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Solistatinol, a novel phenolic compactin analogue from *Penicillium solitum*

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Abstract—Solistatinol, a novel phenolic compactin analogue, has been isolated from *Penicillium solitum* using a UV-guided strategy. The structure and relative stereochemistry were determined by NMR spectroscopy and mass spectrometry. The absolute stereochemistry was determined by chemical degradation and comparison of CD data with literature data.

Fungal statin compounds such as compactins produced by *Penicillium* species,[1] and mevinolins (also called monacolins) produced by *Aspergillus* and *Monascus* species,[2,3] are polyketide derived metabolites of great importance to mankind due to their cholesterol lowering effects.

First generation natural or semisynthetic drugs such as Mevastatin (compactin or ML-236B) and Simvastatin (methylated mevinolin) have now been on the market for more than a decade and second generation synthetic statins such as Lipitor (atorvastatin) and Crestor (rosuvastatin) are now top selling drugs.[4] The fact that the health of a growing number of people in the Western world benefit from taking these antilipidemics probably means that statins will continue to be among the top 10 selling drugs for many years to come. Another reason for the continued interest in these compounds is the fact that statins apparently have pleiotropic effects indicating that they might be used for the treatment of osteoporosis,[5] inflammations, autoimmune diseases[6,7] and cancer.[8]

Today high resolution mass spectrometry (HR-MS) is the most important analytical tool for detection and dereplication of natural products. Structural information, however, can also be deduced from UV-spectroscopy since several metabolites in a biosynthetic family often have very similar and relatively characteristic UV-spectra.[9] This is the case for ML-236A–C, which are three of the later compounds in the compactin biosynthetic pathway (see spectrum of ML-236B in Fig. 1).[10] Other classes of compounds can likewise readily be detected by HPLC–DAD analysis, and a number of groups working with natural products have established their own in-house databases of UV–vis spectra.[11,12] Hansen et al. even developed a new algorithm called *X-hitting* allowing automated searches of full UV-spectra in a learning-based data base.[13] New natural products can therefore easily be detected using either a knowledge-based UV-guided approach, or by use of the *X-hitting* algorithm tracking compounds with UV-spectra very similar to the already known target compounds as demonstrated for a number of alkaloids.[14–17]

A UV-guided approach for the discovery of novel compounds of course has its limitations in all the cases where the introduction of one or more double bonds into an intermediate metabolite leads to a significant change in the chromophore and thereby the UV spectra of later intermediates or end products. This is also the case for the UV spectrum of solistatin (Fig. 1), which was the first aromatic compactin analogue to be reported from *Penicillium solitum*.[18]

Having been involved in the discovery of solistatin we were motivated to search for further new metabolites

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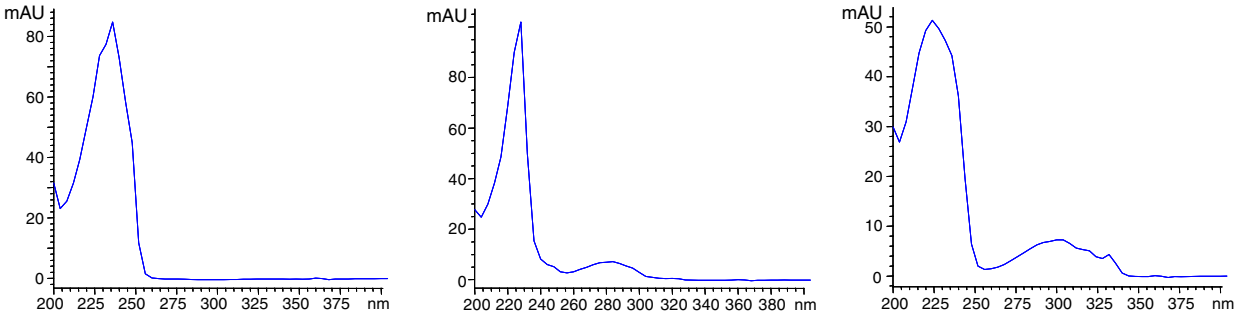

Figure 1. UV spectra of compactin (ML-236B) (left), solistatin (middle) and solistatinol **1** (right).

related to the compactin biosynthetic pathway. Our interest soon focused on a minor compound produced by *P. solitum* with a UV-spectrum somewhat similar (but with broader absorptions at slightly higher wavelengths) to that of solistatin (Fig. 1), since this compound always seemed to co-occur in extracts together with compactins and solistatin. This compound indeed turned out to be a novel aromatic compactin analogue differing from solistatin by an additional phenol group, which is why we have named the compound solistatinol (**1**) (Fig. 2).

