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FULL PAPER

A New Secondary Metabolite from *Alternaria Alternata*: Structure Elucidation and Total Synthesis of Altenuic Acid IV

Dominik Kohler[a] and Joachim Podlech*[a]

To the memory of Robert Thomas

Abstract: A putative intermediate in the biosynthesis of altenuic acids I–III was isolated from *Alternaria alternata* in the 1950ies and a sample survived over the decades. This resorcylic lactone named altenuic acid IV is a composite of a resorcylic and a muconic acid. Its structure was confirmed by NMR spectroscopy and by total synthesis. Altenuic acid IV was here obtained starting with 2-bromo-4,6-dimethoxybenzene and 4-methylbenzene-1,2-diol with a total yield of 20%. The synthesis was achieved in ten steps where the longest sequence consists of seven steps. Key steps were an oxidative ring opening of a bromocresol furnishing a bromomuconate and its Suzuki coupling with a resorcylate-derived boronate.

Introduction

Mycotoxins, i.e. toxins produced by fungal molds,[1] are a serious health problem for humans and cattle. Alternariol (ALT), alternariol 9-methyl ether (AME),[2] and further resorcylic lactones or resorcylic acids are major secondary metabolites of *Alternaria* fungi (Figure 1).[3] Although the toxicity of these mycotoxins is lower than others (e.g., aflatoxins or ochratoxins),[4] infestation with *Alternaria* spp. leads to significant crop shortfalls by fouling of fruits, vegetables, juices, and other products[5] and can lead to intoxications and deseases.[6] The altenuic acids (AA I–III) are known since 1957 when they were isolated by Rosett et al. from *Alternaria tenuis* (obsolete synonym of *A. alternata*) together with altenusin.[7] The altenuic acids have identical molecular formula and are not optically active, but show different melting points, differ in the number of acidic functionalities, and give specific color reactions, e.g., in the reaction with ferric chloride. It turned out that AA I and AA II are converted into AA III when dissolved in mild alkali (NaOH) and subsequently re-acidified. Unambiguous information on the structure of AA II became available through a crystallographic analysis; it shows a unique spirocyclic structure and is present in nature as racemate (a mixture of the *R*,*R* and the *S*,*S* enantiomers).[8] Further structural information on the other altenuic acids was not accessible until 2013 when our group acquired original samples from the 1950ies, stored by the late Robert Thomas, who obtained these from the heritage of the late C. Ewart Stickings. Among these was a sample of AA III whose structure could then unambiguously be elucidated by NMR spectroscopy and by total synthesis.[9] Unfortunately, no sample of AA I survived over the decades. Its structure is still unknown, but R. Thomas made a suggestion in a personal correspondence for its constitution (Figure 1) which is based on the structural and chemical features elucidated in the original publication[7] and on the catalytic mechanism of 4-methylmuconolactone methyl-isomerase.[10] In the last years the isolation of AA II from A. alternata could be repeated[11] and methods for the chromatographic separation[12] and the analysis[12,13] of AA III have furthermore been developed.

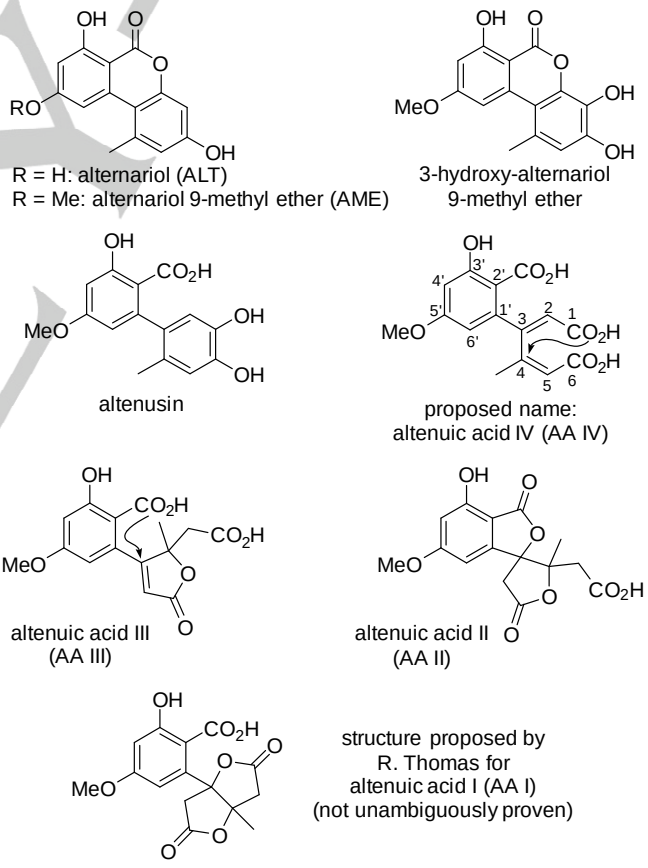

Figure 1. Selected *Alternaria* toxins.

