Absolute Configuration of Sporotricale and Structure of 6-Hydroxysporotricale

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The absolute configuration of the two stereocenters of (+)-sporotricale (**2a**), a bioactive phthalide secondary metabolite, was determined through circular dichroism and by applying Mosher's method. The structure of 6-hydroxysporotricale (**2c**), isolated from cultures in vitro of *Sporotrichum laxum*, was also elucidated.

(+)-Spirolaxine (**1**) and (+)-sporotricale (**2a**) are the major metabolites extracted from cultures of the white rot fungus *Sporotrichum laxum* (Basidiomycetae),[1] now *Phanerochaete pruinosum*. They are structurally similar, both containing a 5-hydroxy-3-methoxyphthalide moiety linked through a polymethylene chain to a spiroketal group in compound **1** and to a hemiacetal group in sporotricale **2a**. The latter exists as an equilibrium of the two epimeric hemiacetals and the hydroxyketone, depending on the solvent.[1]

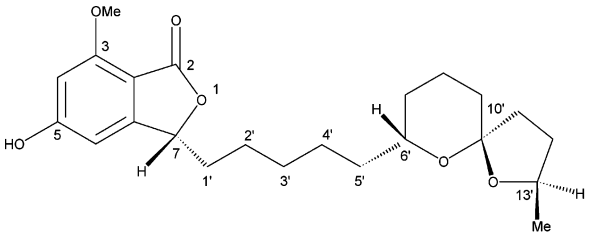
Spirolaxine **1** has been reported to possess cholesterol-lowering activity,[2] and more recently we reported its activity on some endothelial cells and on a variety of tumor cell lines (e.g., LoVo and HL60).[3] The interest in this type of compounds prompted us to determine the absolute configuration of the four stereocenters of spirolaxine on the basis of X-ray crystallographic analysis and a series of CD experiments.[4]

Sporotricale **2a** belongs to a small group of fungal metabolites produced by *Phanerochaete* sp. that have received attention for their activity against *Helicobacter pylori*.[5] They contain the same phthalide moiety, but have different aliphatic chains with respect to sporotricale. However, no configurational data of these compounds were reported.[5] Recently a synthetic approach to (±)-sporotricale methylether (**2b**) was described.[6]

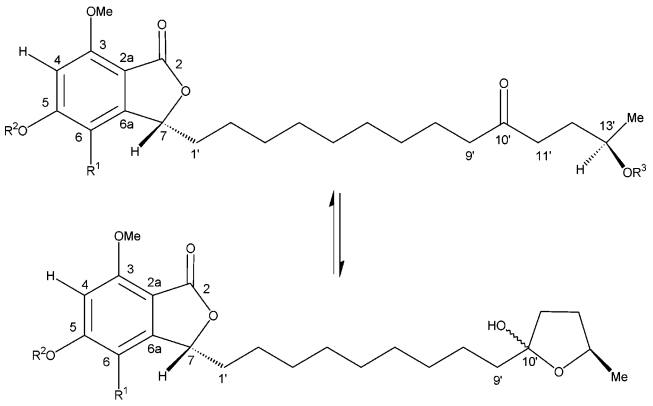
This paper deals with the absolute configuration of the two stereocenters (C-7 and C-13′) of sporotricale, which was determined by CD measurements and Mosher's method carried out on 5-*O*-methylsporotricale **2b** and on 6-hydroxysporotricale (**2c**), a new metabolite isolated from cultures of *S. laxum* grown on rice medium.

In order to predict the absolute configuration at C-7 in compound **2a**, we measured the CD spectrum: (EtOH, *c*, 0.022) λ = 206, 220, 233, 243, 257, 284, 300 nm ($\Delta\epsilon$ +6.3, −4.2, +0.5, −0.1, +0.6, −1.2, +0.3 L mol^{-1} cm^{-1}). The values and the shape of the curve are similar to those measured for spirolaxine **1** (Figure 1). In particular, the strong UV transitions at 206 and 220 nm, typical of "benzoate chromophores",[4] confirm that the stereogenic center at C-7 of compound **2a** is *R*-configured.

