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Alternethanoxins A and B, Polycyclic Ethanones Produced by *Alternaria sonchi*, Potential Mycoherbicides for *Sonchus arvensis* Biocontrol

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Alternaria sonchi is a fungal pathogen isolated from *Sonchus arvensis* and proposed as a biocontrol agent of this noxious perennial weed. Different phytotoxic metabolites were isolated from solid culture of the fungus. Two new polycyclic ethanones, named alternethanoxins A and B, were characterized using essentially spectroscopic and chemical methods. Tested by leaf disk-puncture assay on the fungal host plant and a number of nonhost plants, alternethanoxins A and B were shown to be phytotoxic, whereas they did not possess antimicrobial activity tested at 100 μg/disk. Hence, alternethanoxins A and B have potential as nonselective natural herbicides. Some structure−activity relationship observations were also made.

KEYWORDS: ***Sonchus arvensis*; *Alternaria sonchi*; phytotoxins; polycyclic ethanones; alternethanoxins A and B; bioherbicides**

INTRODUCTION

Perennial weeds are common problems in different crops. They are especially harmful in agricultural systems with reduced herbicide usage because of their tolerance to traditional mechanical control methods. Such a typical plant species is *Sonchus arvensis* L., commonly called perennial sowthistle (*1*, *2*). This weed grows vigorously and forms dense patches. It spreads with underground shoots growing horizontally that give rise to new aerial shoots. Prolific seed production by *S. arvensis* serves for the occupation of new area. This plant species is considered to be an important weed in Europe and North America as it infests many habitats such as cultivated fields, roadsides, pastures and rangelands, railway embankments, and lawns. It is not easy to eradicate *S. arvensis*, and all control methods require follow-up: combinations of mechanical, cultural, and chemical methods are more effective than any single method used alone (*3*). Herbicides recommended for chemical control of *S. arvensis* in nonorganic cropping systems are restricted to a few active substances (clopyralid, dicamba, chlorsulfuron, bentazon, phenoxy acids) (*2*,*4*,*5*). Obviously, new compounds should be actually developed as herbicides against this weed.

Microbial phytotoxins or their synthetic analogues could be used for the development of new agrochemicals against weeds (*6*, *7*). Many plant pathogens, especially necrotrophic and hemibiotrophic fungi, are capable of producing phytotoxins responsible for disease development (*8*). Therefore, appropriate weed pathogens can be a source of such useful metabolites. For instance, potent phytotoxins were isolated from culture filtrate or mycelia of some mycoherbicidal fungi belonging to the genera *Alternaria*, *Ascochyta*, *Drechslera*, *Ophiobolus*, *Phoma*, and many others (*9*, *10*). Recently, the fungus *Alternaria sonchi* has been evaluated as a possible biocontrol agent of sowthistle (*11*). Species belonging to the genus *Alternaria* are known to produce bioactive metabolites, including nonhost phytotoxins, for example, solanapyrones, isolated from cultures of *A. solani*, the causal agent of early blight of tomato and potato (*12*); dextrusins and cyclodepsipeptides, isolated from *A. brassicae*, which causes diseases on numerous oil-yielding, vegetable, condiment, ornamental, and wild and some cultivated and wild noncruciferous plants (*13*); brefeldin and α,β-dehydrocurvularin isolated from *A. zinniae* (*14*) and several phytotoxins belonging to different groups of natural compounds including toxic tetramic acids, dibenzo[α]pyrone moiety containing compounds, and alternatoxins I and II (*15*, *16*). Phytotoxins produced by *A. sonchi* have not been studied so far. Considering the interest in bioactive metabolites produced by weed pathogens as sources of novel natural herbicides, it seemed interesting to investigate the production of toxins by this species of *Alternaria*.

This study was undertaken to isolate, elucidate the structures of, and characterize the biological activity of two new phytotoxic polycyclic ethanones produced in solid culture by *A. sonchi*, named alternethanoxins A and B (**1** and **2**). Their structure was determined by extensive use of spectroscopic (essentially NMR and MS techniques) and chemical methods.