Solistatinol was extracted with EtOAc from a sucrose and yeast extract based medium and purified by standard semi-preparative methods.[19] Mass spectrometric analysis of **1** indicated the formula $C_{18}H_{20}O_4$, different from solistatin by the presence of an additional oxygen.[20] The ^1H, ^{13}C and the C,H-COSY NMR data revealed the presence of one methyl, four methylene and two methine groups, together with five aromatic protons, five quaternary aromatic carbon atoms and finally a carboxy group at 170.2 ppm indicating an ester or lactone.[21] The ^1H and H,H-COSY spectra revealed the presence of one aliphatic (–CH_2–CHOH–CH_2–CH–CH_2–CH_2–) and two aromatic spin systems (AB and ABC) together with a single methyl group and two likely hydroxy protons at 5.20 and 9.95 ppm. Interpretation of the heteronuclear multiple bond coherence spectrum (HMBC) established the proposed structure of solistatinol with the phenol OH group positioned at C-8′ similar to the oxygenation pattern seen in ML-236B.[22] The chemical shift values of the carbons in the naphthalene part of the molecule were shown to be very similar to the values of the model compound 7,8-dimethyl-1-naphthol.[23] Similarly, the chemical shift values of the aliphatic part of the molecule were shown to be very similar to those reported for solistatin and other statins.[18,22] These data strongly supporting the proposed structure (Fig. 2).

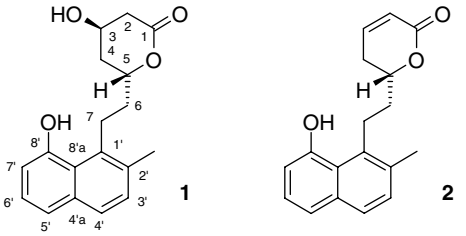

Figure 2. Structures of solistatinol **1** and 2,3-dehydro-solistatinol **2**.

In order to assign the absolute stereochemistry, solistatinol was dehydrated with TsOH in benzene to generate 2,3-dehydro-solistatinol (Fig. 2). LC–DAD–MS analysis indicated a protonated molecular ion of 2,3-dehydro-solistatinol appearing at *m*/*z* 283.1319 corresponding to the composition $C_{18}H_{19}O_3$ (Δ −5.9 ppm) with 10 DBE. The ^1H NMR spectrum of **2** showed two new downfield signals at 7.08 ppm and 5.98 ppm,[24] whereas the 3-OH proton at 5.20 ppm and H-3 at 4.16 ppm seen in **1**[23] were lacking which proved the introduction of the expected double bond. Analysis of the H,H-COSY spectrum proved the double bond to be in the 2,3-position. The patterns of the wavelengths, signs and relative intensity of the CD curve of dehydro-solistatinol was shown to be very similar to the corresponding data obtained for dehydro-solistatin.[18,24] The data therefore clearly supports the view that solistatinol has the same stereochemistry at C-5 as solistatin isolated from the same species, as expected from a biosynthetic point of view.

As for other statins it was possible to detect the open lactone ring form of **1** in the raw extract and in many fractions as a slightly earlier eluting compound than **1**. This was evident from LC–DAD–MS analysis in the negative mode since a smaller chromatographic peak with an identical UV spectrum to **1** had a base peak at *m*/*z* 317.1389 corresponding to the composition $C_{18}H_{21}O_4$ (Δ −6.3 ppm) as expected for $[M-H]^-$.

The fact that the stereochemistry of the lactone ring of solistatinol is identical not only to that of solistatin but also to that of all other known fungal statins means they all share the same pharmacophore. It is therefore highly likely that solistatinol will also have cholesterol lowering properties. Solistatinol can be easily acetylated, for example, by reaction with acetic acid anhydride giving a mixture of primarily the (C-8′) mono-acetate, as evident from LC–DAD–MS analysis showing the major reaction product to have lost the typical broad phenolic UV spectrum of solistatinol in combination with having gained 42 μm (results not shown). Work is in progress in our laboratories to investigate the cholesterol lowering potential of solistatinol and semisynthetic ester analogues.

Whether solistatinol is biosynthesized by oxygenation at the C-8′ position of solistatin in a way similar to that proposed for the biosynthesis of ML-236A from ML-236C,[24] or whether **1** is biosynthesized by the aromatization of ML-236A needs to be clarified. This could be done by molecular studies targeting the genes involved in the biosynthesis of these aromatic compactins.

Furthermore, cloning of the relevant polyketide synthase genes into an appropriate cell factory, such as *Emericiella nidulans*,[25] *Aspergillus niger* or *Saccharomyces cereviseae* could lead to an overexpression and large scale production of solistatinol should the pharmaceutical potential of this presently minor fermentation product turn out to be promising.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.12.038.

