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Biotransformations of *ent*-18-acetoxy-6-ketomanoyl oxides epimers at C-13 with filamentous fungi

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Abstract

Two *ent*-18-acetoxy-6-oxomanoyl oxides, epimers at C-13, have been prepared from *ent*-6α,8α,18-trihydroxylabda-13(16),14-diene (andalusol), isolated from *Sideritis foetens*, by means of several chemical pathways and a regioselective acylation with *Candida cylindracea* lipase (CCL). Biotransformation of these 13-epimeric *ent*-manoyl oxides by *Fusarium moniliforme* and *Neurospora crassa* produced mainly *ent*-1β- or *ent*-11α-hydroxylations, as well as their deacetylated derivatives, in both epimers. In addition, with the 13-*epi* substrate *N. crassa* originated other minor hydroxylations by the *ent*-α face at C-1 or at C-12, whereas an *ent*-11β-hydroxyl group, probably originated by reduction of an 11-oxo derivative also isolated, was achieved with the 13-*normal* substrate.

Keywords: *Fusarium moniliforme*; *Neurospora crassa*; Biotransformation; *ent*-Manoyl oxides; Diterpenoids; Filamentous fungi; Biohydroxylation

1. Introduction

Regio- and stereoselective hydroxylation of non-activated carbon atoms is a very useful methodology in organic chemistry (Azerad, 2001; Holland, 1999; Li and Chang, 2004), and, as these processes are difficult to carry out by chemical means, whole-cells fermentation is the procedure most often employed in such fungal hydroxylation (Lehman and Stewart, 2001; Ishige et al., 2005). The main problem for the biohydroxylation of a certain substrate at a specific position is to find the appropriate microorganism, therefore customarily; one of the most widely used techniques is screening with several fungal strains. In this context, the microbial transformation of *ent*-manoyl oxides – labdane-type diterpenoids – by filamentous fungi constitutes one line of our research. We are currently exploring an extensive series of chemical-microbiological pathways to semi-synthesise diversely functionalized *ent*-manoyl oxides, with both configurations at C-13. These biotransformation processes are used to introduce hydroxyl groups, at positions difficult to achieve by classical chemical methods, onto substrates. The main interest of biotransformation of manoyl oxides is to produce new poly-functionalized compounds, due to the wide variety of biological properties described for these compounds, including, anti-inflammatory (Alcaraz et al., 1989), anti-hypertensive (Tandon et al., 1992), anti-leishmanial (García-Granados et al., 1997a), antibacterial (Demetzos et al., 1998), enzyme stimulation (García-Granados et al., 1994a,b, 1995a), cytotoxic (Chaichantipyuth et al., 2005; Demetzos et al., 1994; Dimas et al., 1999, 2001; Konishi et al., 1998), phytotoxic (Rivero-Cruz et al., 2000) and insecticidal (Ybarra et al., 2005).

In previous papers, we have reported the incubations of several *ent*-manoyl oxides epimers at C-13, with functions at C-6, C-16 and C-18 or C-6 and C-16, with the filamentous fungi *Rhizopus nigricans* and *Curvularia lunata* yielding

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new poly-oxygenated *ent*-manoyl oxides (Arias et al., 1988; García-Granados et al., 1990, 1995a,b, 1997b). In the present work, the fungi *Fusarium moniliforme* (CECT 2152) – a synonym of *Fusarium verticillioides* (EAN 337) and the anamorphic form of *Gibberella fujikuroi* (ATCC 12616) – and *Neurospora crassa* (CECT 2261, ATCC 10336) are used to complete the earlier biotransformation studies and obtain new highly oxygenated *ent*-manoyl oxides analogues of *ent*-forskolin.

2. Results and discussion

The phytochemical study of *Sideritis foetens* Clem. ex Lag. revealed an abundant diterpenic content with structures of *ent*-labda-13(16),14-diene (García-Alvarez and Rodríguez, 1980; García-Granados et al., 1994a). In an earlier paper, a method was described for the in vitro micropropagation of this plant (García-Granados et al., 1994a). These *ent*-labdadienic diterpenoids can be transformed into manoyl oxides with or without a 16-hydroxyl group (Amate et al., 1991; Arias et al., 1988; García-Granados et al., 1990, 1997b). The starting material used in this work was andalusol (**1**). Acetylation of this compound yielded 6,18-diacetylandalusol (**2**) (López et al., 1977). Treatment of this diacetate (**2**) with $TiCl_4$ produced *ent*-manoyl oxides, epimers at C-13, without functionalization at C-16 (**3** and **4**). Chemical deacetylation of **3** and **4** gave diols **5** and **6**, respectively. Regioselective acetylation of these diols (**5** and **6**) with *Candida cylindracea* lipase (CCL) rendered monoacetyl derivatives at C-18 (**7** and **8**, respectively) in high yield. This enzymatic acetylation improved the results of chemical acetylation, so that acetylation with Ac_2O/Pyridine of the epimeric diols (**5** and **6**) yielded diacetates **3** and **4**, monoacetates **7** and **8**, and the acetyl derivatives at C-6 (**9** and **10**), respectively. The individual oxidation of monoacetates **7** and **8** with Jones' reagent produced the corresponding 6-oxo derivatives (**11** and **12**), which were used as substrates in the biotransformation processes. The overall yields of these compounds (**11**, 80%; **12**, 80%) from diols **5** and **6** were considerably higher than those obtained using chemical acetylation (38% and 34%, respectively). These yields were also superior to those achieved in another chemical procedure (**11**, 65%; **12**, 67%), based on the oxidation of diols **5** and **6** at C-6 with pyridinium dichromate (PDC), and subsequent chemical acetylation (García-Granados et al., 1997b).