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Table 1. ^{1}H and ^{13}C NMR Data of Alternethanoxins A (**1**) and B (**2**)[a,b]

	1			2		
compd position	δC m[c]	δH	HMBC	δC m[c]	δH	HMBC
1	148.8 s		MeCO	148.8 s		MeCO
2	128.6 s			112.4 s		HOC-C(3), H-3, MeCO
3	109.5 d	6.21 s	MeCO	107.2 d	6.73 s	HOC-C(3), MeCO
4	160.0 s		H-3	161.1 s		HOC-C(3)
5	160.0 s			155.6 s		H-3
6	109.5 d	6.21 s		111.4 d	6.62 s	
6a	153.0 s		H-10, H-9	150.8 s		H-8, H-9
7	167.3 s		H-8, OMe	169.2		H-8, OMe
8	122.2 d	7.46 d (*J* = 7.5 Hz)	H-10, H-9	125.4 d	7.36 d (*J J*=9.0 Hz)	H-9
9	131.0 d	7.34 dd (*J* = 7.5 and 7.1 Hz)	H-8	122.2 d	7.47 d (*J* = 9.0 Hz)	H-8
10	121.4 d	7.11 d (*J* = 7.1 Hz)	H-8	152.2 s		
10a	130.4 s		H-9	155.6 s		H-9
10b	109.8 s		H-6 and/or H-3	118.6 s		
11						
12						
13						
MeO	52.5 q	3.62 s		53.2 q	4.00 s	
MeCO	198.7 s		H-3	180.1 s		H-3
MeCO	22.0 q	2.23 s	H-3	22.7 q	2.43 s	H-3

[a] The chemical shifts are in δ values (ppm) from TMS. [b] 2D ^{1}H, ^{1}H (COSY) ^{13}C, ^{1}H (HSQC) NMR experiments delineated the correlations of all the protons and the corresponding carbons. [c] Multiplicities were assigned by DEPT spectra.

MATERIALS AND METHODS

General Experimental Procedure. Optical rotation was measured in $CHCl_3$ solution on a JASCO (Tokyo, Japan); IR spectra were recorded as neat on a Perkin-Elmer (Norwalk, CT) Spectrum One FT-IR spectrometer, and UV spectra were taken in MeCN solution on a Perkin-Elmer spectrophotometer. ^{1}H spectra were recorded at 600 and 400 MHz, in $CDCl_3$ on Bruker (Kalsrhue, Germany) spectrometers. ^{13}C NMR spectra were recorded at 150, 100, and 75 MHz, in the same solvent and using the same instruments. The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT experiments (*17*). DEPT, COSY-45, TOCSY, HSQC, HMBC, and NOESY experiments (*17*) were performed using Bruker microprograms. ESI and HRESI MS spectra were recorded on Waters Micromass and Agilent coupled to JEOL AccuTOF (Milford, MA) instruments. Analytical and preparative TLC was performed on silica gel (Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively, Merck, Darmstadt, Germany) or reverse phase (Whatman, KC18 F_{254}, 0.20 mm, Maidstone, U.K.) plates; the spots were visualized by exposure to UV light or by spraying first with 10% H_2SO_4 in methanol and then with 5% phosphomolybdic acid in ethanol, followed by heating at 110 °C for 10 min. For column chromatography (CC) Kieselgel 60, 0.063−0.200 mm, was used (Merck, Darmstadt, Germany).

Fungus. The fungus *A. sonchi* Davis was isolated from diseased leaves of *S. arvensis*, and monoconidial isolate (S-102) was deposited in the culture collection of All-Russian Research Institute of Plant Protection, Pushkin, Saint Petersburg, Russia. The isolate was maintained in sterile tubes containing potato dextrose agar.

