Cercospora beticola Phytotoxins: Cebetins That Are Photoactive, Mg^{2+}-Binding, Chlorinated Anthraquinone–Xanthone Conjugates

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Abstract: Two *Cercospora beticola* toxins, isolated from cultures of the sugar beet pathogen, were identified as tautomeric compounds and named cebetin A (**1**) and cebetin B (**2**). The structure of cebetin B (**2**) and its absolute configuration were determined by single-crystal X-ray diffraction. The structure of cebetin A (**1**) was elucidated on the basis of UV–visible, NMR, and mass spectral data. The molecular backbone of cebetins is totally different from that of the known phytotoxin cercosporin and comprises partially hydrogenated xanthone and anthraquinone moieties connected through two seven-membered rings. The xanthone and anthraquinone systems contain respectively a chlorine atom and an epoxide function. Cebetin B crystallizes in the orthorhombic space group $P22_12$, with a = 14.449 (3) Å, b = 22.269 (2) Å, c = 14.061 (2) Å, V = 4524.3 Å^3, Z = 4, and D_x = 1.369 g/cm^3. The structure was determined from 3670 diffractometer data and refined to a final R = 0.066. In the crystal, cebetin B molecules exist as a dimeric complex, $(C_{31}H_{21}O_{13}Cl{\cdot}2CH_3OH)_2Mg_2$, where the two molecules of cebetin B share two Mg^{2+} ions and are related by a 2-fold axis. Spectroscopic data showed that cebetin A is a metal-free keto–enol tautomer of cebetin B. Both cebetins A and B at a concentration of 1 ppm were lethal to sugar beet cells in suspension culture in the presence of light. This effect was not observed in the absence of light.

Introduction

Cercospora beticola Sacc. is the causal agent of sugar beet leaf spot.[1-3] The disease has a devastating effect on beet sugar production worldwide, despite the routine implementation of costly control measures such as multiple applications of fungicides and slow and difficult breeding programs. The fungus produces a red hydroxyperylenequinone derivative called cercosporin (**3**) (Figure 1) which acts as a photoactivated phytotoxin. The structure and activity of this compound have been studied in detail.[4-8] Light transforms cercosporin to an electronically excited state, leading to production of singlet oxygen and superoxide,[9-11] which cause damage and death to the cells.[12,13] From a strain of *C. beticola*, Frandsen[14] isolated a yellow compound, which was found by Schlösser[15] to be toxic to higher plants and exhibit antibiotic activity against many species of bacteria. The latter author named this compound *C. beticola* toxin (CBT) and reported that it contained a tropolone nucleus and phenolic hydroxyl groups and formed complexes with Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, and Mg^{2+} ions.[2,16-18] In contrast, Balis and Payne[19] suggested that the material Schlösser isolated was a mixture of triglycerides and cercosporin. However, Assante and co-workers in 1977 isolated CBT from Schlösser's original strain of *C. beticola* and from other strains obtained from infected sugar beet.[20] In recent years, various forms of biological activity have been attributed to CBT. These include inhibition of H^+ transport, K^+ uptake, and ATPase activity and depolarization of transmembrane potential in plant tissues.[21-23] Notwithstanding these studies, a chemical structure of CBT has not been proposed to date, and the elucidation of its structure has remained unresolved.[24]

In an attempt to elucidate the structure of CBT, we have isolated two yellow compounds in pure form from cultures of *C. beticola* IPV-F573.[15] both compounds have spectral features in common with CBT as previously described.[2,15-18,20] The compounds have been named cebetin A and cebetin B,[25] **1** and **2**, respectively (Figure 1). The compounds are photoactivated phytotoxins and, in this respect, are similar to cercosporin and certain other quinone derivatives.[8]

Structure elucidation of the cebetins by spectroscopic and chemical means proved to be a formidable task in our hands. The compounds did not yield plausible or reproducible data from elemental analyses. Mass spectral analyses with various ionization techniques, such as FAB, DCI, and LDFT methods, failed to

