Tuscolid and Tuscoron A and B: Isolation, Structural Elucidation and Studies on the Biosynthesis of Novel Furan-3(2*H*)-one-Containing Metabolites from the Myxobacterium *Sorangium Cellulosum*[‡]

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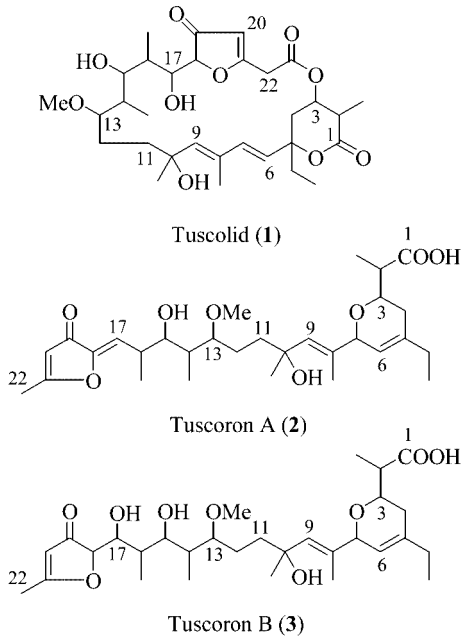
Three novel metabolites, tuscolid (**1**) and tuscoron A (**2**) and B (**3**), were isolated from the myxobacterium *Sorangium cellulosum* (strains So ce1401 and So ce1383). The structures were elucidated by detailed NMR spectroscopic analysis, and their biosynthesis was studied by feeding ^{13}C-labelled precursors. The results revealed that the macrolide **1** and the acyclic derivative **2** are closely related polyketides containing, as a characteristic structural feature, a furan-3(2*H*)-one ring system. The related minor component tuscoron B (**3**) was identified as an unstable structural analogue of tuscoron A. The relative stereochemistry of the tetrahydropyran ring and of C-13 to C-17 of tuscolid (**1**), and of the dihydropyran ring of tuscoron A (**2**), was determined on the basis of ^{1}H-^{1}H-coupling constants and NOE correlations. A tentative mechanism for the conversion of **1** into **2** is discussed.

Introduction

In the course of our screening program for new secondary metabolites from myxobacteria, we isolated a new class of closely related compounds named tuscolid (**1**) and tuscoron A and B (**2**, **3**) from cultures of *Sorangium cellulosum*, strains So ce1401 and So ce1383 (Scheme 1). These compounds were discovered due to their prominent peaks in the HPLC diode-array-trace of the culture extracts. As a characteristic structural feature, both tuscolid and tuscoron A and B contain a functionalised 3(2*H*)-furanone ring system. Furan-3(2*H*)-ones are found in various naturally occurring antitumour agents of plant origin, such as jatrophone,[2] geiparvarin,[3] the eremantholides,[4] or lychnophorolide[5] and in the inhibitor of bacterial tumour cell heparanase, trachyspic acid.[6] Herein, we report the isolation, structural elucidation and studies on the biosynthesis of **1** and **2**. The unstable minor component tuscoron B (**3**) was identified as a structural analogue of tuscoron A.



Scheme 1

Results and Discussion

Isolation

We isolated *Sorangium cellulosum*, strains So ce1401 and So ce1383, from soil samples collected near Tucson (So

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ce1401) and Snow Flake (So ce1383), Arizona. Both strains were found to be comparably good producers of tuscolid (**1**) and tuscoron A (**2**). In the production procedure, strain So ce1401 was grown on a 70 L scale for 11 days in the presence of 1% adsorber resin XAD-16. The adsorber resin was recovered from the fermentation broth by sieving, and was eluted with methanol. Extraction with ethyl acetate, followed by partition between methanol and heptane to remove lipophilic by-products, gave a crude extract, which was separated by consecutive chromatography on Sephadex LH-20 and C-18 reverse-phase silica gel to yield tuscolid (**1**) (41.0 mg) and tuscoron A (**2**) (46.6 mg). From another 70 L fermentation, tuscolid (27.5 mg) and tuscoron A (68.6 mg) were isolated together with tuscoron B (**3**) (6.0 mg); in this case, eluents which were adjusted to pH 7 instead of pH 6 were used for the MPLC and HPLC separations.