Production, Extraction, and Purification of Alternethanoxins A and B (1 and 2). *A. sonchi* was grown on autoclaved pearl barley in 10 1000-mL Erlenmeyer flasks (pearl barley, 100 g; water, 60 mL) for 21 days in darkness. Fungal metabolites were extracted from dry mycelium according to a slightly modified protocol of Evidente et al. (*18*). The dried material was extracted with a mixture of acetone/2% NaCl (1:1, 2 L). After evaporation of acetone, the aqueous residue was extracted with EtOAc (3 × 500 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure, yielding a brown oily residue (975 mg). The organic extract, showing high phytotoxicity, was purified by silica gel column chromatography eluted with the $CHCl_3$/*i*-PrOH (9:1, v/v), to give 11 groups of homogeneous fractions. Fractions were tested for bioactivity against *S. arvense* as described below, and those showing phytotoxicity were further purified. The residue (174.7 mg) of the fourth fraction was purified by silica gel column, eluted with $CHCl_3$/*i*-PrOH (95:5, v/v), to yield five fractions. The residue (88 mg) of the second fraction was purified by preparative TLC on silica gel [eluent $CHCl_3$/*i*-PrOH (95:5, v/v)], to yield eight fractions. The residue (51 mg) of the sixth fraction appeared to be a homogeneous yellow solid, which was named alternethanoxin A (**1**, R_f 0.38; 51.0 mg). The residue of the fourth fraction was purified by preparative TLC on reverse phase [eluent EtOH/H_2O (6:4, v/v)] to yield an amorphous solid, which was named alternethanoxin B (**2**, R_f 0.47; 2.2 mg).

Alternethanoxin A (1). was obtained as an amorphous solid: $[\alpha]^{25}_{D}$ − 16° (*c* 0.2); IR ν_{max} 3341, 1697, 1635, 1583, 1515, 1291 cm^{-1}; UV λ_{max} (log ε) nm 381 (sh), 299 (3.82), 241 (4.07); ^{1}H and ^{13}C NMR spectra, see **Table 1**; HRESIMS (+), *m*/*z* 627 $[2M + Na]^+$, 325.0701 [$C_{16}H_{14}NaO_6$ calcd 325.0688, M + Na]$^+$, 287 [M − Me]$^+$.

Alternethanoxin B (2). was obtained as an amorphous solid: $[\alpha]^{25}_{D}$ − 32.5° (*c* 0.1); IR ν_{max} 3232, 1688, 1656, 1608, 1589, 1519, 1291, 1259 cm^{-1}; UV λ_{max} (log ε) nm 381 (3.6), 294 (3.8), 262 (4.4), 237 (4.3); ^{1}H and ^{13}C NMR spectra, see **Table 1**; HRESI MS, (+) *m*/*z* 623 $[2M + Na]^+$ 323.0541 [$C_{16}H_{12}NaO_6$ calcd 323.0532, M + Na]$^+$.

Triacetylalternethanoxin A (3). Alternethanoxin A (**1**, 10.0 mg) was acetylated with acetic anhydride (70 μL) and pyridine (70 μL) at room temperature overnight. The reaction was stopped by the addition of MeOH and evaporated by a N_2 stream. The residue (11.0 mg) was purified by preparative TLC on silica gel [(eluent $CHCl_3$/*i*-PrOH (98:2, v/v)], yielding the triacetyl derivative of alternethanoxin A (**3**) as an amorphous solid (R_f 0.56, 8.0 mg): $[\alpha]^{25}_{D}$ −15° (*c* 0.2); IR ν_{max} 1770, 1724, 1670, 1620, 1575, 1433, 1176 cm^{-1}; UV λ_{max} (log ε) nm 287 (sh), 253 (3.93); ^{1}H NMR, δ 7.82 (1H, d, *J*=7.7 Hz, H-8), 7.50 (1H, dd, *J*=8.0, 7.7 Hz, H-9), 7.40 (1H, d, *J*=8.0 Hz, H-10), 6.86 (2H, each s, H-3 and H-6) 3.72 (3H, s, OMe), 2.40 (3H, MeCO), 2.02 (3H, MeCOO), 1.95 (6H, 2 × MeCOO); ^{13}C NMR, δ 188.4 (MeCO), 168.8 (2 × MeCOO), 168.5 (MeCOO), 165.9 (C-7), 150.5 (2C, s, C-4 and C-5), 147.6 (C-1), 144.2 (2C, s, C6a and C-10a), 136.2 (C-10b), 130.6 (C-2), 129.0 (d, C-9), 127.3 and 127.2 (2C, d, C-8 and C-10), 122.0 (2C, d, C-3 and C-6), 52.5 (OMe), 21.55 (MeCOO), 20.5 (3 × MeCOO); ESIMS (+), *m*/*z* 879 $[2M + Na]^+$, 451[M + Na]$^+$.