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(24) Since the submission of this paper, structures of two yellow toxins from *C. beticola* have been reported (Milat, M.-L.; Prange, T.; Ducrot, P.-H.; Tabet, J.-C.; Einhorn, J.; Blein, J.-P.; Lallemand, J.-Y. *J. Am. Chem. Soc.* **1992**, *114*, 1478–1479). The compounds were shown to be isomeric and were named beticolins 1 and 2. The structures of the beticolins have basic features similar to those of the cebetin structures, but there are very distinct and significant differences. In the beticolins, the anthraquinone and xanthone groups are connected through C(2) and C(11) rather than C(4) and C(11) as in the cebetins. Also the Cl atom is at C(4) in beticolins, while it is bonded to C(2) in cebetins. These differences are also reflected in the spectral data of the two groups of compounds. It is likely that the production of cebetins or beticolins in this fungus is strain-dependent or dependent on culture condition.
(25) The term CBT has been used in the literature in an abbreviation for the unrelated compound cembratriene-4,6-diol (Saito, Y.; Takayawa, H.; Konishi, S.; Yoshida, D.; Mizusaki, S. *Carcinogenesis* **1985**, *6*, 1189. Marshall, J. A.; Robinson, E. D. *Tetrahedron Lett.* **1989**, *30*, 1055–1058). To avoid confusion the new term "cebetin" is introduced.

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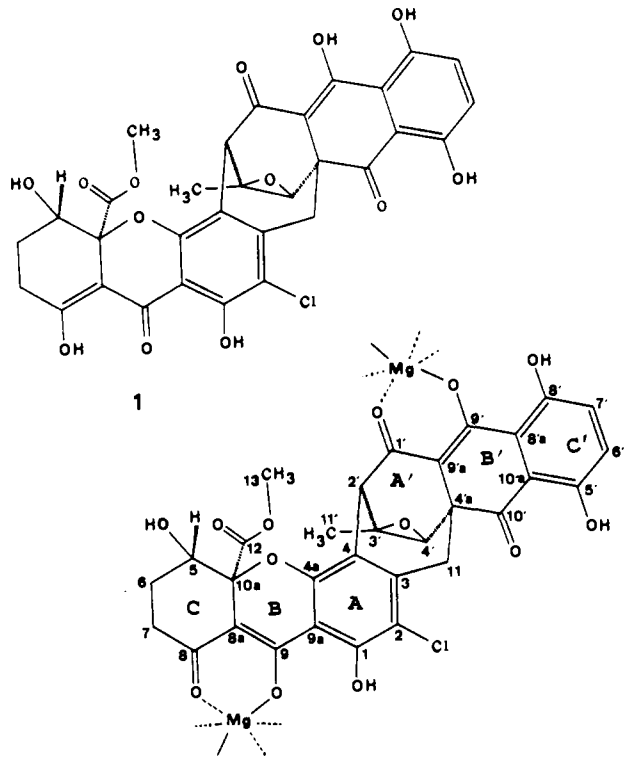

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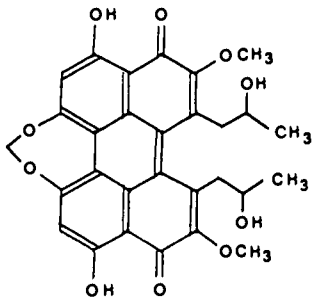

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Figure 1. Structures of cebetin A (**1**), cebetin B (**2**), and cercosporin (**3**) showing the atom and ring numbering system.

provide the high-resolution data required to obtain a molecular formula. NMR spectroscopy revealed that only 10 out of a total of 31 carbon atoms were bonded to protons and that the molecule had six asymmetric centers. Both compounds yielded well-formed but extremely unstable single crystals. After many attempts, a suitable X-ray diffraction data set for cebetin B (**2**) was obtained, from which both the structure and the absolute configuration of the molecule were determined. The structure of cebetin A (**1**) is based on the comparison of its UV–visible, ^1H and ^{13}C NMR, and mass spectral data with those of cebetin B.