Although the biosynthesis of the altenuic acids has never been investigated in detail, some proposals have been made over the years: AME is oxidized to 3-hydroxy-alternariol 9-methyl ether,[14] which has been isolated from *Alternaria* spp. and other fungi[14,15]

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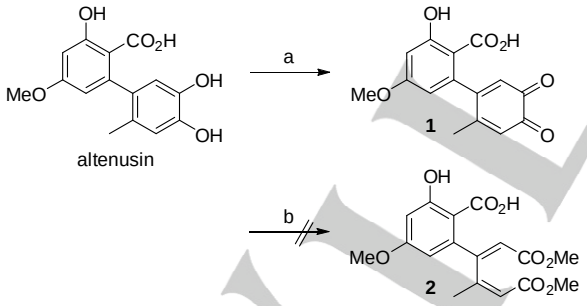
and was observed in the human metabolization of ALT and AME.[16] Reductive cleavage should in the further course lead to altenusin.[14,17] Among the above mentioned samples was a vial with about 25 milligrams of a compound which had been isolated from *A. tenuis* (*A. alternata*) together with the altenuic acids I–III. Robert Thomas suggested in a personal conversation the constitution given in Figure 1 (altenuic acid IV) and suspected it to be the biosynthetic successor of altenusin and a precursor of the altenuic acids I–III. We propose the name altenuic acid IV (AA IV) for this natural product. This compound has not been published by the original investigators since it was only obtained in small amounts not sufficient for further investigations at that time. AA IV might be biosynthesized in the fungi by oxidative cleavage of the catechol moiety in altenusin.[18] The absence of optical activity in altenuic acids I–III supports the possibility that their chiral centers might be built up by oxidation with a peroxidase[10] to free radical intermediates, which can undergo spontaneous radical coupling or cyclisation without accumulation of an excess of one enantiomer. Herein, we present the structure elucidation of AA IV and its total synthesis.

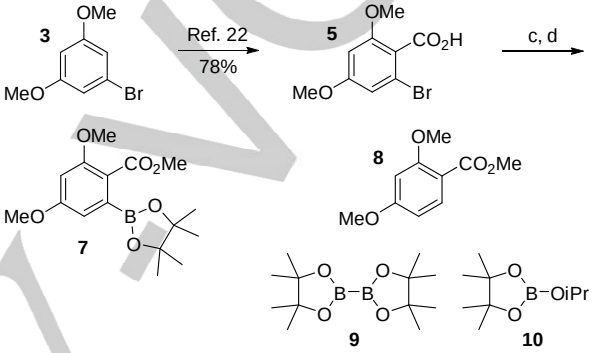
Results and Discussion

Inspired by the putative biosynthesis of the altenuic acids, we at first tried a biomimetic approach featuring an oxidative ring opening of the catechol moiety in altenusin. For this we tested a published method developed by Kaschabek et al. who transformed catechols into the corresponding muconates.[19] According to this protocol, altenusin[20] was oxidized with sodium periodate under phase transfer catalytic conditions to *ortho*-quinone **1**, which was not isolated but immediately reacted with lead(IV) acetate (Scheme 1). Nevertheless, these conditions led to unspecific decomposition of the substrate, the expected muconate **2** could not be detected in the complex product mixture.



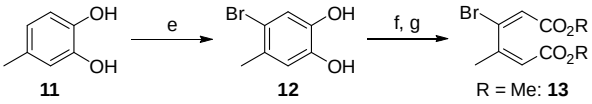
Scheme 1. Intended two-step oxidation of altenusin to altenuic acid IV. Reagents and conditions: (a) $NaIO_4$, Bu_4NBr (cat.), CH_2Cl_2/H_2O, rt, 10 min (36%); b) $Pb(OAc)_4$, MeOH, 0 °C, 1 h.

Due to the laborious synthesis of altenusin[20] used as starting material, we abandoned this approach and aimed for a different strategy, in which suitable resorcylic acid and muconic acid derivatives should be connected in a cross coupling reaction. Bases on our prior work[21] we considered a Suzuki coupling to be most promising for this key reaction. We envisaged the pinacol-derived boronate **7** which was easily obtained from 2-bromo-4,6-dimethoxybenzene **3** by Vilsmeyer-Haack formylation (→**4**), oxidation (→**5**),[22] esterification (→**6**)[23] and subsequent palladium-catalyzed Miyaura boronylation with bis(pinacolato)diboron (**9**).[24] Boronate **7** was thus accessible in 95% yield over two steps from the known resorcylic acid **5** (Scheme 2). An alternative approach using a metal-halogen interchange with butyl lithium followed by transmetalation with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**10**) was unsuccessful and led to the formation of significant amounts of defunctionalized substrate **8**.[25]