Alternethanoxin A Dimethyl Ether (4). To alternethanoxin A (**1**, 4.0 mg), dissolved in MeOH (0.5 mL), was added an ethereal solution of diazomethane. The reaction was carried out overnight at room temperature in the dark. The reaction was stopped by evaporation under N_2 stream. The residue (4.2 mg) was purified by preparative TLC on silica gel [(eluent petroleum/Me_2CO (8:2, v/v)], yielding alternethanoxin A dimethyl ether (**4**) as an amorphous solid (R_f 0.31, 2.0 mg): $[\alpha]^{25}_{D}$−17 (*c* 0.2); IR ν_{max} 2923, 1721, 1628, 1600, 1574, 1464, 1277 cm^{-1}; UV λ_{max} (log ε) nm 337 (sh), 285 (3.84); ^{1}H NMR, δ 12.95 (OH, s), 7.61 (1H, d, *J* = 7.8 Hz, H-8), 7.36 (1H, dd, *J*=7.8, 7.7 Hz, H-9), 7.11 (1H, d, *J*=7.7 Hz, H-10), 6.46 (1H, s, H-3), 6.05 (1H,s, H-6), 3.74 (3H, s, OMe), 3.72 (3H, s, OMe), 3.31

(3H, s, OMe), 2.29 (3H, s, MeCO); ESIMS (+), *m*/*z* 683 $[2M+Na]^+$, 353 $[M+Na]^+$.

(*S*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Alternethanoxin A (5). (*R*)-(−)-MPTA-Cl (20 μL) was added to alternethanoxin A (**1**, 2.0 mg) and dissolved in dry pyridine (40 μL). The mixture was kept at room temperature. After 12 h, the reaction was complete, and MeOH was added. The pyridine was removed by a N_2 stream. The residue was purified by preparative TLC on silica gel [(eluent petroleum/Me_2CO (7:3, v/v)] yielding **5** as a homogeneous solid (R_f 0.39, 2.0 mg): $[\alpha]^{25}_D$ −12.7 (*c* 0.15); IR ν_{max} 3374, 1771, 1725, 1637, 1595, 1284, 1214 cm^{-1}; UV λ_{max} log (ε) 290 (3.9), 225 (sh) nm; ^1H NMR, δ 7.97 (1H, d, *J* = 7.5 Hz, H-8), 7.51 (1H, dd, *J* = 8.0, 7.5 Hz, H-9), 7.46−7.31 (5H, m, Ph), 7.39 (1H, d, *J* = 8.0 Hz, H-10), 6.37 (1H, s, H-6), 5.93 (1H, s, H-3), 3.76 (3H, s, OMe), 3.44 (3H, s, OMe), 2.23 (3H, s, MeCO); ESIMS (+), *m*/*z* 541 $[M+Na]^+$.

(*R*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Triester of Alternethanoxin A (6). (*S*)-(+)-MPTA-Cl (20 μL) was added to alternethanoxin A (**1**, 2.0 mg) and dissolved in dry pyridine (40 μL). The reaction was carried out under the same conditions used for preparing **5** from **1**. Purification of the crude residue by preparative TLC on silica gel [(eluent petroleum/Me_2CO (7:3, v/v)] yielding **6** as homogeneous solid (R_f 0.55, 1.7 mg): $[\alpha]^{25}_D$ −33.6 (*c* 0.13); IR ν_{max} 1769, 1728, 1670, 1621, 1452, 1265, 1211, 1168 cm^{-1}; UV λ_{max} (log ε) nm 287 (sh), 256 (4.62); ^1H NMR, δ 7.58−7.25 (15H, m, Ph), 7.49 (1H, d, *J* = 7.6 Hz, H-8), 7.19 (1H, dd, *J* = 8.0, 7.6 Hz, H-10), 6.92 (1H, d, *J* = 8.0 Hz, H-10), 6.76 (1H, s, H-6), 6.72 (1H, s, H-3), 3.55 (3H, s, OMe), 3.52 (3H, s, OMe), 3.45 (3H, s, OMe), 3.35 (3H, s, OMe), 2.38 (3H, s, MeCO); ESIMS (+), *m*/*z* 973 $[M+Na]^+$.