To determine the configuration of the stereogenic center of the side chain, (+)-sporotricale was methylated to give compound **2b**.[6] The absolute configuration at C-13′ of **2b** was elucidated by applying the modified Mosher's method[7] to (*S*)- and (*R*)-MTPA (α-methoxy-α-trifluoromethylphenylacetic acid) esters (**3a** and **3b**), obtained by reacting **2b** with the corresponding MTPAs. The *R* configuration at C-13′ was deduced from the $\Delta\delta$ values (Figure 2) obtained by subtracting the chemical shift (δR) of each proton of the (*R*)-MTPA ester from that (δS) of the (*S*)-MTPA ester. In fact,



1



2a $R^1 = R^2 = R^3 = H$
2b $R^1 = H$; $R^2 = Me$; $R^3 = H$
2c $R^1 = OH$; $R^2 = R^3 = H$
2d $R^1 = OAc$; $R^2 = R^3 = Ac$

all the protons lying on the left-hand side of the plane containing the ester group of MTPA had negative $\Delta\delta$ values as a consequence of the shielding effect due to the phenyl ring of the Mosher's acid (Figure 3). Formula **2a** shows the absolute configuration of (+)-(7*R*,13′*R*)-sporotricale.

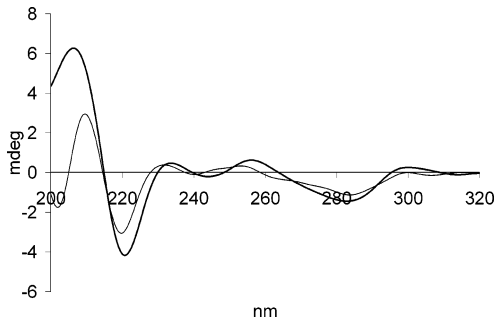


Figure 1. Comparison of CD spectra between sporotricale (bold line) and **1** (regular line).

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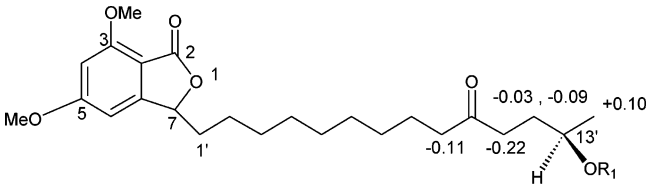

3a R_1 = S(-) MTPA

3b R_1 = R(+) MTPA

Figure 2. Differences of the proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$) of the MTPA ester derivations of compound **2b**.

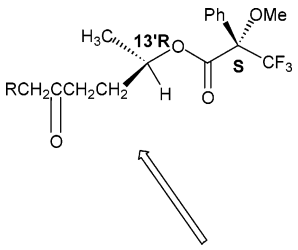

Figure 3. MTPA plan for the (*S*)-MTPA ester used to assign the absolute configuration at C-13′ for compound **2b**.

Compound **2c** was isolated in low yield as an oil, when the fungal strain was grown on ground rice agar instead of MPGA (malt-peptone-glucose agar).[1] The ESIMS spectrum established the molecular formula as $C_{23}H_{34}O_7$ (MH^+; $m/z = 423$), while the EIMS spectrum, although lacking the expected (M^+), showed a fragment at m/z 404 due to ready loss of water from the molecular ion. In addition, a strong peak was observed at m/z 195, which suggested the presence of a phthalide moiety with an additional hydroxy function with respect to sporotricale **2a**.

The ^{1}H NMR spectrum of **2c** (see Experimental Section) showed the presence of a single aromatic CH resonance at 6.62 ppm, confirming that the additional hydroxy group is located on the aromatic ring. This assignment was proven by the proton and carbon spectra of the *O*-acetyl derivative (**2d**), showing the occurrence of only one aromatic CH at 6.79 and 107.4 ppm, respectively.