Structural Elucidation

HR-DCI mass spectrometry and elemental analysis of **1** furnished the elemental composition $C_{31}H_{46}O_{10}$, which implies nine double-bond-equivalents (DBEs). Six DBEs are accounted for by three carbonyl signals and three pairs of olefinic signals in the ^{13}C NMR spectrum. The remaining DBEs indicate the presence of three ring systems. The signals in the ^{1}H and ^{13}C NMR spectra were assigned by $^{1}H,^{1}H$-COSY, direct $^{1}H,^{13}C$-correlation (HMQC) and long-range $^{1}H,^{13}C$-HMBC correlation spectroscopy (Figure 1). The substituted 3(2*H*)-furanone ring system was deduced from the chemical shifts and long-range HMBC correlations of 20-H (δ = 5.77 ppm, s) to C-19 (δ = 207.4 ppm), C-21 (δ = 188.0 ppm) and C-18 (δ = 88.5 ppm), and from 18-H (δ = 4.79 ppm, d) to C-19 and C-21. The separation of the ring-oxygen and the α,β-unsaturated C-19 carbonyl functionality to form a non-lactonic ring system was evident from the ^{13}C carbonyl resonance at δ = 207.4 ppm, which is clearly in the ketone region. The significant HMBC cross-peak from 3-H to the C-23 carbonyl (δ = 168.8 ppm) revealed the presence of a macrocyclic lactone. Due to H/D-exchange of the acidic C-22 methylene group, only 30 carbon atoms appeared in the ^{13}C NMR spectrum measured in CD_3OD (Table 2). The presence of an additional 22-CH_2 group was indicated only by a very weak HMBC correlation of 20-H to C-23, and was finally confirmed by

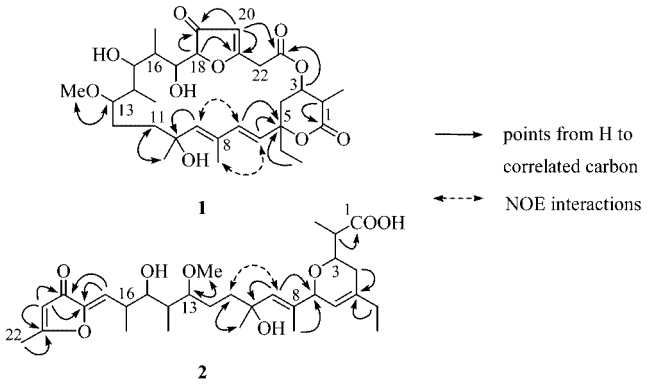

Figure 1. Selected NOE and $^{1}H,^{13}C$-long-range correlations

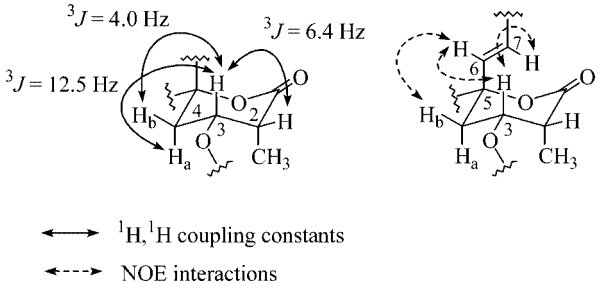

Figure 2. Selected $^{1}H,^{1}H$ coupling and NOE interactions in the tetrahydro-2*H*-pyran-2-one ring of tuscolid (**1**)

proton- and carbon-signals appearing in the spectra measured in $CDCl_3$ (δ_H = 3.54 and 3.78 ppm, J = 17.0 Hz; δ_C = 36.1 ppm). The structure of the tetrahydro-2*H*-pyran-2-one moiety was derived from the $^{1}H,^{13}C$-long-range correlation of 2-H to the lactonic C-1 carbonyl carbon at δ = 176.5 ppm, and from 4-H, 6-H, 7-H, and 25-H_3 to the quaternary C-5 at δ = 85.9 ppm. The relative stereochemistry of the tetrahydropyran ring could be deduced from the vicinal-proton-couplings and from NOE experiments (Figure 2). It follows from the fact that the coupling constant between 3-H and 4-H_a is J = 12.5 Hz that the substituent at C-3 must be equatorial. The coupling constant between 3-H and 2-H of J = 6.4 Hz indicates that the CH_3 group at C-2 is axial. The equatorial orientation of the ethyl substituent at C-5 was deduced from the NOE interactions between 6-H and 4-H_b, and between 7-H and 3-H. The configuration of the $\Delta^{6,7}$ double bond was assigned as *trans* on the basis of the vicinal coupling constant of $J_{6,7}$ = 15.6 Hz. The (*E*) configuration of the $\Delta^{8,9}$ double bond was derived from nuclear Overhauser enhancements (NOEs) between 7-H and 9-H, and between 6-H and 8-CH_3. Analysis of coupling constants and NOE-difference-spectra revealed the relative stereochemistry of C-13 to C-17 of the macrolide as shown in Figure 3. NOE correlations from 14-Me to 13-OCH_3 and 15-H, together with an NOE enhancement observed between 13-H and 14-H and a large coupling between 14-H and 15-H (10.0 Hz), established a *syn* relationship between 13-H and 14-H and an antiperiplanar arrangement of 14-H and 15-H. A NOE correlation from 15-H to 16-H together with a small vicinal coupling (2.5 Hz) indicates a *syn* relationship of these two protons. A large coupling between 16-H and 17-H of 10.2 Hz together with an NOE correlation observed between 16-Me and 17-H established an antiperiplanar relationship between 16-H and