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19. The isolate (IBT 21545) was cultured for 2 weeks in the dark at 20 °C as three point mass inoculations on 1000 Petri dishes (9 cm) on about 20 L of yeast extract sucrose agar (YES) (yeast extract 20 g, sucrose 150 g, agar 20 g, distilled water). The total content of the Petri dishes was extracted twice with 10 L of EtOAc to give ca. 25 g of crude extract which was partitioned between a total of 1 L of heptane and 1 L of 90% MeOH. The MeOH fraction was diluted to 60% and extracted with 1 L of CH_2Cl_2 to give a total of 8 g of solistatinol rich extract. This extract was then coated onto 8 g of Celite and divided into three portions which subsequently were separated by vacuum liquid chromatography on a C_{18} SPE column (10 g) into four fractions using 100 mL of H_2O/MeOH in the following amounts: A (75:25), B (50:50), C (25:75) and D (0:100). The C fraction (4.6 g) rich in solistatinol was further separated on a Sephadex LH20 column (25×900 mm) using MeOH at 1 mL/min to give 8 fractions (CA–CF). Finally, the CE fraction (96 mg) was subjected to HPLC separation on a Waters Prep Nova-Pak C18 cartridge (25×100 mm, 6 μm) using H_2O/CH_3CN (65:35) as the mobile phase to give 30 mg of pure solistatinol.
20. Accurate mass analysis was performed on an Agilent HP 1100 liquid chromatograph with a diode array detector (DAD) coupled to a Waters LCT time-of-flight instrument with Z-spray electrospray source (ESI) and a lockspray probe. Sample (1 μL) was injected on an Agilent Hypersil BDS-C_{18} 125×2 mm column with 3 μm particles. A water–acetonitrile gradient, starting with 15% acetonitrile–water going to 100% acetonitrile in 40 min, maintaining 100% acetonitrile for 5 min, before returning to the start conditions in 8 min equilibrating for 5 min. TFA, 50 ppm was added to the water. The MS was operated in the positive ESI mode using leucineenkephalin as the lockmass ($[M+H]^+$ ion at m/z 556.2771).
21. *Solistatinol* (**1**): $[\alpha]_D^{20}$ +28.7 (*c* 1.2 MeOH); UV (MeOH) λ_{max} ($\log\varepsilon$) 223 (4.20), 232 (4.15), 303 (3.53), 319 (3.42), 332 (3.39); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.95 (1H, br s, 8′-OH), 7.54 (1H, d, $J = 8.2$ Hz, H-4′), 7.24 (1H, d, $J = 8.2$ Hz, H-3′), 7.24 (1H, d, $J = 7.9$ Hz, H-5′), 7.16 (1H, dd, $J = 7.9$ and 7.5 Hz, H-6′), 6.83 (1H, d, $J = 7.5$ Hz, H-7′), 5.20 (1H, br s, 3-OH), 4.72 (1H, m, 5-H), 4.16 (1H, br s, 3-H), 3.45 (1H, br s, H-7a), 3.36 (1H, br s, H-7b), 2.68 (1H, dd, $J = 17.2$ and 4.7 Hz, H-2a), 2.44 (3H, s, Me), 2.43 (1H, d, 17.2 Hz, H-2b), 1.92 (2H, m, H-6), 1.91 (1H, m, H-4a), 1.79 (1H, ddd, 14.3, 11.7 and 3.3 Hz, H-4b). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.2 (C-1), 154.8 (C-8′), 135.1 (C-1′), 135.1 (C-4′a), 132.1 (C-2′), 129.0 (C-3′), 125.8 (C-4′), 124.7 (C-6′), 123.1 (C-8′a), 119.5 (C-5′), 109.9 (C-7′), 75.5 (C-5), 61.0 (C-3), 38.5 (C-2), 36.5 (C-6), 34.8 (C-4), 27.0 (C-7), 19.5 (Me). The protonated molecular ion of solistatinol appeared at 301.1421 corresponding to the composition $C_{18}H_{21}O_4$ (Δ −6.3 ppm) corresponding to nine DBE.
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24. *2,3-Dehydro-solistatinol* (**2**): $[\alpha]_D^{20}$ −62.3 (*c* 1.2, EtOH); UV (MeOH) λ_{max} ($\log\varepsilon$) 224 (4.57), 233 (4.47), 304 (3.89), 319 (3.57), 332 (3.57); CD (CHCN, *c* 0.01) $\Delta\varepsilon$ (λ nm) 207

(−6.0), 258 (−2.0); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.95 (1H, br s, 8′-OH), 7.54 (1H, d, $J = 8.2$ Hz, H-4′), 7.25 (1H, d, $J = 8.2$ Hz, H-3′), 7.25 (1H, d, $J = 7.9$ Hz, H-5′), 7.16 (1H, dd, $J = 7.9$ and 7.5 Hz, H-6′), 7.08 (1H, ddd, $J = 10.0$, 6.0 and 2.5 Hz, H-3), 6.84 (1H, d, $J = 7.5$ Hz, H-7′), 5.98 (1H, dd, $J = 10.0$ and 2.0 Hz, H-2), 4.55 (1H, m, 5-H), 3.48 (1H, br s, H-7a), 3.31 (1H, br s, H-7b), 2.55 (1H, m, H-4a), 2.44 (3H, s, Me), 2.42 (1H, m, H-4b), 2.01 (1H, m, H-6a), 1.95 (1H, m, H-6b). The protonated molecular ion of 2,3-dehydro-solistatinol appeared at m/z 283.1319 corresponding to the composition $C_{18}H_{19}O_3$ (Δ −5.9 ppm) corresponding to 10 DBE.

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