Experimental Section

Organism Source and Culture. Strain IPV-573 of *C. beticola* was kindly provided by E. G. Ruppel of USDA, ARS, Fort Collins, CO. This strain originated from Schlösser[2,15] and was examined by Assante et al.[20] Potato dextrose agar (PDA) plates (9-cm diameter) were inoculated with a mycelial suspension of the fungus in sterile water, by spreading 0.5 mL of suspension per plate. The fungus was grown for 7 days at 25 °C in both light and darkness.

Extraction and Purification. The agar plus mycelium mixture was extracted with ethanol to give a yellow solution, solvent was removed in vacuo at <40 °C, and the remaining aqueous extract was partitioned against ethyl acetate until all yellow pigment was transferred to the organic phase. The ethyl acetate extract was dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The crude extract was chromatographed in a column of Sephadex LH20 (25 × 450 mm) with ethanol. Cebetin B eluted earlier than cebetin A. Cercosporin eluted separately as a minor component before cebetin B. Fractions rich in cebetin A or B were identified by UV–visible spectroscopy, pooled, and rechromatographed in columns of Sephadex LH20 (25 × 450 mm) with ethanol–chloroform, 70:30. Pure fractions of cebetin A or B were pooled and dried as bright yellow powder. A large number of extractions were carried out at different times on various quantities of the culture medium. Yields of **1** and **2** were highly variable, with cebetin B as the major component. Light conditions and the incubation period affected the production of cebetins. From a large batch of culture (approximately 9 L of solidified agar from 360 Petri dishes), 1.54 g of cebetin B was isolated. Cercosporin, which was a minor component, was not quantified. In some cases, cebetin B was further purified in a reversed-phase column (Whatman, Partisil 40, 25 × 450 mm under gravity) using a gradient of ethanol–water (60–100% ethanol). In silica gel TLC with chloroform–methanol–water, 40:10:1, both compounds migrated to an R_f value of 0.46. The two compounds comigrated in a few other solvent systems in both silica gel and cellulose layers.

Spectroscopic Measurements. Electronic absorption spectra (UV–visible) were recorded on a Perkin-Elmer Lambda 5 spectrophotometer, in ethanolic solution with or without a drop of 0.1 N NaOH. NMR spectroscopy was carried out at 300 MHz for ^1H and 75.4 MHz for ^{13}C nuclei at 22 °C in an Varian XL300 FT NMR spectrometer. Assignment of the NMR signals was based on homo- and heteronuclear selective decoupling, 2D COSY spectroscopy, and correlation with chemical shifts of similar structural groups in other polyketide compounds (e.g. xanthones[26–28] and anthraquinones[29,30]). In addition, $-CH_3$, $-CH_2-$, $-CH-$, $=C-$, and $-C=O$ carbon atoms were distinguished by the attached proton test (APT) pulse sequence program.[31] Desorption chemical ionization (DCI) mass spectra were obtained in both negative- and positive-ion modes with ammonia and methane. Laser desorption Fourier transform (LDFT) mass spectra were obtained in the negative-ion mode at three energy levels. Fast atom bombardment (FAB) mass spectra were obtained in the positive-ion mode with *p*-nitrobenzyl alcohol and glycerol as the matrices. Elemental analyses were carried out by Galbraith and Huffman Laboratories.

Biological Assay. Phytotoxicity tests were carried out on sugar beet cell cultures in the absence and the presence of light. Ten-milliliter aliquots of sugar beet cell suspension cultures were dispensed into glass Petri dishes (10 × 60 mm). One hundred microliters of a 100 μg/mL stock solution of cebetin A or cebetin B in DMSO was added to aliquots of cells in quadruplicate. An equal volume of DMSO was added to control cultures. Half of the Petri plates in each treatment were doubly wrapped with aluminum foil before incubation at ca. 29 °C in the presence of light (270 $\mu E \cdot m^{-2} \cdot s^{-1}$, General Electric, Cool White fluorescent). After 24 h, cultures were stained with Trypan Blue.[32] Cell viability was scored during microscopic examination.