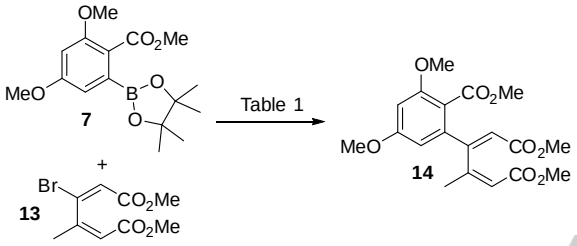
Scheme 2. Synthesis of a boronate **7** suitable for Suzuki coupling. Reagents and conditions: (c) K_2CO_3, MeI, DMF, 85 °C, 45 min (99%); (d) B_2Pin_2 (**9**) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (cat.), KOAc, dioxane, 80 °C, 20 h (96%).

A general approach to simple muconates was established via the protocol, which has already been applied by us in the unsuccessful oxidative transformation of altenusin (Scheme 3).[19] A suitable building block **13** was obtained by bromination of 4-methylcatechol (**11**) with *N*-bromosuccinimide (NBS).[26] Performing the bromination with elemental bromine led to considerable amounts (20%) of the hardly separable 3,5-dibrominated catechol side product.[27] Using the above mentioned two-step protocol with sodium periodate and lead(IV) acetate in methanol furnished the corresponding brominated dimethyl muconate **13** with 64% yield (Scheme 3).[19] Unfortunately, the analogous reaction in *tert*-butanol did not supply the respective di-*tert*-butyl muconate which could have been useful in the final liberation of the carboxylic acids with alternative reaction conditions. However, the thus obtained methyl ester groups in the resorcylic ester and in the muconate part of the aimed coupled substrate should be cleavable with base, i. e., with conditions which were expected to not harm the final altenuic acid IV.



Scheme 3. Synthesis of a bromo muconate **13**. Reagents and conditions: (e) NBS, MeCN, 0 °C → rt (quant.); (f) $NaIO_4$, Bu_4NBr (cat.), CH_2Cl_2/H_2O, rt, 20 min; g) $Pb(OAc)_4$, ROH, 0 °C, 1 h (R = Me: 64%, 2 steps; R = *t*Bu: 0%).

To achieve the cross coupling of boronate **7** and bromomuconate **13**, we tested a method which had been successfully applied in the total synthesis of altenusin.[20] Nevertheless, utilization of palladium acetate as a catalyst with the ligand 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos) and cesium carbonate as a base did not yield the coupled product **14** but furnished debrominated product **8**. Only a poor 6% yield was obtained with potassium phosphate as a base[28] and the catalysts 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride,[29] bis(triphenylphosphine)palladium(II) dichloride,[30] or tris(dibenzylideneacetone)dipalladium(0)[31] gave either no conversion or a poor yield not exceeding 12 % (Scheme 4 and Table 1). An acceptable yield of 36% could only be achieved when the boronate **7** was transferred into the respective trifluoroborate. Palladium(II) acetate was utilized as a catalyst in this protocol.[32]



Scheme 4. Cross coupling to protected altenuic acid IV.

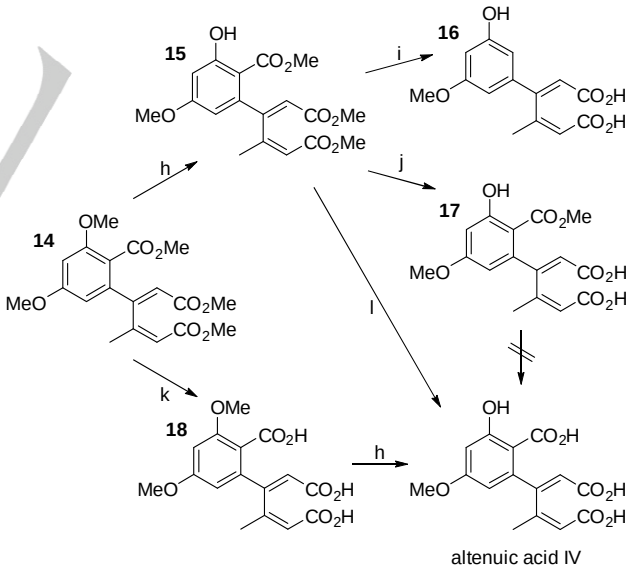
Table 1. Conditions used in the intended cross coupling.