Leaf Disk-Puncture Assay. Culture filtrates of *A. sonchi*, its organic extract, the chromatographic fractions, and pure compounds **1**−**4** were assayed by leaf disk-puncture bioassay on *S. arvensis* and a number of nonhost plants. The plants were produced from pieces of underground shoots or seeds and grown in a greenhouse. The disks (10 mm diameter) were cut off well-expanded leaves with a cork borer, placed on moistened filter paper, and punctured by a sharp needle in the center. Crude organic extract, chromatographic fractions, and pure compounds were dissolved in a small amount of EtOH and then brought up to desirable concentration with distilled H_2O. The final concentration of EtOH in test solutions was 5% v/v, which is nontoxic to leaves of all plants in the control. Droplets (10 μL) of the test solution were applied on the disks and then incubated in transparent plastic boxes at 24 °C under a 12 h photoperiod. After 2 days of incubation, the diameter of the necrotic lesions (mm) was measured.

Antimicrobial Assay. Antifungal activity of the alternethanoxins A and B was assayed on *Saccharomyces cerevisiae*, and their antibacterial activity was tested on *Xanthomonas campestris*, *Escherichia coli*, and *Bacillus subtillis* at the concentration 100 μg per disk according to the method previously described (*19*).

RESULTS AND DISCUSSION

The organic extract obtained from the solid culture of *A. sonchi*, showing a high phytotoxic activity on *S. arvensis*, was purified by a combination of column chromatography and preparative TLC on silica gel and reverse phase to obtain two pure phytotoxic metabolites. Their close relationship was shown by ^1H and ^{13}C NMR investigations, and they were named alternethanoxins A (**1**) and B and (**2**) (**Figure 1**) (51.0 and 2.2 mg/kg, respectively) on the basis of fungus source and their carbon skeleton.

Alternethanoxin A showed a molecular weight of 302 associated with a molecular formula of $C_{16}H_{14}O_6$, consistent with 10 unsaturations, 9 of which were due to a 1,2,3-trisubstituted (A) and a pentasubstituted (C) aromatic ring and to a carbonyl group. In fact, the ^1H NMR (**Table 1**) and COSY (*17*) spectra showed two doublets (*J* = 7.5 and *J* = 7.1 Hz) and a double doublet (*J* = 7.5 and *J* = 7.1 Hz) at the typical chemical shift values for a suitable trisubstituted aromatic ring at δ 7.46 (H-8), 7.11 (H-10), and 7.34 (H-9) (*20*). The same spectrum showed three singlets due to the proton (H-3) of the pentasubstituted aromatic ring and another proton, a methoxy, and an acetyl group at δ 6.21, 3.62, and 2.23 (*20*). The singlet at δ 6.21, which integrated for two protons, was due to the overlapping of the H-3 signal and

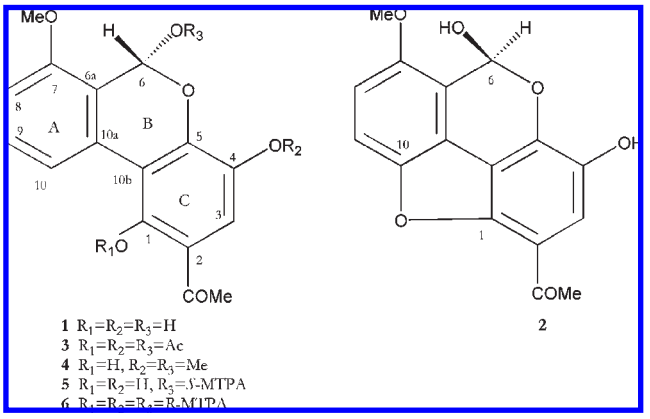

Figure 1. Structures of alternethanoxin A (**1**), its derivatives (**3**−**6**), and alternethanoxin B (**2**).