Crystallization and X-ray Diffraction. Cebetin A crystallized as long needles from chloroform, on slow evaporation. Cebetin B crystallized from a large number of solvent systems; the best single crystals were obtained when benzene was diffused into a methanolic solution. Crystals of both compounds were extremely unstable outside the mother liquor. A number of attempts, over a period of 2 years, to use one of these crystals for X-ray diffraction failed. Finally, a large plate-shaped crystal of cebetin B (0.56 × 0.40 × 0.03 mm), which crystallized from deuteriomethanol solution inside an NMR tube, was mounted directly under cold conditions and transferred to the diffractometer. The cell parameters were obtained by a least-squares fit to $\pm 2\theta$ of 48 reflections measured at 163 K using Cu $K\alpha_1$. The crystals are orthorhombic: space group $P22_12$; a = 14.449 (3), b = 22.269 (2), c = 14.061 (2) Å; V = 4524.3 Å^3; Z = 4; D_x = 1.370 g/cm^3; fw ($C_{31}H_{21}O_{13}ClMg$ + 8.5CH_3OH) = 933.6; $F(000)$ = 1960; μ(Cu Kα) = 16.7 cm^{-1}. The intensities of all the unique reflections within the range $0 < 2\theta < 150°$ were measured at 163 ± 2 K by employing the θ–2θ scan mode on an Enraf-Nonius CAD-4 diffractometer. A variable scan width of $(1.0 + 0.20 \tan \theta)°$ and a variable horizontal aperture of $(4.50 + 0.86 \tan \theta)$ mm were used. Intensities of three standard reflections, monitored every 2 h of X-ray

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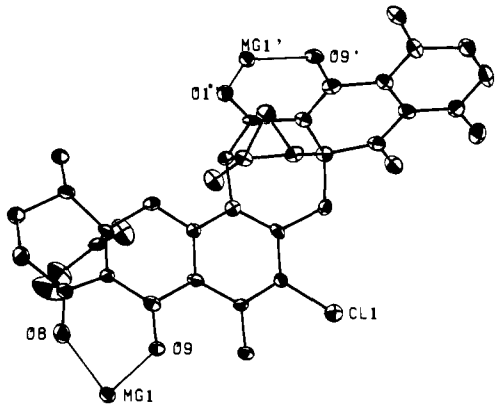

Figure 2. Perspective view of a single molecule of cebetin B.

exposure, showed a maximum variation of 4.5%. A total of 5171 reflections were measured, out of which 3670 reflections were considered observed on the basis of $I > 3\sigma(I)$. The intensities were corrected for Lorentz and polarization factors and for absorption (maximum/minimum transmission 0.9561/0.5029).[33]

Structure Determination and Refinement. The complete structure was determined by a combination of direct methods by using the computer programs MITHRIL[34] and SHELXS-86[35] and successive difference Fourier analyses. The atom identifications were carried out by carefully scrutinizing the isotropic temperature factors and interatomic bond distances and angles involving the atoms concerned and by refining in a few cases the atomic occupancy factors. The structure analysis led to the structure of cebetin B and nine methanol solvate molecules (one of which lies on a 2-fold axis and has disordered hydrogen). Two additional peaks were refined as a fractional methanol (25%). The structure was refined by a full-matrix least-squares routine in SHELX-76[33] using anisotropic thermal parameters for the non-hydrogen atoms. Sixteen hydrogen atoms were located from the difference Fourier maps, and the remaining hydrogen atoms in the cebetin B molecule were placed in their calculated positions. The refinement converged to final $R = 0.066$, $R_w = 0.096$, and $S = 1.1$ for 3670 observed reflections and 632 variables. The maximum and minimum peaks in the final difference map were ± 0.5 e/Å^3.