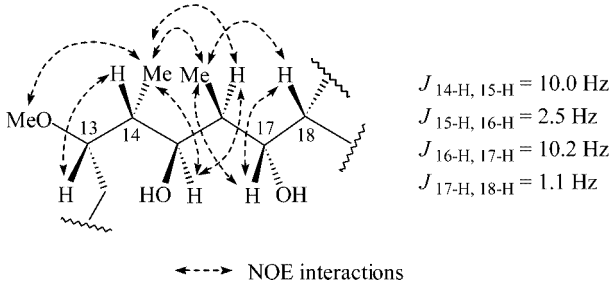

Figure 3. Relative stereochemistry of C-13 to C-17 of tuscolid (**1**)

17-H. The relative stereochemistry at C-17 was deduced from a small coupling constant of 1.1 Hz between 17-H and 18-H together with an NOE correlation observed between 18-H and both 17-H and 16-Me. Thus, the macrolide skeleton forms an extended zig-zag conformation from C-13 to C-18 of the furan-3-(2*H*)-one ring with a turn of the carbon backbone at C-13.

The molecular formula of **2** was elucidated to be $C_{30}H_{46}O_8$ from HR DCI-MS and elemental analysis, which implies eight double-bond-equivalents, and which differs from the molecular formula of tuscolid (**1**) only by loss of CO_2. The ^{13}C NMR spectrum shows two carbonyl signals and four double bonds as structural elements. The two remaining DBEs indicate the presence of two ring systems. A comparison of the ^1H and ^{13}C NMR spectroscopic data of **2** with those of **1** revealed that the structures are, in part, closely related. The structure of tuscoron A (**2**) differs from that of tuscolid (**1**) between C-8 and C-21 only by the $\Delta^{17,18}$ double bond, which is formed due to the elimination of H_2O. Instead of a macrocyclic ring, the carboxylic acid **2** possesses an open-chain structure, which is terminated by the C-21 methyl-substituted 3(2*H*)-furanone, as indicated by the $^1H,^{13}C$-long-range correlation of 22-H_3 (δ = 2.38 ppm, s) to the quaternary C-21 at δ = 184.7 ppm. Moreover, the tetrahydro-2*H*-pyran-2-one moiety of **1** is replaced by a 3,6-dihydro-2*H*-pyran ring structure. The NOE interactions between 3-H and 7-H suggested the *cis* relative configuration of the substituents at C-3 and C-7 of the dihydropyran ring as shown in Figure 4. The absence of an NOE interaction between 9-H and the methyl substituent at C-8 confirmed the (*E*) configuration of the $\Delta^{8,9}$ double bond.

Tuscoron B (**3**), with the elemental composition $C_{30}H_{48}O_9$, was isolated as an unstable component in another fermentation in minor quantities and was characterised by mass spectrometry and by ^1H and ^{13}C NMR spectroscopy. Its molecular formula differs from that of tuscoron A (**2**) only by the addition of H_2O, and the close relationship between tuscoron A and B was evident from a comparison of the ^1H and ^{13}C NMR spectroscopic data (Table 1 and 2). The only significant differences appeared in the chemical shifts of C-17 (δ = 73.0 ppm) and C-18 (δ = 87.6 ppm), and in the coupling patterns and chemical shifts

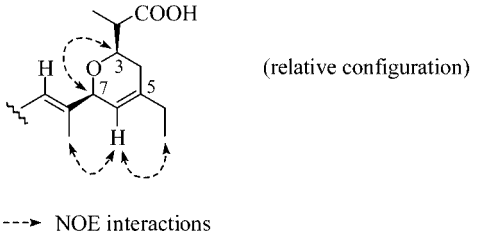

Figure 4. Selected NOE interactions in the dihydro-2*H*-pyran ring of tuscoron A (**2**)

Table 1. ^1H NMR spectroscopic data of tuscolid (**1**) and tuscoron A (**2**) and B (**3**)