#	Conditions	Yield [%]	Ref.
1	$Pd(OAc)_2$, SPhos,[a] Cs_2CO_3, dioxane/H_2O, 80 °C, 20 h	–[b]	[20]
2	$Pd(OAc)_2$, SPhos, K_3PO_4, THF, 80 °C, 72 h	6	[28]
3	$Pd(dppf)Cl_2 \cdot CH_2Cl_2$, NEt_3, THF/H_2O, 60 °C, 18 h	traces	[29]
4	$PdCl_2(PPh_3)_2$, Na_2CO_3, THF/H_2O, 80 °C, 72 h	–	[30]
5	$Pd_2(dba)_3$, K_2CO_3, toluene, 80 °C, 72 h	12	[31]
6	$Pd(OAc)_2$, KF, dioxane, 80 °C, 72 h	36	[32]

[a] SPhos: 2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl; [b] Only defunctionalized product **8** was obtained.

We further considered a vice versa approach in which bromide **5** should be cross coupled with a boronate accessible from bromomuconate **13** by boronylation. Nevertheless, no attempts for the replacement of the bromine in **13** with a boronate group turned out to be successful.[33]

Three methyl esters and one methyl ether had finally to be cleaved in the coupled product **14** to obtain altenuic acid IV (Scheme 5). Especially the high polarities of the natural product and of possible intermediates made this endeavor more challenging than expected. An attempt for simultaneous cleavage of all four protecting groups in **14** with nine equivalents of boron tribromide lead to a statistical cleavage of the methyl groups, which could not be improved with more harsh conditions or longer reaction times.[34] When the methyl ether was initially deprotected with boron tribromide (2.2 equivalents), it turned out that the subsequent trial for the complete saponification of the ester groups in phenol **15** was not successful:[35] Either the mono methyl ester **17** or a decarboxylated product **16** was observed when methanolic sodium or potassium hydroxide, respectively, were used as reagents. A completion of the reaction by further application of saponification conditions to mono methyl ester **17** failed – most probably due to a poor reactivity of deprotonated carboxylate **17**, a trianion. To avoid the observed decarboxylation, we decided to at first perform the threefold saponification (→**18**) followed by ether cleavage. This sequence could be performed successfully with standard conditions and yielded the deprotected tricarboxylic acid, albeit with a poor 28% yield over two steps. Nevertheless, altenuic acid IV was finally obtained by saponification of phenol **15** with 6N aqueous sodium hydroxide, where this final step was now achieved with an excellent 92% yield.



Scheme 5. Liberation of altenuic acid IV. Reagents and conditions: (h) BBr_3, CH_2Cl_2, –78 °C → rt, 75 min (**15**: 82%, **AA IV**: 33%); (i) KOH, MeOH, 50 °C, 18 h, then 90 °C, 24 h (52%); (j) NaOH, MeOH, 50 °C, 18 h, then 90 °C, 24 h (quant.); (k) NaOH, MeOH, 50 °C, 24 h (86%); (l) 5M NaOH, 55 °C, 15 h (92%).

Altenuic acid IV was thus obtained starting with commercially available 2-bromo-4,6-dimethoxybenzene and 4-methylbenzene-

1,2-diol with a total yield of 20%. The total synthesis was achieved in ten steps while the longest sequence has consisted of seven steps.

The target compound synthesized turned out to be identical with the isolated natural product altenuic acid IV, which could be proven by comparison of the NMR spectra and by measurement of a 1:1 mixture of synthesized material and the natural product. The NMR-spectroscopic data are given in Table 2. The ^{13}C signal of the quaternary C-2' carbon was not detected in standard NMR experiments, obviously due to a prolonged relaxation time T_1. Nevertheless, the signal could be exposed as cross peak in an HMBC experiment with higher relaxation delay d_1 and suppression of ^1J couplings.[36] While the constitution of the compound is unambiguously derived after evaluation of ^1H, ^{13}C, ^1H-^{13}C HSQC, and ^1H-^{13}C HMBC spectra, the double bonds' configuration kept inconclusive. If the atom's positions are maintained during the oxidation process (Scheme 3), altenuic acid IV should have a 2*E*,4*Z* configuration, which admittedly could be foiled by concomitant or subsequent double bond isomerizations, where this must have similarly happened during the synthesis *and* the biosynthesis. Nevertheless, Kaschabek et al. clearly showed that the oxidation of the catechols is obtained with preservation of the configuration.[17] An nOe from 4-CH_3 to the vicinal proton 5-H gave further evidence at least for the preservation of the 4*Z* configuration.