that of the proton (H-6) of the aldehyde group bonded at C-6 of the aromatic A ring and hemiacetalized with a phenolic group at C-5 of the aromatic C ring. These results were in full agreement with the absorption bands for hydroxy, conjugated carbonyl, and aromatic groups observed in the IR spectrum (*21*), as well as with the absorption maxima exhibited in the UV spectrum at 381, 299, and 241 nm (*20*). These partial structures were supported by the data of the ^{13}C NMR spectrum (**Table 1**) and the couplings observed in the HSQC spectrum (*17*). The aromatic protonated carbons as well as the methoxy and acetyl groups were observed at the typical chemical shift values of δ 131.0, 122. 2, 121.4, 109.5, 52.5, and 22.0 for C-9, C-8, C-10, C-3, MeO, and MeCO, respectively (*22*). The same spectrum also showed significant signals for the carbonyl and the hemiacetalic carbon (C-6) at δ 198.7 and 109.5, with the latter overlapped to the C-3 signal. The signals of the three and five quaternary carbons of the aromatic A and C rings resonated at very typical chemical shift values of δ 167.3, 153.0, and 130.4 for C-7, C-6a, and C-10a and at δ 148.8, 160.0 (double signals), 128.6, and 109.8 for C-1, C4 and C-5, C-2, and C-10b and were essentially assigned on the basis of the couplings observed in the HMBC spectrum (*17*) (**Table 1**). The couplings reported in **Table 1** also allowed us to deduce the presence of a 2,6-pentasubstituted-2*H*-4-dehydropyran ring (B) accounting for the remaining unsaturation, which was joined to the other two rings (A and C) by the bridge-head carbons C-6a and C-10a and C-5 and C-10b, respectively. These findings allowed us to assign the chemical shift to all of the carbons and the corresponding protons (**Table 1**) as well as to alternethanoxin A the structure of a 1-(1,4,6-trihydroxy-7-methoxy-6*H*-benzo-(*d*)chromen-2-yl)ethanone (**1**, **Figure 1**).

This structure was supported by other couplings observed in the HMBC spectrum (**Table 1**) and the data from the HRESIMS spectrum, recorded in positive modality, which showed sodium clusters formed by the toxin itself and the corresponding dimer at *m*/*z* 325.0701, $[M+Na]^+$, and 627 $[2M+Na]^+$ and the fragmentation peak at *m*/*z* 287 $[M-Me]^+$, which was generated by the molecular ion by loss of a methyl residue.

The structure of alternethanoxin A was confirmed by preparing two key derivatives having spectroscopic properties that were fully consistent with structure **1**. By usual acetylation with acetic anhydride and pyridine, alternethanoxin A was converted into the corresponding triacetyl derivative **3** (**Figure 1**), the IR spectrum of which showed the significant absence of hydroxy groups and the presence of bands due to more ester carbonyl groups. Its ^1H and ^{13}C NMR spectra differed from those of **1** for the significant presence of the signals of the three acetoxy groups at δ 2.02 and 1.95 (two MeCOO) and at δ 168.8 (two MeCOO),

168.5 (MeCOO), and 20.5 (three MeCOO). In the same spectra also the downfield shifts ($\Delta\delta = 0.65$) of the overlapped signals of H-3 and H-6 at δ 6.86 and ($\Delta\delta = 12.5$) of C-3 and C-6 at δ 122.0 were observed. The ESIMS spectrum showed sodium clusters formed by triacetylalternethanoxin A itself and the corresponding dimer at m/z 451 $[M+Na]^+$ and 879 $[2M+Na]^+$.