H-Atom	**1**[a] δ	*J* (Hz)	Proton	**2**[a] δ	*J* (Hz)	**3**[b] δ	*J* (Hz)
2-H	2.95 (dq)	6.4, 7.3	2-H	2.49 (dq)	7.0, 7.0	2.55 (m)	
3-H	5.07 (ddd)	4.0, 6.4, 12.5	3-H	3.79 (m)		3.75 (m)	
4a-H	2.11 (m)		4a-H	1.97 (m)		1.93 (m)	
4b-H	2.31 (dd)	4.0, 13.4	4b-H	1.97 (m)		1.93 (m)	
6-H	5.67 (d)	15.6	6-H	5.47 (d)	1.5	5.39 (br. s)	
7-H	6.10 (d)	15.6	7-H	4.43 (br. s)		4.47 (br. s)	
9-H	5.35 (s)		9-H	5.41 (d)	0.9	5.41 (br. s)	
11a-H	1.89 (m)		11a-H	1.79 (m)		1.70 (m)	
11b-H	1.56 (m)		11b-H	1.63 (m)		1.70 (m)	
12a-H	1.57 (m)		12a-H	1.63 (m)		1.68 (m)	
12b-H	1.33 (m)		12b-H	1.49 (m)		1.53 (m)	
13-H	3.48 (m)		13-H	3.45 (m)		3.48 (m)	
14-H	2.03 (m)		14-H	2.06 (m)		2.03 (m)	
15-H	3.65 (dd)	2.5, 10.0	15-H	3.48 (m)		3.97 (br. d)	10.2
16-H	1.95 (m)		16-H	3.06 (ddq)	4.1, 6.8, 10.2	2.09 (m)	
17-H	3.85 (dd)	1.1, 10.2	17-H	6.15 (d)	10.2	4.08 (dd)	7.6, 2.5
18-H	4.79 (d)	1.1	18-H			4.63 (d)	2.3
20-H	5.77 (s)		20-H	5.76 (s)		5.49 (s)	
22-H_2	nd[c][d]		22-H_3	2.38 (s)		2.28 (s)	
			23-H_2	2.10 (m)		2.04 (m)	
24-H_2	1.84 (m)		24-H_3	1.09 (t)	7.5	1.03 (t)	7.6
25-H_3	0.99 (t)	7.3					
2-Me	1.33 (d)	7.3	2-Me	1.13 (d)	7.0	1.13 (d)	7.1
8-Me	2.10 (s)		8-Me	1.89 (s)		1.86 (s)	
10-Me	1.37 (s)		10-Me	1.34 (s)		1.33 (s)	
14-Me	0.75 (d)	7.0	14-Me	0.90 (d)	7.0	0.79 (d)	6.6
16-Me	0.95 (d)	6.8	16-Me	1.15 (d)	6.8	0.98 (d)	7.0
13-OCH_3	3.38 (s)		13-OCH_3	3.33 (s)		3.34 (s)	

[a] In CD_3OD at 300 MHz. [b] In $CDCl_3$ at 400 MHz. [c] nd = not detected. [d] δ_H = 3.54 and 3.78 ppm (CH_2, 2 × d, *J* = 17.0 Hz) in $CDCl_3$ at 300 MHz.

Table 2. ^{13}C NMR spectroscopic data of tuscolid (**1**) and tuscoron A (**2**) and B (**3**) and incorporation of ^{13}C label from acetate