Table 2. NMR data of altenuic acid IV.[a]

Position[b]	δ[ppm] [c,d]	δ[ppm][c,e]	Position[b]	δ[ppm][c,e]
4-C*H*$_3$	2.32 (d, ^4J = 0.9)		C-6'	109.1
OC*H*$_3$	3.78 (s)		C-2	119.8
5-H	5.29 (d, ^4J = 0.9)		C-5	121.8
6'-H	6.03 (d, ^4J = 2.5)		C-1'	142.1
2-H	6.18 (s)		C-3	152.8
4'-H	6.47 (d, ^4J = 2.5)		C-4	155.3
			C-5'	162.9
4-*C*H$_3$		15.4	C-3'	164.9
O*C*H$_3$		55.5	C-1	166.7
C-4'		100.0	C-6	167.4
C-2'		106.9[f]	2'-*C*O$_2$H	171.9

[a] Assignment was made according to ^1H, ^{13}C, ^1H-^{13}C HSQC, and ^1H-^{13}C HMBC spectra. [b] Numbering see Figure 1. [c] Signals are given in increasing order. [d] ^1H NMR (400 MHz, DMSO-d$_6$); multiplicities and coupling constants [Hz] are given in parentheses. [e] ^{13}C NMR (100 MHz, DMSO-d$_6$). [f] Signal is not detected in standard experiments due to a high T_1 relaxation time. It was visible as cross peak in an HMBC experiment with higher relaxation delay d_1 and suppression of ^1J couplings.

Conclusions

The structure of an authentic sample of a fungal metabolite called altenuic acid IV (AA IV) was elucidated by NMR spectroscopy and by total synthesis. It seems quite obvious (albeit not proven) that AA IV is a metabolic intermediate resulting from oxidative cleavage of altenusin. Intramolecular oxy-Michael-type additions would further lead to altenuic acids I–III.

Experimental Section

2-Bromo-4,6-dimethoxybenzaldehyde (4).[22] Freshly distilled $POCl_3$ (3.15 mL, 5.29 g, 34.5 mmol) was added dropwise at 0 °C under Ar to a solution of 2-bromo-4,6-dimethoxybenzene (**3**, 3.00 g, 13.8 mmol) in anhydrous DMF (8 mL). The mixture was stirred for 4 h at 100 °C, poured on ice water (500 mL), and left for 16 h. The precipitate was collected by filtration, washed with H_2O (50 mL) and dried in high vacuum. The crude product was recrystallized (hexane/EtOAc, 2:1) to yield **4** (2.26 g, 9.22 mmol, 67%) as a colorless solid; R_f 0.40 (hexane/EtOAc, 2:1); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.87 (s, 3 H, Me), 3.89 (s, 3 H, Me), 6.43 (d, ^4J = 2.1 Hz, 1 H, Ar-H), 6.78 (d, ^4J = 2.1 Hz, 1 H, Ar-H), 10.31 (s, 1 H, CHO).

2-Bromo-4,6-dimethoxybenzoic Acid (5).[22] A solution of $KMnO_4$ (1.42 g, 8.99 mmol) in H_2O (75 mL) was added at 75 °C within 40 min to an emulsion of carbaldehyde **4** (1.37 g, 5.59 mmol) in H_2O (50 mL). The mixture was stirred for 2 h at that temperature and the pH was raised to 13 by slow addition of aqueous KOH solution (20%). The mixture was filtered (Celite), the filtrate was acidified with 2N HCl and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield **5** (1.16 g, 4.44 mmol, 79%) as a colorless solid; R_f 0.59 (CH_2Cl_2/MeOH/AcOH, 100:10:1); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.82 (s, 3 H, OMe), 3.86 (s, 3 H, OMe), 6.44 (d, ^4J = 2.1 Hz, 1 H, Ar-H), 6.74 (d, ^4J = 2.1 Hz, 1 H, Ar-H).

Methyl 2-Bromo-4,6-dimethoxybenzoate (6).[23] A solution of benzoic acid **5** (420 mg, 1.61 mmol), K_2CO_3 (334 mg, 2.42 mmol), and MeI (0.150 mL, 342 mg, 2.41 mmol) in DMF (5 mL) was stirred for 45 min at 85 °C. 1N HCl (10 mL) was added and the organic layer was separated and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield **6** (438 mg, 1.59 mmol, 99%) as a dark red oil; R_f 0.18 (hexane/EtOAc, 2:1); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.79 (s, 6 H, 2×OMe), 3.91 (s, 3 H, CO_2Me), 6.40 (d, ^4J = 2.1 Hz, 1 H, Ar-H), 6.68 (d, ^4J = 2.1 Hz, 1 H, Ar-H).

Methyl 2,4-Dimethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (7). Benzoate **6** (304 mg, 1.11 mmol), KOAc (271 mg, 2.76 mmol), and bis(pinacolato)diboron (**9**, 309 mg, 1.21 mmol) were dissolved under argon in anhydrous and degassed dioxane (6 mL). $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (39 mg, 0.048 mmol) was added under an argon flow. The mixture was stirred at 80 °C for 20 h, filtered (Celite), and purified by column chromatography (silica gel, hexane/EtOAc, 4:1) to yield **7** (345 mg, 1.07 mmol, 96%) as a yellow oil which contained traces of pinacol; R_f 0.54 (hexane/EtOAc, 2:1); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.33 (s, 12 H, Me_2CCMe_2), 3.81 (s, 3 H, OMe), 3.83 (s, 3 H, OMe), 3.86 (s, 3 H, OMe), 6.49 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 6.71 (d, ^4J = 2.3 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 24.9 (*Me*$_2$C*C*Me$_2$), 52.4 (OMe), 55.6 (OMe), 56.1 (OMe), 84.1 (Me$_2$*C*CMe$_2$), 101.0 (CH), 109.2 (CH), 119.1 (C), 158.8 (C), 162.4 (C), 169.2 (C); IR (ATR): \tilde{\nu } (cm^{-1}) = 2977 (w), 1735 (w), 1591 (w), 1452 (w), 1422 (w), 1356 (m), 1325 (w), 1296 (w), 1265 (w),