By reaction with an ethereal solution of diazomethane overnight at room temperature **1** was converted into the dimethyl ether derivative **4** (**Figure 1**). Its ^{1}H NMR spectrum differed from that of **1** only for the presence of two more singlets due to the new methoxy groups at δ 3.74 and 3.31. Probably the phenolic hydroxy group at C-1 was not methylated as it was hydrogen bonded with the carbonyl group at C-2, generating a stable six-membered ring as shown by the singlet observed at a typical chemical shift value of δ 12.95 (*20*). The ESI mass spectrum of **5** showed sodium clusters formed by dimethyl alternethanoxin A itself and the corresponding dimer at m/z 353 $[M + Na]^+$ and 683 $[2M+Na]^+$.

Alternethanoxin B showed a molecular weight of 300 associated with a molecular formula of $C_{16}H_{12}O_6$ as deduced from its HRESIMS spectrum and consistent with 11 unsaturations. It differs from alternethanoxin A in that it lacks two hydrogens and one unsaturation more. They showed very similar IR and UV spectra, and comparison of their ^{1}H and ^{13}C NMR spectra (**Table 1**) showed very close structures with the only difference in the substitution of the aromatic A ring. In fact, its ^{1}H NMR spectrum showed two ortho-coupled aromatic protons resonating as doublets ($J = 9.0$ Hz) at δ 7.47 and 7.36 and assigned to H-9 and H-8, which coupled in the HSQC spectrum with the aromatic protonated carbons at δ 122.2 and 125.4, and the absence of H-10 (*20*, *22*). The ^{13}C NMR spectrum also showed a significant downfield shift ($\Delta\delta = 30.8$) of C-10 attributable to the presence of a tetrasubstituted furan ring, accounting for the additional unsaturation. This new ring probably was generated by the attachment of the oxygen at C-1 of the C ring to the carbon C-10 of the A ring. This partial structure was also consistent with the couplings observed in the COSY and HSQC spectra and essentially with those of the quaternary carbons recorded in the HMBC spectrum (**Table 1**). Furthermore, examination of the ^{1}H NMR and COSY spectra also showed different chemical shift values for the protons (H-3) of the pentasubstituted aromatic C ring and of the hemiacetalized aldehyde group (H-6) resonating at δ 6.73 and 6.62, respectively, which coupled in the HSQC with the signals at δ 107.2 and 111.4 (C-3 and C-6), respectively. These findings suggested an opposite stereochemistry at C-6 in **2** with respect to **1**, which was also supported by the presence in the ^{1}H NMR spectrum of **2** of a singlet at δ 12.20 due to the hemiacetalic hydroxy group, which is probably hydrogen bonded to the methoxy group at C-7 and generating a stable six-membered cycle. This result was confirmed by the couplings observed in the NOESY spectra (*17*) of **1** and **2**. In fact, the NOESY spectrum of **1**, besides the expected effect observed between H-3 and the methyl of the acetyl group at C-2, also showed an effect between H-6 and the methoxy group at C-7. This latter effect was significantly absent in the NOESY spectrum of **2**.

These findings allowed us to assign the chemical shift values to all of the carbons and the corresponding protons (**Table 1**) and to alternethanoxin B the structure of 1-(7,9-dihydroxy-1-methoxy-9*H*-4,8-dioxacyclopenta[*def*]phenanthren-5-yl)ethanone (**2**, **Figure 1**). This structure was supported by the other couplings observed in the HMBC spectrum (**Table 1**) and by the data of the HRESIMS, recorded in positive modality, which showed sodium clusters formed by the toxin itself and the corresponding dimer at m/z 323.0541 $[M+Na]^+$ and 623 $[2M+Na]^+$.