C-Atom	δ (ppm) **1**[b]	**2**[b]	**3**[c]	[1-^{13}C]acetate **1**[d]	**2**[d]	[2-^{13}C]acetate **1**[e]	**2**[f]	[1,2-$^{13}C_2$]acetate[a] **1**	**2**
C-1	176.5	179.1	178.5	3.9	nd[g]	nd	nd		
C-2	39.9	46.9	45.0	nd	nd	3.4	6.7	37.7	
C-3	70.1	70.9	70.1	5.7	7.2	0.0	−0.1	37.0	39.2
C-4	33.0	32.6	31.5	nd	0.5	3.0	7.1	36.2	40.2
C-5	85.9	139.5	137.6	5.3	3.3	nd	−0.3		40.2
C-6	129.8	120.8	119.4	0.0	0.0	1.8	7.6	74.0	44.3
C-7	138.0	79.4	78.2	6.1	7.2	nd	0.2	74.0	46.3
C-8	134.5	137.0	136.4	nd	nd	1.3	3.8		
C-9	139.6	137.1	134.2	7.1	7.5	nd	0.2	74.7	
C-10	75.0	74.2	73.4	nd	−0.3	2.5	5.0		37.2
C-11	41.9	40.6	39.1	7.2	8.0	0.0	0.0	38.5	37.2
C-12	25.7	24.9	23.3	0.0	0.0	2.8	6.8	40.0	39.2
C-13	83.2	83.8	84.1	6.8	7.1	nd	0.4	40.0	39.2
C-14	39.4	39.1	37.0	nd	0.3	5.0	6.5	38.5	38.2
C-15	70.8	76.3	72.7	6.2	7.3	0.0	0.6	37.8	39.2
C-16	38.2	35.2	36.8	0.3	−0.2	3.9	5.7	40.0	42.3
C-17	74.5	122.2	73.0	6.4	6.9	nd	0.0	39.2	43.3
C-18	88.5	149.0	87.6	0.0	nd	3.0	1.0	42.3	
C-19	207.4	189.5	204.5	2.1	1.2	nd	nd		
C-20	108.4	107.1	105.5	−0.1	0.0	2.9	6.7	74.0	69.4
C-21	188.0	184.7	192.2	2.2	2.3	nd	nd		
C-22	nd[h]	16.1	16.9	nd	nd	nd	4.8	nd	
C-23	168.8	30.9	29.9	0.9	1.3	nd	4.3		35.2
C-24	35.1	12.5	11.9	0.4	0.4	2.8	3.3	37.7	34.2
C-25	7.7	–	–	0.3	–	1.6	–	35.5	
2-Me	13.3	13.5	13.3	0.0	nd	−0.3	0.2		
8-Me	13.4	16.0	15.5	0.9	nd	0.0	nd		
10-Me	32.1	29.3	29.7	0.0	0.2	nd	0.5		
14-Me	10.0	11.2	10.7	nd	0.2	nd	nd		
16-Me	9.1	13.8	9.2	0.0	nd	nd	0.2		
13-OMe	57.2	57.0	56.8	0.0	0.3	0.4	0.9		

[a] $^1J_{C\text{-}C}$ (Hz). [b] In CD_3OD at 75.5 MHz. [c] In $CDCl_3$ at 100.6 MHz. [d] Percentage enrichment relative to the natural abundance of C-6. [e] Percentage enrichment relative to the natural abundance of C-11. [f] Percentage enrichment relative to the natural abundance of C-17. [g] nd = not detected. [h] δ_C = 36.1 ppm in $CDCl_3$ at 75.5 MHz.

of the proton signals 17-H (δ = 4.08 ppm, dd) and 18-H (δ = 4.63 ppm, d) indicating that the $\Delta^{17,18}$ double bond of tuscoron A (**2**) could be hydroxylated as in tuscolid (**1**) to give tuscoron B (**3**). HPLC analysis with diode-array-detection of the sample of **3** after the NMR measurements in $CDCl_3$ revealed a nearly complete conversion of tuscoron B (**3**) to tuscoron A (**2**) by elimination of H_2O under acidic conditions (HCl in $CDCl_3$). This was evident from the maxima at 203 nm, 248 nm and 310 nm in the UV spectrum of tuscoron A, which were in contrast to those at 205 nm and 260 nm in the spectrum of tuscoron B. As tuscoron A had already been detected as a major component during the fermentation (pH 7.2), we can assume that it is not formed as an artifact of tuscoron B during fermenter workup or the isolation process.

The absolute stereochemistry of tuscoron A and tuscolid remains so far unresolved. Experiments were performed to synthesise the corresponding C-15 α-methoxy-α-(trifluoromethylphenyl)acetic acid (MTPA) esters of tuscoron A in order to apply Mosher's method.[7] Whereas treatment of **2** with (+)-(*S*)-methoxy(trifluoromethylphenyl)acetyl chloride (DMAP, Et_3N in CH_2Cl_2) gave the (*R*)-MTPA ester, isolated in 61% yield by preparative HPLC, the synthesis of the (*S*)-MTPA ester was, due to unknown reasons, unsuccessful under the same conditions.

Biosynthesis

The biosynthesis of tuscolid (**1**) and tuscoron A (**2**) was investigated by feeding experiments with ^{13}C-labelled precursors. The ^{13}C NMR spectra of both [1-^{13}C]acetate-derived **1** and **2** showed incorporation into the odd-numbered carbon atoms from C-3 to C-21. The even-numbered carbon atoms from C-2 to C-20 were enriched by feeding [2-^{13}C]acetate. In most instances, ^{13}C-enrichments[8] were between 2.1 and 8%, with the exceptions of C-6 (1.8%), C-8 (1.3%), and C-23 (0.9%) of tuscolid and C-18 (1.0%) and C-19 (1.2%) of tuscoron A. Moreover, C-1 and C-23 of tuscolid were labelled by [1-^{13}C]acetate and C-22 of tuscoron A by [2-^{13}C]acetate. The incorporation of intact C_2-units from [1,2-$^{13}C_2$]acetate was observed for C-2/C-3, C-6/C-7, C-12/C-13, C-14/C-15, and C-16/C-17 of tuscolid and for C-4/C-5, C-6/C-7, C-10/C-11, C-12/C-13, C-14/C-15, and C-16/C-17 of tuscoron A as indicated by the $^1J_{c\text{-}c}$ coupling constants. The explicit incorporation of intact acetate units could be only partly identified, due to low specific incorporation. Nevertheless, these results indicate the direction of

