture at 25° in 250 ml conical flasks (100 ml medium per flask) using the following medium (per litre): glucose (20 g), peptone (5 g), yeast extract (3 g), potassium dihydrogen phosphate (5 g). The incubations were carried out as follows: (a) \textit{en}19-hyd-
Table 1. $^{13}$C NMR signals of the gibberellin 20-19 lactones

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**These assignments may be interchanged.

* Determined in MeOD.
culture for 10 days. The broth was filtered and the metabolites recovered in EtOAc and chromatographed on silica gel. Elution with 15% EtOAc-petrol gave unchanged substrate (60 mg). Further elution with 20% EtOAc-petrol gave ent-15,18,19-dihydroxystigberrin-16-en-7,20-dione and 20,19-lactone "methyl ester (13) (100 mg) which crystallized from CHCl₃, as prisms, mp 163°C. (Found: C, 66.5; H, 7.7; C₁₉H₂₂O₅. H₂O requires C, 66.6; H, 8.0%). IR (ν = cm⁻¹) 3489, 1716, 1650.

¹H NMR: δ 0.86 (3H, s, H-18), 2.59 (1H, d, J = 10.2 Hz, H-61), 3.68 (3H, s, OMe), 4.14 (1H, dd, J = 11.8 and 1.5 Hz) and 4.36 (1H, dd, J = 11.8 and 2.2 Hz) reach H-19, 4.17 (1H, s, H-15), 5.11 and 5.29 (each 1H, s, H-17) (I) ent-7,19-Dihydroxystigberrin-20-one and 20,19-lactone (14) (500 mg) in EtOH (20 ml) was evenly distributed between 40 flasks and incubated in shake culture for 8 days. The broth was filtered and the metabolites were recovered in EtOAc and chromatographed on silica gel. Elution with 40% EtOAc-petrol gave the unchanged substrate (52 mg). Further elution gave a mixt. (A18 mg) and then elution with EtOAc gave mixt. B31 mg. Each mixt. was acetylated (Ac₂O-pyridine) and the products were chromatographed on silica gel in EtOAc-petrol (1:4). A afforded a compound, tentatively assigned the structure of ent-7,12-diacetoxy-19-hydroxystigberrin-20-one and 20,19-lactone (15) (3 mg) as a gum. (Found: C, 41.0; H, 4.0; requires C, 41.0; H, 4.0%). IR (ν = cm⁻¹) 3489, 1716, 1650.

¹H NMR: δ 0.99 (3H, s, H-18), 2.41 (1H, d, J = 6 Hz, H-17), 2.04 and 2.07 (each 1H, s, OAc), 3.97 (1H, dd, J = 11.6 and 6.6 Hz) and 4.25 (1H, dd, J = 11.6 and 3 Hz) reach H-7, 4.10 (1H, dd, J = 11.8 Hz and 4.53 (1H, dd, J = 11.8 and 2.1 Hz) reach H-19, 5.87 (1H, d, J = 7.4 Hz, H-12). MS m/z: 418 [M⁺], 376, 375, 358, 298, 269, 225. Further elution gave ent-7,12-diacetoxy-19-hydroxystigberrin-20-one and 20,19-lactone (16) (17) as a gum. (Found: C, 41.0; H, 4.0; requires C, 41.0; H, 4.0%). IR (ν = cm⁻¹) 3489, 1716, 1650.

¹H NMR: δ 0.97 (3H, s, H-18), 2.09 (1H, d, J = 11.2 Hz, H-3), 2.49 (1H, d, J = 11.2 Hz, H-61, 3.23 (1H, s, H-15), 3.74 (3H, s, OMe), 3.76 and 4.04 (each 1H, d, J = 12.7 Hz, H-17), 4.10 (1H, dd, J = 11.9 and 1.3 Hz) and 4.24 (1H, dd, J = 11.9 and 2.1 Hz, each H-19).

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