Results and Discussion

Structure of Cebetin B. A perspective drawing of a single molecule of cebetin B is shown in Figure 2. The polycyclic backbone of the cebetin B molecule is completely different from that of cercosporin and consists of two tricyclic systems cross-linked by two C–C covalent bonds, as has been observed in other fungal metabolites such as rugulosin,[36] luteoskyrin,[37] and the secalonic acids.[26,38] The distinctive feature of cebetin B is the combination of two dissimilar tricyclic units: a highly substituted xanthone system, which has a Cl at C(2), and a partially hydrogenated anthraquinone system, which has an epoxide function at C(3′)–C(4′). In contrast to cebetin B, most previously known dimeric xanthones and anthraquinones have two similar tricyclic units forming symmetric or pseudosymmetric structures.[36-38] The nature of the linkage of the two tricyclic systems of cebetin B is also unique. The molecule has one sp^2–sp^3 linkage, C(4)–C(2′), directly connecting rings A and A′ and a bridging sp^3–sp^3 bond, C(11)–C(4′a), between the two rings. Such a linkage of the two groups leads to the formation of two seven-membered rings at the center and gives rise to a twist in the polycyclic backbone of cebetin B. The two planar sections of cebetin B are almost perpendicular to each other, with the dihedral angle between them being 78°. The dihedral angle in other dimeric polyketides is much smaller, e.g., 33° in luteoskyrin and 37° in secalonic acid A. Ring C is in a half-chair and ring B is in a sofa conformation, while ring A is essentially planar. Ring A′, which has the epoxide function at C(3′)–C(4′), assumes a boat conformation. The two seven-membered rings are both in a twist-boat conformation. The methyl ester group at C(10a) is in an axial position and lies on the same side of the molecular backbone as the epoxide function.

Absolute Configuration. The absolute configuration of the cebetin B molecule was determined by applying the *R* method of Hamilton.[39] The weighted *R* factor for the correct absolute configuration was 0.096 and that for the alternate absolute configuration was 0.103, giving an *R* factor ratio of 1.0729. This was compared with the *R* factor ratio $R_{1,3000,0.005} \approx 1.0013$ from ref 40. Thus the two refinements differed at much higher than 99.5% confidence level. The asymmetric centers in cebetin B have the following absolute configurations: 10a*S*, 5*S*, 2′*R*, 3′*R*, 4′*S*, and 4′a*S*. It may be noted that the secalonic acids, which contain xanthone groups, as in the present structure, are reported to exist in both enantiomeric forms.[26]

Dimer. In the crystalline state, cebetin B molecules exist as a dimeric complex, represented by $(C_{31}H_{21}O_{13}Cl{\cdot}2CH_3OH)_2Mg_2$, where the two molecules are related by a crystallographic 2-fold axis (Figure 3). The dimer of cebetin B is shaped like a slightly twisted but open rectangular ($\sim 5 \times 10$ Å) cage, in which the two metal atoms and the two epoxide functions occupy the corners and the planar sections of the molecule constitute the walls. The arrangement brings the two xanthone systems into nearly parallel positions (dihedral angle between the two xanthone sections is 12°) and the two chlorine atoms close to each other (3.240 Å).

Mg Coordination. Metal-chelated structures of dimeric polyketides have not been reported in the literature heretofore. Each Mg^{2+} ion binds to the xanthone system of one molecule and the anthraquinone system of the other molecule of the dimer. The ion is neutralized by the deprotonation of atoms O(9) and O(9′). The bond lengths of C(8)–O(8) (1.275 Å), C(8)–C(8a) (1.426 Å), C(8a)–C(9) (1.397 Å), and C(9)–O(9) (1.293 Å) indicate that, in the crystalline state, this ligand group is a resonating enolate system conjugated to ring A. Two oxygen atoms from the solvent methanol molecules complete the near-perfect octahedron around the Mg^{2+} ion. Mg–O distances range from 2.002 to 2.074 Å, and the three axial O–Mg–O angles are 171.7, 173.7, and 177.0°. The major distortions of the octahedral geometry are due to the two six-membered chelate rings (the angles O(8)–Mg–O(9) and O(1′)–Mg–O(9′) are 84.8 and 82.4°, respectively). The ring containing O(1′) and O(9′) is significantly nonplanar (the Mg ion deviates from the mean plane through atoms C(1′), O(1′), C(9′), C(9′a), and O(9′) by 0.673 Å), while the one containing O(8) and O(9) is more planar (the Mg ion deviates from the mean plane of the five atoms by 0.185 Å).