1215 (w), 1191 (w), 1145 (m), 1098 (w), 1050 (w), 850 (w); MS (ESI, pos.): *m/z* (%) = 345 (100) [M^++Na], 344 (24), 340 (21), 339 (87), 245 (38).

4-Bromo-5-methylbenzene-1,2-diol (12). A solution of NBS (7.16 g, 40.2 mmol) in MeCN (100 mL) was added slowly at 0 °C to a solution of 4-methylbenzene-1,2-diol (**11**, 5.00 g, 40.3 mmol) in MeCN (200 mL). The mixture was warmed to room temperature overnight and poured in 1N HCl (200 mL). The aqueous layer was extracted with EtOAc (3×200 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield **12** (8.18 g, 40.3 mmol, quant.) as a brownish solid; R_f 0.64 (hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, acetone-d_6): δ (ppm) = 2.19 (s, 3 H, Me), 6.79 (s, 1 H, Ar-H), 6.99 (s, 1 H, Ar-H).

Dimethyl (2*E*,4*Z*)-3-Bromo-4-methylhexa-2,4-dienedioate (13). A solution of $NaIO_4$ (3.32 g, 15.5 mmol) in H_2O (150 mL) was added to a solution of cresol **12** (2.72 g, 13.4 mmol) and Bu_4NBr (TBAB, a spatula tip) in CH_2Cl_2 (100 mL). The mixture was stirred vigorously for 20 min, the phases were separated and the organic layer was dried ($MgSO_4$). The *ortho*-quinone (still dissolved in CH_2Cl_2) was added immediately at 0 °C to a solution of $Pb(OAc)_4$ (7.30 g, 16.5 mmol) in anhydrous MeOH (150 mL) and the mixture was stirred for 1 h in the dark. The solvents were removed under reduced pressure and the remnant was extracted with hexane/EtOAc (1:1, 4×100 mL). The combined organic layers were stirred with an excess of a saturated $NaHCO_3$ solution (evolution of CO_2), washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure to yield **13** (2.27 g, 8.63 mmol, 64%) as a red oil; R_f 0.65 (hexane/EtOAc, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.10 (d, ^3J = 1.5 Hz, 3 H, Me), 3.67 (s, 3 H, CO_2Me), 3.69 (s, 3 H, CO_2Me), 5.72 (d, ^3J = 1.4 Hz, 1 H, CH), 6.35 (s, 1 H, CH); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 22.3 (4-Me), 51.2 (OMe), 51.5 (OMe), 117.5 (CH), 122.0 (CH), 140.0 (C), 152.6 (C), 163.7 (C), 164.6 (C); IR (ATR): \tilde{\nu } (cm^{-1}) = 3278 (w), 2951 (w), 1724 (m), 1656 (m), 1609 (m), 1487 (w), 1431 (m), 1367(w), 1280 (m), 1223 (m), 1191 (m), 1168 (s), 1131 (m), 1065 (m), 1042 (m), 1005 (m), 954 (m), 918 (m), 865 (m), 806 (w), 759 (m), 699 (w), 666 (m), 605 (w), 539 (w), 453 (w); MS (EI, 20 °C): *m/z* (%) = 263 (1) [M^+(^{81}Br)], 262 (1) [M^+(^{80}Br)], 261 (1) [M^+(^{79}Br)], 233 (10), 206 (6), 205 (73), 203 (76), 183 (100) [$C_9H_{11}O_4^+$], 151 (12) [$C_8H_7O_3^+$], 137 (22) [$C_8H_9O_2^+$], 95 (11), 93 (14) [$C_6H_5O^+$], 69 (14), 59 (13); HMRS (EI, 20 °C): M^+, found 261.9836. $^{12}C_9{}^1H_{11}{}^{16}O_4{}^{79}Br$ requires 261.9835.