The absolute stereochemistry of the secondary hydroxylated carbon C-6 of alternethanoxin A (**1**) was determined by applying Mosher's method (*23*−*25*). By reaction with the *R*-(−)-α-methoxy-α-trifluorophenylacetate (MTPA) and *S*-(+)MTPA chlorides, alternethanoxin A was converted in the corresponding diastereomeric *S*-MTPA ester and *R*-MTPA triesters (**5** and **6**, respectively), the spectroscopic data of which were consistent with the structure assigned to **1**. The comparison between the ^{1}H NMR data of the *S*-MTPA ester (**5**) and those of the *R*-MTPA triester (**6**) of **1** [$\Delta\delta$ (**5** − **6**): H-3, −0.79; H-8, +0.48; H-9, +0.42; H-10, +0.49; MeO, +0.21; and MeCO, −0.15] allowed us to assign an *R*-configuration at C-6. In alternethanoxin B C-6 has the opposite stereochemistry with respect to **1**, so that an *S*-configuration could be assigned to this chiral carbon in **2**.

Compounds **1**−**4** were tested at a range of concentrations from 0.1 to 4 mg/mL on leaf disks of *S. arvense*. Only alternethanoxins A and B (**1** and **2**) were shown to be phytotoxic. Small necrotic lesions were seen at concentrations of 1 and 2 mg/mL for **1** and **2**, respectively. At the highest concentration of **1** and **2** (4 mg/mL) lesions reached 4 and 2 mm in diameter, respectively. When tested at the concentration of 2 mg/mL on leaf disks of a number plant species (*Cirsium arvense*, *Calendula officinalis*, *Taraxacum officinalis*, *Amaranthus rethroflexus*, *Convolvulus arvensis*, *Aegopodium podagraria*, *Elytrigia repens*, *Phleum pratense*), alternethanoxins A and B showed similar nonspecific activities (lesions ∼ 1−2 mm diameter).

Furthermore, the inactivity of derivatives **3** and **4** demonstrated that the phenolic hydroxy group at C-4 of C ring is a structural feature important for the phytotoxicity, whereas the activity of alternethanoxin B showed that the other one at C-1 and the hemiacetal hydroxyl group at C-6 are nonessential. The reduction of both the hemiacetal and the acetyl groups at C-6 and C-2 and the eletrophilic substitution of one or more hydrogens of rings A and C with a suitable group could contribute to demonstrating the importance of the benzo(*d*)crhomene moiety and the role of the acetyl group. Neither **1** nor **2** demonstrated antibiotic or antifungal activity when tested at 100 μg/disk on *Bacillus subtilis*, *Xanthomonas campestris*, *Escherichia coli*, and *Saccharomyces cerevisiae*.

Alternethanoxins A and B are two fungal metabolites in which an ethanone group was bonded to an original polysubstituted benzo(*d*)chromene and dioxacyclopenta[*def*]phenanthrene residue, respectively, and occur for the first time as natural compounds with interesting biological activity. In particular, the main fungal metabolite alternethanoxin A (**1**) and also alternethanoxin B (**2**) showed potential herbicidal properties.

A number of well-known fungal metabolites (alternariol, its monomethyl ether, altenuene, and altenuisol), which belong to a class of toxic metabolites containing a dibenzo[α]pyrone moiety, are structurally close to alternethanoxin A. These compounds are produced by different *Alternaria* species isolated from plant material, and their antibiotic, cytotoxic, and teratogenic activities are usually stressed (*15*). Interestingly, alternethanoxins A and B did not demonstrate antimicrobial activity. Furthermore, the closest compounds to alternethanoxin A from the group of ethanones appeared to be the acetophenones, namely, cynandiones A−D, cynanchone, and analogues, isolated from the roots of different *Cynanchum* plant species and showing potential pharmacological applications (*26*). Compounds close to alternethanoxin B are those belonging to the cylopenta[*d*,*e*,*f*]-phenathrene group including the steriols, toxic metabolites produced by some *Fusarium sporotrichiella* strains, isolated from naturally infected grain (*27*).

Because of the structural relationship of alternethanoxins A and B to some mycotoxins of *Alternaria* spp., their antimicrobial

activity should be assayed on more species of microorganisms. It seems to be important also to elucidate the role of these metabolites in the life cycle of their producer. If there is a correlation between the production of these toxins and the virulence of the fungus, the toxin production can be used as a marker for selection of strains of *A. sonchi* with higher herbicide potential, hence increasing their commercial value.

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