Finally, an NOE experiment showed that the *O*-methyl group at C-3 produced a strong enhancement (25%) of the adjacent H-4, thus confirming that the additional hydroxy group is linked to carbon C-6.

From a biological point of view, sporotricale **2a** and compound **2c** were studied in comparison to spirolaxine **1**, which demonstrated a good antiproliferative activity both in vitro and in vivo. The cytotoxic activity was evaluated on an endothelial cell line (BMEC) and on a few tumor cell lines. In a CAM (chorioallontoic membrane of fertilized chick eggs) assay, widely used as a model to examine angiogenesis and anti-angiogenesis activity, all the compounds inhibited angiogenesis induced by growth factors such as bFGF. None of these compounds showed a better cytotoxic profile than spirolaxine, and among them compound **2c** was slightly more active than sporotricale.[8]

Experimental Section

General Experimental Procedures. Optical rotations were obtained using a Jasco-500 DIP-181 polarimeter; UV spectra were measured for solutions in 95% EtOH; CD spectra were recorded on a Jasco 500A dichrograph. Mass spectra were obtained with a Finnigan-MATT-TSQ 70 eV, a Bruker Esquire 3000, and for HRMS a Bruker APEX-QZT ICR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DMX 500 or ARX 400 instruments at 305 K. The proton signals were assigned from the chemical shift correlation experiments (COSY) and the carbon nuclei from the heteronuclear correlation experiments via one-bond (HSQC) and long-range (HMBC) coupling constants. The nuclear Overhauser effects were determined by monodimensional NOE difference spectra.

HPLC analyses were performed using a LiChroCART column RP-18 250-4 (Merck) on an Agilent 1100 instrument; flow rate = 0.5 mL min^{-1}. Flash CC was performed on Merck silica gel; TLC and PLC with Merck HF_{254} silica gel. The purity of products was checked by TLC, NMR, and MS and deemed sufficient for the purpose of structural determination.

Biological Assay. CAM: chorioallontoic membrane of fertilized chick eggs; BMEC: bovine microvascular endothelial cells; bFGF: basic fibroblast growth factor.

Isolation and Purification of Compounds 2a and 2c. The strain of *Sporotrichum laxum* CBS 578.63,[1] now *Phanerochaete pruinosum*, was inoculated in 40 Roux flasks containing RA (ground rice agar 90: 20 g/L). After 30 days at 24 °C, the flasks were extracted twice with EtOAc containing 1% MeOH. The crude extracts were washed with hexane to obtain 8 g of the residue. The mixture of metabolites, spirolaxine **1**, sporotricales **2a**[1] and **2c**, and phanerosporic acid[1] was chromatographed on a column of flash silica gel filled with hexane−EtOAc (1:1) and using a mixture of hexane−EtOAc−MeOH as eluent. With hexane−EtOAc (1:2) sporotricale **2a** (250 mg) was eluted, and with EtOAc−MeOH (2:1) a mixture of **1**, **2c**, and phanerosporic acid was eluted. The fractions containing the last metabolites were successively purified by flash chromatography with CH_2Cl_2−MeOH (9:1) to obtain **1** (400 mg) and **2c** (35 mg).

Compound 2b. A 100 mg sample of **2a** was dissolved in dry acetone (15 mL), and K_2CO_3 (200 mg) and MeI (0.5 mL) were added; the mixture was refluxed until the starting material disappeared on TLC. The reaction was filtered and the solvent evaporated; PLC with hexane−EtOAc (1:1) gave 80 mg of pure **2b**,[6] crystallized from acetone−hexane: mp 94−97 °C; $[\alpha]_D^{25}$ +75.2 (*c* 0.08, MeOH); ^{1}H NMR δ (acetone-d_6) 6.71 and 6.59 (2H, br d, J = 1.7 Hz, H-4 and H-6), 5.35 (1H, br dd, J = 7.6 and 3.6 Hz, H-7), 3.92 and 3.93 (3H, s, 2×OMe), 3.66 (1H, m, H-13′), 2.53 and 2.44 (4H, t, J = 7.5, H_2-9′ and -11′), 2.0−1.2 (18H, m, 9×CH_2), 1.10 (3H, d, J = 7.2, H_3-14′); EIMS, m/z 403 [$MH^+ - 18$].