Hydrogen Bonding and Packing. Cebetin B exhibits three short intramolecular O–H···O hydrogen bonds: O(1)–H···O(9) = 2.546 Å, O(5′)–H···O(10′) = 2.542 Å, and O(8′)–H···O9(′) = 2.480 Å. There are no intermolecular hydrogen bonds. Cebetin B interacts with only three of the nine solvent molecules. The hydroxyl group O(5) is involved in two of the four hydrogen bonds. The methanol oxygen O(5S) forms a bridge between O(5) and O(1′), and the methanol oxygen O(6S) forms the only linkage between the neighboring dimers. Lack of intermolecular hydrogen bonding and limited solvent interactions are probably the direct results of the presence of internal hydrogen bonding. They also explain the unstable nature of the crystals of cebetin B. The structural features indicate that the hydroxyl group O(5) is probably the most significant interacting site of the cebetin B molecule. The dimeric cages of cebetin B stack on top of each other along the crystallographic *a* axis.

Structure of Cebetin A (1). The structure of cebetin A is determined on the basis of the comparison of its spectral data with

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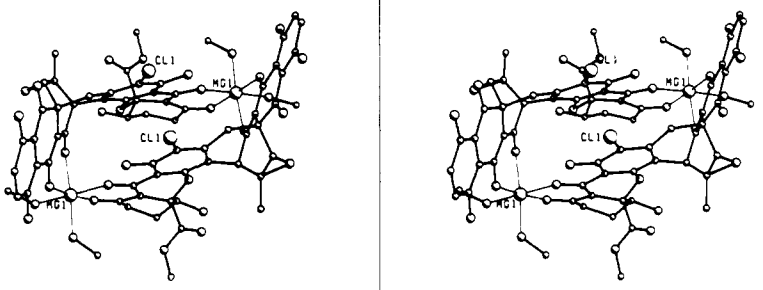


Figure 3. Stereoview of the dimeric unit, $(C_{31}H_{21}O_{13}Cl{\cdot}2CH_3OH)_2Mg_2$, of cebetin B.

Table I. ^1H (300 MHz) and ^{13}C NMR (75.4 MHz) Chemical Shifts (ppm) of Cebetins A and B in Deuteriomethanol[a]

	cebetin A		cebetin B	
position	^1H (J, Hz)	^{13}C	^1H (J, Hz)	^{13}C
1		156.8		156.9
2		116.0		113.6
3		146.1		141.4
4		117.2		116.5
4a		156.1		154.2
5	4.55 (dd, 11.6, 5.6)	72.0	4.22 (dd, 12.6, 5.3)	74.0
6	2.07 (m)	25.5	2.05 (td, 13.3, 5.3, 6.2)	26.4
			2.28 (qd, 13.3, 6.7, 11.7, 12.6)	
7	2.47 (dd, 20.2, 6.2)	28.7	2.55 (dd, 18.5, 6.7)	36.2
	2.76 (ddd, 20.2, 9.9, 6.9)		2.68 (ddd, 18.5, 11.7, 6.2)	
8		180.4		193.1
8a		100.9		99.7
9		186.8		176.8
9a		106.2		105.9
10a		86.6		87.5
11	2.90 (AB, 18.6)	43.2	2.98 (AB, 18.4)	43.0
	3.11		3.17	
12		171.7		173.2
13	3.66 (s)	53.3	3.59 (s)	52.8
1′		193.8		193.7
2′	4.88 (d, 0.1)	50.9	4.51 (d, 1.3)	50.6
3′		60.0		60.2
4′	4.19 (d, 0.1)	61.3	3.99 (d, 1.3)	62.7
4′a		52.0		51.6
5′		157.1		157.6
6′	6.92 (d, 9.3)	125.0	7.22 (d, 9.2)	124.9
7′	6.69 (d, 9.3)	130.3	7.12 (d, 9.2)	130.2
8′		155.4		156.0
8′a		116.1		117.6
9′		177.3		177.6
9′a		101.3		103.1
10′		204.1		204.9
10′a		112.5		113.6
11′	1.48 (s)	19.8	1.38 (s)	19.9

