Biotransformation of 3α,4α-Dihydroxy-dihydro-β-agarofuran by *Rhizopus nigricans*

Julio Alarcón* and Sergio Águila

Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bío-Bío, Chillán Chile. Fax: +56-42-203046. E-mail: jalarcon@pehuen.chillan.ubiobio.cl

* Author for correspondence and reprint requests

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3α,4α-Dihydroxy-dihydro-β-agarofuran (**1**), prepared from (+)-dihydrocarvone by a traditional synthetic method, the Robinson annelation, was biotransformed by *R. nigricans* to afford a new metabolite characterized as 1α,3α,4α-trihydroxy-dihydro-β-agarofuran (**2**) by spectroscopic method. An acetylated derivative was prepared.

Key words: Celastraceae, Sesquiterpenes, Agarofuran Synthesis

Introduction

From plants of the Celastraceae family a variety of compounds has been isolated such as: maytansinoids, triterpenes, diterpenes and sesquiterpenes. These compounds are thought to be responsible for the biological activity attributed to these plants (Tu, 1991; Brüning and Wagner, 1978). The Celastraceae sesquiterpenoids generally occur as polyesters of variously poly-oxygenated tricyclic scaffolds all based on a core C_{15} skeleton known as dihydroagarofuran. This kind of compounds exhibits a wide spectrum of biological properties, including significant cytotoxic, immunosuppressive, anticancer, insecticidal, insect antifeedant and potent anti-HIV activity (Zhou *et al*., 2001; Cespedes *et al*., 2001).

Their highly oxygenated tricyclic frameworks, comprising a number of contiguous stereocenters, pose a formidable synthetic challenge and have attracted immense interest from synthetic chemists. Several total synthesis of agarofurans have been reported since 1970 (Boyer *et al*., 2003; White *et al*., 1995, 1997; Kelly, 1972; Tu and Sun, 1998; Alarcón *et al.*, 1998a).

The objective of our work is to obtain agarofuran sesquiterpenoic polyol combining synthetic strategies with microbiological hydroxylation, for which we previously prepared a compound **1** by means of Robinson annelation. Then the compound was hydroxylated with *R. nigricans.* The use of fungi for hydroxylations of terpenoid substrates is amply documented in the literature (Chaney and Hertzog, 1967; Lamare and Furstoss, 1990).

Material and Methods

Synthesis of 3α,4α-dihydroxy-dihydro-β-agarofuran (**1**)

Compound **1** was prepared from dihydrocarvone by means of reaction of Robinson annelation with ethyl vinyl ketone according to Alarcón *et al.* (1998b) (Fig. 1). The structure of compound **1** was determined by NMR spectroscopy. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm) δ = 3.61–3.56 (dd, 1H, J = 5.07, 10.14 Hz), 1.32 (s, 3H), 1.28 (s, 3H), 1.23 (s, 3H), 1.15 (s, 3H). – ^{13}C NMR (65 MHz, $CDCl_3$, δ in ppm): δ = 88.50, 82.44, 76.29, 73.53, 43.68, 38.33, 37.55, 35.41, 32.40, 30.05, 26.59, 24.35, 23.79, 22.79, 21.56, 21.12. – MS (70 eV): *m/z* (rel. int.) = 254 (30), 239 (100), 221 (36), 203 (11), 193 (18), 177 (52), 154 (13), 125 (20), 123 (21), 109 (22), 81 (12), 69 (18), 55 (19).

Fermentation conditions

R. nigricans (LSPN001, 'authors' laboratory) was grown in shaked culture medium comprising (g per liter): $CaCl_2$ (0.05), KH_2PO_4 (0.025), $(NH_4)_2HPO_4$ (0.25), $MgSO_4 \times 7H_2O$ (0.15), 1.3 ml of $FeCl_3$ (1%), malt extract (3.0) and glucose (10.0). The culture was grown in 250 ml conical flasks each containing 100 ml medium for 36 h at 25 °C prior to the addition of the substrate.

Incubation of 3α,4α-dihydroxy-dihydro-β-agarofuran with R. nigricans

3α,4α-dihydroxy-dihydro-β-agarofuran (**1**) (0.5 g) in ethanol (5 ml) was evenly distributed in 10 flasks of *R. nigricans* and the fermentation was