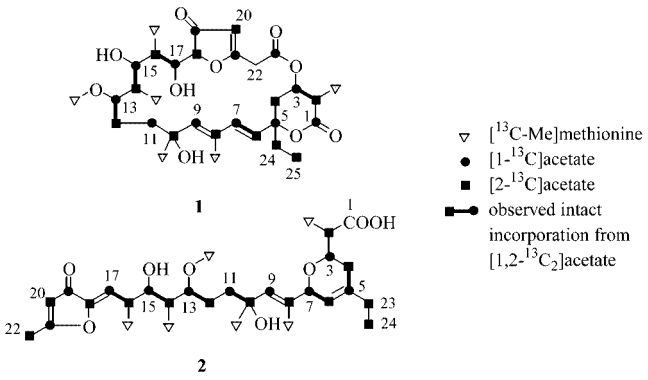

Figure 5. Incorporation of ^{13}C label from acetate and methionine

the polyketide synthesis starting at carbon atom C-1 and ending at C-23 of tuscolid (**1**) and C-22 of tuscoron A (**2**). Figure 5 summarises the labelling patterns derived from the described feeding experiments. Besides both C-25 of **1** and C-24 of **2** and the terminal C-22 methyl group of tuscoron A, all other methyl groups, including the C-13 methoxy group, showed high enrichments by feeding [methyl-^{13}C]methionine. No incorporation of label from [1-^{13}C]propionate into either **1** or **2** was found. In the [2-^{13}C]acetate feeding experiment C-24 and C-25 of tuscolid and C-23 and C-24 of tuscoron A were enriched to about 2−4% and appear as doublets with $^1J_{C\text{-}C}$ coupling constants of 35−37 Hz in the [1,2-$^{13}C_2$]acetate experiment. The origin of the ethyl group at C-5 of both **1** and **2** is most probably identical to the C-13 ethyl appendage in myxovirescin A.[9,10] Like in myxovirescin A, the ethyl groups are presumably derived from intact 2,3-C_2-units of succinate via the succinate/methylmalonate pathway.[10] Thus, feeding experiments demonstrated that tuscolid (**1**) and tuscoron A (**2**) are acetate-derived polyketides whose methyl groups originate from methionine.

The intact incorporation of a supposed three-carbon starter unit C-1 to C-3 in **1** and **2** was investigated by feeding [U-$^{13}C_3$]malonic acid. Malonate starter units were previously found for the macrolide-polyether antibiotic sorangicin A[11] produced by *Sorancium cellulosum*, strain So ce12, and for the lysolipins,[12] cycloheximide,[13] and tetracycline.[14] But neither the incorporation of [^{13}C]sodium hydrogen carbonate nor malonic acid was successful. Either no incorporation was detected, or tuscolid and tuscoron A could not be isolated in sufficient amounts.

Thus, structural and biosynthetic investigations revealed a close relationship between tuscoron A and B and tuscolid. A tentative mechanism for the conversion of tuscolid (**1**) to tuscoron A (**2**) is shown in Figure 6. The molecular formula of **2** differs from that of **1** only by CO_2. We assume that hydrolysis of the macrocyclic lactone bond of **1** and subsequent decarboxylation of the vinylogous β-keto acid provides the C-22 methyl group of the 3(2*H*)-furanone ring system of tuscoron B (**3**).[15] Nucleophilic substitution with allylic rearrangement[16] of the $\Delta^{6,7}$ double bond by an intramolecular attack of the C-3 oxygen at C-7 then results in the formation of the 3,6-dihydro-2*H*-pyran ring structure. Subsequent β-elimination of H_2O generates the $\Delta^{17,18}$