Compound 2c: $[\alpha]_D^{25}$ +71.4 (*c* 0.05, MeOH); IR (Nujol) ν_{max} 3300-(OH) and 1705(CO ester) cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.62 (1H, s, H-4), 5.41 (1H, dd, J = 7.8 and 3.1 Hz, H-7), 3.80 (3H, s, OMe), 3.70 (1H, m, H-13′), 2.54 (2H, t, J = 7.5 Hz, CH_2-11′), 2.42 (2H, t, J = 7.5 Hz, CH_2-9′), 2.40−1.20 (18H, m, 9 CH_2 groups), 1.12 (3H, d, J = 6.6 Hz, H_3-14′); *anal.* C 65.29%, H 7.95%, calcd for $C_{23}H_{34}O_7$, C 65.38%, H 8.11%; HREIMS m/z 422.2327 (calcd for $C_{23}H_{34}O_7$ 422.2304).

Compound 2d. 2c (15 mg) was acetylated with pyridine−Ac_2O at rt; workup as usual gave 8 mg of **2d**, crystallized from CH_2Cl_2−hexane: mp 77−80 °C; $[\alpha]_D^{25}$ +56.5 (*c* 0.4; MeOH); IR (KBr) ν_{max} 1750 (CO ester) and 1730 (CO ketone); ^{1}H NMR ($CDCl_3$) δ 6.79 (1H, s, H-4), 5.37 (1H, dd, J = 7.7 and 3.2 Hz, H-7), 4.87 (1H, m, H-13′), 3.94 (3H, s, OMe), 2.42 (2H, t, J = 7.5 Hz, CH_2-11′), 2.37 (2H, t, J = 7.5 Hz, CH_2-9′), 2.31 (3H, s, COMe), 2.30 (3H, s, COMe), 2.00 (3H, s, COMe), 1.99−1.21 (18H, m, 9 CH_2 groups), 1.20 (3H, d, J = 6.5 Hz, H_3-14′); ^{13}C NMR ($CDCl_3$) δ 210.5 (s, CO-10′), 170.9 (s, CO-2), 167.7, 167.6, 167.3 (s, 3 OCOMe), 156.7, 148.5, 145.2, 129.8 (s, C-3, C-5, C-6, C-6a), 112.4 (s, C-2a), 107.4 (d, CH-4), 79.1 (d, CH-7), 70.5 (d, CH-13′), 56.8 (q, OMe), 43.0 (t, CH_2-9′), 38.7 (t, CH_2-11′), 32.9, 29.9, 29.5, 29.47, 29.42, 29.36, 29.32, 24.5, 24.0 (t, 9 CH_2 groups), 21.5, 20.9, 20.4 (q, 3 COMe groups), 20.2 (q, Me-14′); HREIMS m/z 548.2624 (calc for $C_{29}H_{40}O_{10}$ 548.2621); EIMS, m/z 549 [MH^+] (4%), 489 [$MH - 60^+$] (100), 446 (65) 404 (75); CIMS, m/z 549 (40%), 489 (53), 447 (30).

Compounds 3a and 3b (Mosher's esters of 5-*O*-methylsporotricale **2b**). To two solutions of compound **2b** (10 mg each) in CH_2Cl_2 (2 mL) containing DMAP (few crystals) and DCC (30 mg) was added 20 mg of (*S*)-(−)MTPA or (*R*)-(+)MTPA, respectively. Each mixture was stirred at rt for 6 h, and the products **3a** and **3b** were purified by PLC using hexane−EtOAc (2:1) as eluent.