Dimethyl (2*E*,4*Z*)-3-(3,5-Dimethoxy-2-(methoxycarbonyl)phenyl)-4-methylhexa-2,4-dienedioate (14). KF (83.1 mg, 1.43 mmol) was added to boronate **7** (154 mg, 0.478 mmol) dissolved in anhydrous dioxane (1 mL) in a pyrex tube and the mixture was stirred for 10 min at room temperature. A solution of muconate **13** (83.3 mg, 0.317 mmol) and $Pd(OAc)_2$ (1.7 mg, 7.6 µmol) in anhydrous dioxane (1 mL) was added and the transfer was completed by rinsing with further anhydrous dioxane (0.5 mL). The mixture was stirred at 80 °C for 72 h, filtered (Celite) and rinsed with EtOAc (7.5 mL). The filtrate was concentrated under reduced pressure and purified by column chromatography (hexane/EtOAc, 2:1) to yield **14** (42.7 mg, 0.113 mmol, 36%) as a yellow oil; R_f 0.23 (hexane/EtOAc, 2:1); ^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) = 2.39 (d, ^4J = 1.2 Hz, 3 H, Me), 3.54 (s, 3 H, CO_2Me), 3.61 (s, 3 H, CO_2Me), 3.63 (s, 3 H, CO_2Me), 3.84 (s, 3 H, OMe), 3.85 (s, 3 H, OMe), 5.63 (d, ^4J = 1.2 Hz, 1 H, C = CH), 6.29 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 6.33 (s, 1 H, C = CH), 6.64 (d, ^4J = 2.2 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) = 15.5 (Me), 51.4 (Me), 51.5 (Me), 51.8 (Me), 55.9 (Me), 56.3 (Me), 98.7 (CH), 107.3 (CH), 121.8 (CH), 123.8 (CH), 139.8 (C), 153.6 (C), 155.2 (C), 159.6 (C), 162.5 (C), 165.8 (C), 167.1 (C), 167.3 (C); IR (ATR): \tilde{\nu } (cm^{-1}) = 2950 (w), 1715 (s), 1597 (m), 1431 (m), 1366 (w), 1342 (w), 1264 (m), 1195 (s), 1156 (s), 1097 (m), 1044 (m), 945 (w), 865 (w), 836 (w), 599 (w); *m/z* (%) = 378 (4) [M^+], 377 (7) [M^+–H], 359 (26), 348 (19), 347 (100) [$C_{18}H_{19}O_7^+$], 221 (12); HRMS (ESI, pos.): M^+, found 378.1299. $^{12}C_{19}{}^1H_{22}{}^{16}O_8$ requires 378.1309.

(2*E*,4*Z*)-3-(2-Carboxy-3,5-dimethoxyphenyl)-4-methylhexa-2,4-dienoic Acid (18). 1N NaOH (1.47 mL) was added to a solution of the triester **14** (55.6 mg, 0.147 mmol) in MeOH (5 mL) and the mixture was stirred at 50 °C for 24 h, acidified (pH 1) by addition of 1N HCl, and diluted with H_2O to a total of 10 mL. The aqueous layer was extracted with EtOAc (3×10 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The remnant was dissolved in 2.5N NaOH (1.6 mL), stirred at 90 °C for 24 h, cooled to room temperature, diluted with H_2O to a total of 15 mL, and washed with EtOAc (2×15 mL). The aqueous layer was acidified with 6N HCl and extracted with EtOAc (3×20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield **18** (42.5 mg (0.126 mmol, 86%) as a light brown solid; R_f 0.11 (CH_2Cl_2/MeOH/AcOH, 100:10:1); ^{1}H NMR (400 MHz, methanol-d_4): δ (ppm) = 2.37 (d, ^4J = 1.1 Hz, 3 H, Me), 3.83 (s, 3 H, OMe), 3.89 (s, 3 H, OMe), 5.58 (d, ^4J = 1.1 Hz, 1 H, C = CH), 6.27 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 6.39 (s, 1 H, C = CH), 6.27 (d, ^4J = 2.2 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, methanol-d_6): δ (ppm) = 15.8 (4-Me), 56.1 (Me), 56.6 (Me), 99.1 (CH), 108.2 (CH), 122.2 (CH), 124.6 (CH), 141.4 (C), 153.8 (C), 156.5 (C), 160.6 (C), 164.5 (C), 169.0 (C), 169.7 (C), 169.8 (C); IR (ATR): \tilde{\nu } (cm^{-1}) = 2919 (w), 2849 (w), 1681 (w), 1593 (m), 1459 (w), 1425 (w), 1363 (w), 1334 (w), 1286 (w), 1216 (w), 1157 (w), 1088 (w), 1034 (w), 867 (w), 838 (w), 819 (w), 426 (w); MS (ESI, neg.): *m/z* (%) = 335 (27) [M^-–H], 291 (42) [$C_{15}H_{15}O_6^-$], 248 (15), 247 (100) [$C_{14}H_{15}O_4^-$], 203 (8) [$C_{13}H_{15}O_2^-$]; HMRS (ESI, neg.): M^-–H, found 335.0771. $^{12}C_{16}{}^1H_{15}{}^{16}O_8$ requires 335.0767.