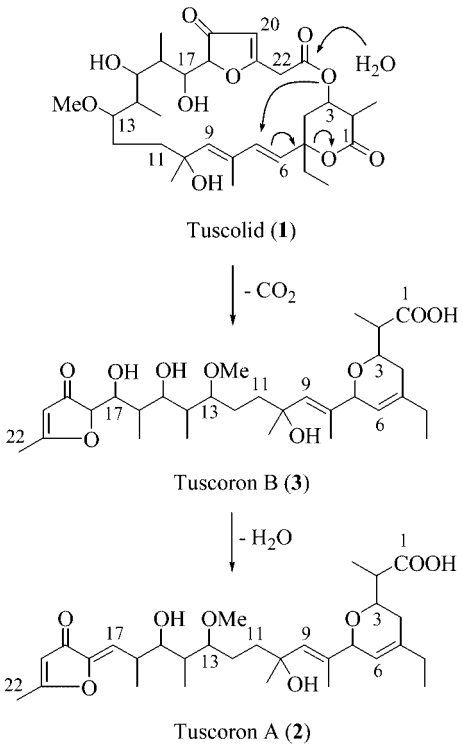

Figure 6. Tentative mechanism for the conversion of tuscolid (**1**) into tuscoron A (**2**)

double bond of **2**. HPLC analysis of the production of tuscolid and tuscoron A revealed that tuscolid formation started three days after inoculation, whereas tuscoron A was produced with a time delay of about one day, thus supporting our hypothesis that tuscolid is the precursor of tuscoron. Stability tests showed that tuscolid is stable in methanol for at least three months, but decomposes to give unidentified products in phosphate buffer solutions in the range from pH 6 to pH 8 (37 °C for 24 h). An in vitro conversion of tuscolid to tuscoron A or B could not be detected by HPLC analysis in these experiments.

1 and **2** were tested for biological activity in agar diffusion assays against a broad spectrum of bacteria, yeasts and fungi, but proved to be inactive. For both compounds no cytotoxicity was observed in assays with L929 mouse fibroblast cell culture (MIC > 5 μg/mL). Thus, a specific function of this new structural class of compounds remains uncertain.

Experimental Section

General: TLC: silica gel Si 60 F_{254} aluminium sheets, Merck; detection: UV absorption at 254 nm and staining with cerium(IV) sulfate/phosphomolybdic acid in sulfuric acid followed by charring. Analytical HPLC: 125 × 2 mm Nucleosil 100−7 C-18 with a pre-column of 11 mm (Machery−Nagel). UV detection at 254 nm and diode-array detection; eluent A: H_2O/CH_3CN, 95:5, 5 mM NH_4OAc, pH 5.5; eluent B: CH_3CN/H_2O, 95:5, 5 mM NH_4OAc, pH 5.5; gradient: 5 min with 25% B, then to 30% B in 10 min, for 5 min 30% B, then to 100% B in 10 min; flow: 0.3 mL/min. MPLC: 7 × 50 cm HD-Sil Labogel 18−30−60, 20−45 μ (Kronlab). Preparative HPLC: 16 × 250 mm Nucleosil 100−7 C-18

(Machery−Nagel). UV: Shimadzu UV/Vis scanning spectrometer UV-2102 PC, solvent methanol [Uvasol (Merck)]. IR: Nicolet FT-IR spectrometer 20DXB. Optical rotation: Perkin−Elmer Polarimeter 245. NMR: Bruker spectrometer ARX 400 (^{1}H: 400 MHz; ^{13}C: 100.6 MHz) or Bruker spectrometer AM 300 (^{1}H: 300 MHz; ^{13}C: 75.5 MHz); internal standard was the solvent signal. ESI-HPLC-MS: ET 125/2 Nucleosil 120−5 C-18 Machery−Nagel, PE Sciex Api-2000 LC/MS/MS. Mass spectrometry (DCI): Finnigan spectrometer MAT 95, resolution $M/\Delta M$ = 1000; high resolution data from peak matching ($M/\Delta M$ = 10000). Elemental analyses were carried out by Mikroanalytisches Laboratorium I. Beetz (Kronach, Germany). Sodium [1-^{13}C, 99%]acetate, sodium [2-^{13}C, 99%]acetate, sodium [1,2-$^{13}C_2$, 99%]acetate, and L-[methyl-^{13}C, 98%]-methionine were obtained from Cambridge Isotope Laboratories.

Isolation

Sorangium cellulosum, strain So ce1401, was cultivated on a 70-L scale in the presence of 1% Amberlite XAD-16 adsorber resin (Rohm & Haas). The adsorber resin was harvested by sieving, transferred to a chromatography column, and eluted with 10 L methanol. The eluate was concentrated and extracted three times with ethyl acetate. The combined organic phases were dried with sodium sulfate and concentrated to dryness. The residue was partitioned between methanol and heptane. The methanol extract was concentrated in vacuo and applied on a Sephadex LH-20 (Pharmacia) column (10 × 80 cm; eluent: methanol; flow rate 25 mL/min). Product-containing fractions were concentrated to dryness. Further fractionation in portions by MPLC (eluent A: CH_3CN/H_2O, 25:75, 25 mM NH_4OAc, pH 6; eluent B: CH_3CN/H_2O, 95:5, 10 mM NH_4OAc, pH 6; gradient: for 30 min 0% B, then to 8% B in 75 min, for 45 min 8% B, then gradient to 36% B in 60 min, and to 100% B in 60 min; flow rate 9 mL/min; detection by UV absorption at 254 nm) to give two fractions. The organic solvent was removed in vacuo and the aqueous layer extracted three times with ethyl acetate. The crude products were further purified by HPLC (eluent: acetonitrile/H_2O, 30:70, 0.05 M NH_4OAc for tuscoron A containing fractions, acetonitrile/H_2O, 40:60, 0.05 M NH_4OAc, pH 6, for tuscolid; flow rate 10 mL/min) to give tuscolid (1) (41.0 mg) and tuscoron A (2) (46.6 mg).