Biotransformation of substrate **11** with *F. moniliforme* produced the deacetylated metabolites **13** (17%), **14** (16%) and **15** (6%). The first metabolite (**13**) was the result of the sole deacetylation of substrate **11**. The molecular formula of metabolite **14** ($C_{20}H_{32}O_4$) suggested the presence of an additional hydroxyl group in the molecule. This hydroxyl group was in an equatorial arrangement, pointed out by the signal of the geminal axial proton (δ 3.61, *dd*, $J = 10.0$, 5.3 Hz), in its ^{1}H NMR spectrum. The position of this hydroxyl group was determined by comparing the ^{13}C NMR spectra of **13** and **14**, the δ-effect on C-11 ($\Delta\delta = +2.5$) being significant due to the spatial proximity of this carbon atom and the equatorial hydroxyl group at C-1, and confirmed by HMBC experiments (cross-peak signals between H-2, H-5, H-9, 3H-20, and C-1). Therefore, metabolite **14** was *ent*-1β,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide. Metabolite **15** had the same molecular formula ($C_{20}H_{32}O_4$) of **14** and its ^{1}H NMR spectrum showed a signal at δ 4.21 (*ddd*, $J = 9.5$, 7.7, 4.8 Hz) due to a geminal proton to a hydroxyl group, that could only be situated at C-11 in this molecule. The arrangement of this hydroxyl group was deduced from the chemical shift and the coupling constants of H-11, which were comparable to those observed for other *ent*-13-*epi*-manoyl oxide derivatives containing an *ent*-11α-hydroyl group (Konishi et al., 1996; Fraga et al., 1999). The position and the stereochemistry of this hydroxylation were confirmed by the analysis of the ^{13}C NMR data of **13** and **15** (δ-effect at C-1 of +2.1), and by HMBC experiments (correlations of C-11 with H-9 and H-12, and of H-11 with C-9, C-10 and C-13). Consequently, metabolite **15** was *ent*-11α,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide.

The biotransformation of substrate **11** with *N. crassa* gave the same metabolites (**13**, 18%; **14**, 22%; and **15**, 9%), previously isolated from the biotransformation of this substrate (**11**) by *F. moniliforme*, together with **16** (3%), **17** (10%), **18** (2%), and **19** (2%). Spectral data of **16** and **17** revealed that these metabolites were the 18-acetyl derivatives of **14** and **15**, respectively, as a consequence of the direct biohydroxylation at C-1 (*ent*-β) or C-11 (*ent*-α) of substrate (**11**). Metabolites **18** and **19** were again 18-deacetylated compounds. In the ^{1}H NMR spectrum of **18** appeared a signal at 3.70 ppm as a broad singlet, similar to that observed for an axial hydroxyl group at C-1 (Fraga et al., 1998a). This *ent*-1α-hydroxylation was confirmed from the analysis of the ^{13}C NMR of **13** and **18**, particularly by the strong γ-gauche effects on C-3 ($\Delta\delta = -6.8$), C-5 ($\Delta\delta = -6.56$) and C-9 ($\Delta\delta = -8.55$), suggesting that the hydroxyl group adopted an axial disposition at C-1. Thus, metabolite **18** was *ent*-1α,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide. In the ^{1}H NMR spectrum of **19**, the equatorial geminal proton to a hydroxyl group appeared at δ 4.14 (*dd*, $J = 3.3$, 3.3 Hz), this hydroxyl group being located at C-3 or C-12. The ^{13}C NMR strong γ-gauche effects on C-9 ($\Delta\delta = -8.75$) and C-16 ($\Delta\delta = -5.6$) positioned, in an axial arrangement, the hydroxyl group at C-12. Therefore, **19** had a structure of *ent*-12α,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide.

Biotransformation of substrate **12** – the 13-epimer compound of **11** – with *F. moniliforme* produced metabolites **20** (21%), **21** (10%), and **22** (9%), in which the first action of this fungus was again the deacetylation of the substrate (**12**). In this way, metabolite **20** was the 18-deacetyl derivative of **12**, whereas the molecular formula of **21** and **22** ($C_{20}H_{32}O_4$) indicated that this microorganism had introduced an additional hydroxyl group into the molecule, respectively. In the ^{1}H NMR spectrum of **21**, the signal