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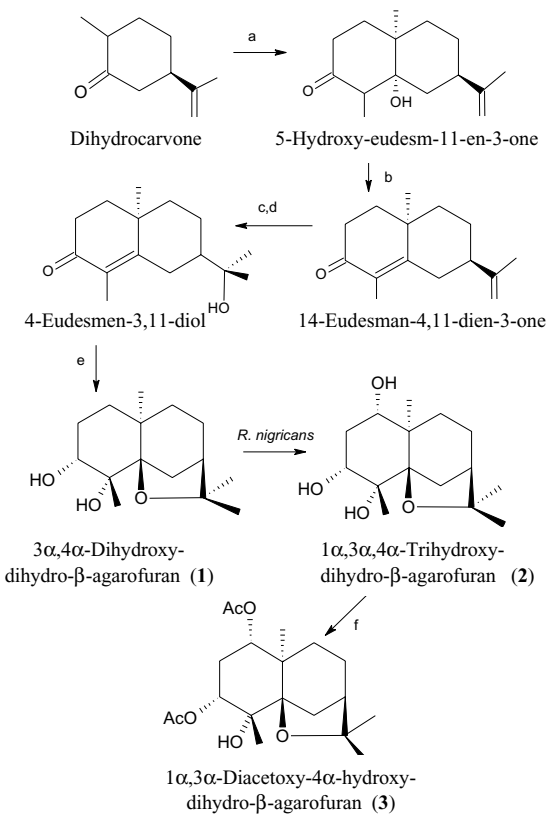

Fig. 1. Synthesis of 3α,4α-dihydroxy-dihydro-β-agarofuran (**1**) and biotransformation with *R. nigricans*. a) EVK, KOH, 0 °C. b) KOH, EtOH, reflux, 1 h. c) *m*-CPBA, $CHCl_3$, room temperature. d) $LiAlH_4$, Et_2O. e) *m*-CPBA, toluene. f) Ac_2O, Pyridine.

subsequently continued for 5 d. The myceliun was filtrated and the broth successively extracted with ethyl acetate. The solvent was evaporated to give a dry residue which was chromatographed on silica gel. Elution with 10% ethyl acetate/light petroleum ether (40–60 °C) gave the starting material (0.11 g) identified by its ^{1}H NMR spectrum. Further elution with 20% ethyl acetate/light petroleum ether gave 1α,3α,4α-trihydroxy-dihydro-β-agarofuran (**2**) (0.31 g, yield 62%; Fig. 1). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ = 4.15 (t, *J* = 4.9 Hz), 3.60–3.51 (m), 1.32 (s, 3H), 1.25 (s, 3H), 1.19 (s, 3H), 1.09 (s, 3H). – ^{13}C NMR (65 MHz, $CDCl_3$, δ in ppm): δ = 87.88, 82.51, 72.38, 70.34, 70.05, 42.24, 33.98, 31.66, 29.44, 29.26, 26.98, 24.68, 22.42, 21.47, 20.15. – MS (70 eV): *m/z* (rel. int.) = 270 (1), 252 (32), 234 (7), 219 (9), 191 (9), 177 (8), 153 (100), 125 (25), 91 (23).

Acetylation of **2**

Pyridine (0.1 ml) was added to a solution of compound **2** (10 mg) in Ac_2O (0.5 ml), and the solution was stirred for 7 h at room temperature (25 °C). The product was isolated in the usual manner and separated by silica gel CC by a hexane/EtOAc gradient. The diacetate **3** was obtained. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ = 4.18 (t, *J* = 4.7 Hz), 3.62 (dd, *J* = 5.01, 10.2 Hz), 2.10 (s, 3H), 2.05 (s, 3H), 1.32 (s, 3H), 1.28 (s, 3H), 1.23 (s, 3H), 1.17 (s, 3H). – ^{13}C NMR (65 MHz, $CDCl_3$, δ in ppm): δ = 171.43, 170.71, 87.88, 82.86, 70.37, 68.96, 63.46, 42.15, 34.03, 31.76, 29.54, 29.17, 28.96, 27.05, 24.73, 22.54, 21.20, 20.85. – MS (70 eV) *m/z* (rel. int.) 354 (1), 339 (14), 313 (4), 294 (15), 219 (36), 173 (48), 133 (100), 69 (65).

Results and Discussion

In a typical aerobic fermentation, compound **1** was incubated with *Rhizopus nigricans* for 5 d. Extraction of the reaction mixture followed by chromatography gave 22% of unconverted **1**, 62% of metabolite **2** (yield calculated from the amount of **1** converted) and a mixture of other, not yet identified hydroxylation products (ca. 16%).

The major metabolite was 1α,3α,4α-trihydroxy-dihydro-β-agarofuran (**2**). The ^{1}H NMR spectrum showed singlets at δ 1.32, 1.25, 1.19 and 1.09 ppm, corresponding to the four methyl groups. The protons at C-1 and C-3 appeared at δ 4.15 and δ 3.60–3.51, respectively. The ^{13}C NMR spectrum confirmed a CH(OH) resonance at δ 72.38, 70.34, 70.05 ppm which was confirmed by DEPT and HETCOR measurements. The C-1 position of the newly introduced hydroxyl was established due to the HMBC correlation of H-1 (δ 4.15) with C-10 (33.98). MS of metabolite **2** showed a molecular ion peak at *m/z* 270 due to the introduction of an oxygen atom.

In conclusion, we have shown that the microbiological hydroxylation of 3α,4α-dihydroxy-dihydro-β-agarofuran (**1**) gave 1α,3α,4α-trihydroxy-dihydro-β-agarofuran (**2**) in a higher yield than by chemical synthesis. This procedure may be a convenient new route to the synthesis of polyhydroxyagarofurans.

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