Tuscolid (1): Colourless amorphous solid: R_f = 0.44 (CH_2Cl_2/MeOH, 90:10); 0.29 (CH_2Cl_2/MeOH, 95:5). R_t = 15.9 min (analytical HPLC). $[\alpha]_D^{20}$ = +74.7 (c = 1.0, MeOH). UV (MeOH): λ_{max} (lg ε) = 231 nm (4.18, sh), 235 (4.22), 243 (4.06, sh), 264 (3.79). IR (KBr): $\tilde{\nu}$ = 3461 cm^{-1}, 2971, 2936, 1728, 1604, 1462, 1381, 1244, 1084, 1017, 974, 949. ^{1}H NMR see Table 1. ^{13}C NMR see Table 2. (−)-ESI-HPLC-MS: m/z = 577.0. (+)-DCI-MS (NH_3): m/z (%) = 578 [M − H_2O + NH_4]$^+$ (100), 596 [M + NH_4]$^+$ (30). $C_{31}H_{46}O_{10}$ requires 578.3329; found 578.3294 (HR-DCI MS). $C_{31}H_{46}O_{10}$ (578.33): calcd. C 64.34, H 8.02; found C 64.05, H 7.76.

Tuscoron A (2): Colourless oil: R_f = 0.40 (CH_2Cl_2/MeOH, 90:10); 0.19 (CH_2Cl_2/MeOH, 95:5). R_t = 17.2 min (analytical HPLC). $[\alpha]_D^{20}$ = +58.3 (c = 1.0, MeOH). UV (MeOH): λ_{max} (lg ε) = 203 nm (3.88), 248 (3.97), 310 (3.76). IR (KBr): $\tilde{\nu}$ = 3418 cm^{-1}, 2967, 2932, 2880, 1706, 1660, 1602, 1460, 1385, 1309, 1277, 1219, 1155, 1113, 1088, 1021, 961. ^{1}H NMR see Table 1. ^{13}C NMR see Table 2. (−)-ESI-HPLC-MS: m/z = 533.2. (−)-DCI-MS (NH_3): m/z (%) = 534 [M]$^-$ (100). $C_{30}H_{46}O_8$ requires 534.3193; found 534.3171 (HR-DCI MS). $C_{30}H_{46}O_8$ (534.32): calcd. C 67.38, H 8.68; found C 67.36; H 8.70.

A second 70-L fermentation provided, besides tuscolid (27.5 mg) and tuscoron A (68.6 mg), the unstable intermediate tuscoron B (6.0 mg). Here, eluents for MPLC were adjusted to pH 7. As HPLC eluent for tuscoron B, acetonitrile/H_2O, 30:70, 0.03 M NH_4OAc, pH 7, was used.

Tuscoron B (3): Colourless oil: R_t = 4.5 min (CH_3CN/H_2O, 35:65, 10 mM NH_4OAc). ^{1}H NMR see Table 1. ^{13}C NMR see Table 2. (−)-DCI-MS (NH_3): m/z (%) = 534 [M − H_2O]$^-$ (100), 552 [M]$^-$ (20). $C_{30}H_{48}O_8$ requires 552.3298; found 552.3254 (HR-DCI MS).

Feeding Experiments with ^{13}C-Labelled Precursors: For the feeding experiments, *Sorangium cellulosum*, strain So ce1401, was cultivated in 250 mL medium E[17] in the presence of 2% Amberlite XAD-16. The labelled precursors (0.1% w/v) were added in two equal portions on the fourth and fifth days. After 10 days of cultivation, the adsorber resin was harvested by sieving, and extracted with methanol. The extracts were evaporated, and partitioned between ethyl acetate and water. The organic solution was dried with sodium sulfate and concentrated. The residue was partitioned between methanol and heptane, and the crude products from the methanol layer were separated by preparative HPLC, eluent CH_3CN/H_2O, 30:70, 0.05 M NH_4OAc for 20 min, then 20 min with acetonitrile/H_2O 40:60, 0.05 M NH_4OAc.

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