Dimethyl (2*E*,4*Z*)-3-[3-Hydroxy-5-methoxy-2-(methoxycarbonyl)phenyl]-4-methylhexa-2,4-dienedioate (15). BBr_3 (1M in CH_2Cl_2, 1.04 mL, 1.04 mmol) was added under argon at –78 °C to a solution of triester **14** (17.9 mg, 47.3 µmol) in anhydrous CH_2Cl_2 (4 mL). The mixture was stirred for 30 min at –78 °C and 45 min at room temperature and poured into saturated aqueous NH_4Cl solution (5 mL). The aqueous layer was extracted with CH_2Cl_2 (3×5 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield **15** (14.1 mg, 38.7 µmol, 82%) as a light brown solid; R_f 0.43 (hexane/EtOAc, 2:1); ^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) = 2.49 (d, ^4J = 1.1 Hz, 3 H, 4-Me), 3.52 (s, 3 H, OMe), 3.60 (s, 3 H, OMe), 3.76 (s, 3 H, OMe), 3.87 (s, 3 H, OMe), 5.48 (d, ^4J = 1.1 Hz, 1 H, 2-H or 4-H), 6.16 (d, ^4J = 2.5 Hz, 1 H, Ar-H), 6.33 (s, 1 H, 4-H or 2-H), 6.53 (d, ^4J = 2.5 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) = 15.6 (4-Me), 51.4 (OMe), 51.5 (OMe), 56.1 (OMe), 101.0 (CH), 110.9 (CH), 119.7 (CH), 122.6 (CH), 142.7 (C), 154.1 (C), 157.7 (C), 165.2 (C), 165.8 (C), 166.1 (C), 167.1 (C), 171.2 (C); IR (ATR): \tilde{\nu } (cm^{-1}) = 2951 (w), 1710 (w), 1652 (w), 1604 (w), 1572 (w), 1433 (w), 1377 (w), 1331 (w), 1248 (w), 1193 (m), 1153 (m), 1037 (w), 1011 (w), 994 (w), 953 (w), 861 (w), 804 (w), 757 (w), 712 (w), 626 (w); MS (ESI, pos.): *m/z* (%) 365 (20) [M^++H], 364 (3) [M^+], 351 (11) 334 (18), 333 (100) [$C_{17}H_{17}O_7^+$], 319 (22) [$C_{16}H_{15}O_7^+$], 221 (19); HMRS (ESI, pos.): M^+, found 364.1143. $^{12}C_{18}{}^1H_{20}{}^{16}O_8$ requires 364.1158.

(2*E*,4*Z*)-3-(2-Carboxy-3-hydroxy-5-methoxyphenyl)-4-methylhexa-2,4-dienoic Acid, Altenuic Acid IV. A solution of phenol **15** (13.3 mg, 36.5 µmol) in 5M aqueous NaOH (3 mL) was stirred for 15 h at 55 °C. The mixture was carefully acidified with 6M HCl (5 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield AA IV (10.8 mg, 33.5 µmol, 92%) as a brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 2.32 (d, ^4J = 0.9 Hz, 3 H, 4-Me), 3.78 (s, 3 H, OMe), 5.29 (d, ^4J = 0.9 Hz, 1 H, 5-H), 6.03 (d, ^4J = 2.5 Hz, 1 H, 6'-H), 6.18 (s, 1 H, 2-H), 6.47 (d, ^4J = 2.5 Hz, 1 H, 4'-H), 12.7 (br s, 3 H, 3 CO_2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) = 15.4 (4-Me), 55.5 (OMe), 100.0 (CH), 109.0 (CH), 119.8 (CH), 121.8 (CH), 142.1 (C), 152.8 (C), 155.3 (C),162.9 (C), 164.9 (C), 166.7 (C), 167.4 (C), 171.9 (C); IR (ATR): \tilde{\nu } (cm^{-1}) = 2945 (w), 1685 (w), 1602 (m), 1442 (w), 1365 (w), 1231 (m), 1200 (m), 1158 (m), 1074 (w), 1037 (m),

867 (w), 838 (w), 704 (w), 606 (w), 427 (w); MS (EI, 220 °C): *m/z* (%) 322 (20) [M^+], 278 (52) [$C_{14}H_{14}O_6^+$], 192 (100) [$C_{10}H_8O_4^+$]; HMRS (EI, 220 °C): M^+, found 322.0684. $^{12}C_{15}{}^{1}H_{14}{}^{16}O_8$ requires 322.0683.

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A missing link in the putative biosynthesis of the altenuic acids I–III has been identified and its structure was confirmed by total synthesis. It could be produced in the fungus *Alternaria alternata* by oxidative ring opening of altenusin.

Mycotoxins

*Dominik Kohler, Joachim Podlech**

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