3a: ^{1}H NMR (acetone-d_6) δ 7.6−7.4 (5H, m, Ph-2″, 6.68 (1H, dd, J = 1.8 and 0.8 Hz, H-6), 6.57 (1H, d, J = 1.8 Hz, H-4), 5.33 (1H, br dd, J = 7.8 and 3.6 Hz, H-7), 5.14 (1H, ddq, J = 8.2, 4.5, and 6.3 Hz, H-13′), 3.92 (6H, s, OMe-3 and-5), 3.57 (3H, q, J = 1.2 Hz, OMe-2″), 2.33 (2H, t, J = 7.4 Hz, H_2-11′), 2.32 (2H, t, J = 7.2 Hz, H_2-9′), 1.86 (1H, ddt, J = 14.2, 4.5, and 7.4 Hz, H-12′$_a$), 1.76 (1H, ddt, J = 14.2, 8.2, and 7.4 Hz, H-12′$_b$), 1.36 (3H, d, J = 6.3 Hz, H_3-14′), the remaining methylene protons resonate between 2.1 and 1.2 ppm.

3b: ^{1}H NMR (acetone-d_6) δ 7.7−7.4 (5H, m, Ph-2″), 6.70 (1H, dd, J = 1.8 and 0.8 Hz, H-6), 6.58 (1H, d, J = 1.8 Hz, H-4), 5.33 (1H, br dd, J = 7.8 and 3.6 Hz, H-7), 5.16 (1H, ddq, 7.4, 5.3, and 6.2 Hz, H-13′), 3.92 and 3.91 (6H, s, OMe-3 and -5), 3.56 (3H, q, J = 1.2 Hz, −OMe-2″), 2.56 (2H, t, J = 7.4 Hz, H_2-11′), 2.43 (2H, t, J = 7.2 Hz, H_2-9′), 1.89 (1H, ddt, J = 14.2, 5.3, and 7.4 Hz, H-12′$_a$), 1.85 (1H, ddt, J = 14.2, 7.4, and 7.4 Hz, H-12′$_b$), 1.26 (3H, d, J = 6.2 Hz, H_3-14′), the remaining methylene protons resonate between 2.1 and 1.2 ppm.

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References and Notes

(1) Arnone, A.; Assante, G.; Nasini, G.; Vajna de Pava, O. *Phytochemistry* **1990**, *29*, 613−616.
(2) Blaser, M. J. *Clin. Infect. Dis.* **1992**, *15*, 386−391.
(3) Giannini, G.; Pisano, C.; Riccioni, T.; Marcellini, M.; Chiarucci, I.; Nasini, G.; Bava, A. *AACR Annual Meeting*; Orlando, FL, March 27−31, 2004; Vol. 45, p 230.
(4) Bava, A.; Clericuzio, M.; Giannini, G.; Malpezzi, L.; Valdo Meille, S.; Nasini, G. *Eur. J. Org. Chem.* **2005**, 2292−2296.
(5) Dekker, K. A.; Inagaki, T.; Gootz, T. D.; Kaneda, K.; Nomura, E.; Sakakibara, T.; Sakemi, S.; Sugie, Y.; Yamauchi, Y.; Yoshikawa, N.; Kojima, N. *J. Antibiot.* **1997**, *50*, 833−839.
(6) Nannei, R.; Dallavalle, S.; Merlini, L.; Bava, A.; Nasini, G. *Synlett.* **2005**, 2676−2678.
(7) Ohtani, I.; Kusumi, T.; Ishitsuku, M. O.; Kakisawa, H. *Tetrahedron Lett.* **1989**, *30*, 3147−3150.
(8) Unpublished results, Sigma-Tau (Pomezia).

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