[a] Key: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

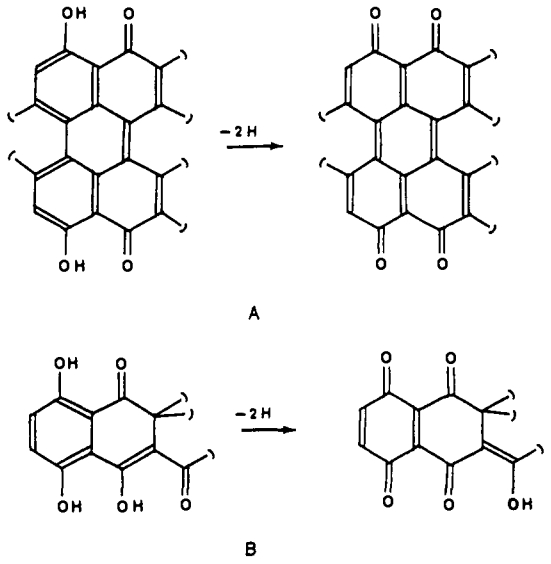
those of cebetin B. Both cebetins A and B showed four spin systems and two methyl singlets. The four spin systems correspond to sp^3 protons in ring C, the aromatic protons in ring C′, the benzylic methylene group at position 11, and the weakly coupled CH protons in ring A′. The assignments and J values of protons were obtained by homonuclear decoupling and a 2D-COSY experiment. ^{13}C signals of the protonated carbons were assigned by the attached proton test (APT),[30] single-frequency heteronuclear decoupling, and one-bond proton–carbon J values. Nonprotonated carbons were assigned by single-frequency decoupling of the neighboring protons and correlation to similar structural groups in xanthones and anthraquinones.[26-30]

^1H and ^{13}C NMR data (Table I) of cebetin A and cebetin B reveal that the former is a keto–enol tautomer of cebetin B with respect to positions 8 and 9. In the case of cebetin B, the ^{13}C atoms at positions 8 and 9 produce signals at 193.1 and 176.8 ppm, respectively, indicating that C(8) in the solution state is an α,β-unsaturated carbonyl and C(9) is a hydroxylated sp^2 carbon. In cebetin A, on the other hand, the ^{13}C signal of C(8) shifts upfield to 180.4 ppm and that of C(9) moves downfield to 186.8 ppm, indicating that C(9)–O(9) has more double-bond character than C(8)–O(8) in this compound. These values for cebetin A are similar to those for secalonic acid A, in which the xanthone C(8) is hydroxylated and C(9) is a carbonyl group.[27,28] The ligand group in the anthraquinone part of the molecule produces similar NMR signals in both cebetins A and B.

Both cebetins produce two UV–visible absorption bands, band I occurring at 430–460 nm and band II at 330–350 nm. Band II arises from the xanthone chromophore and is similar to the one observed in the secalonic acids.[41] The longer wavelength band (I) results from the chromophore in the anthraquinone system.[29] Features of band I are identical in both cebetins A and B, while those of band II are different in the two compounds. The ratio of the intensities of the two peaks (I/II) is also changed from 0.7 in cebetin B to 0.5 in cebetin A due to the increased relative

(41) Kurobane, I.; Vining, L. C.; McInnes, A. G. *J. Antibiot.* **1979**, *32*, 1256–1266.

Scheme I



intensity of band II in the latter compound, reflecting a change in the chromophore in the xanthone part of the molecule.

The low-resolution FAB mass spectra of cebetin B show ion peaks at m/z 1321 ± 3, 1303 ± 3, 1262 ± 3, and 1245 ± 3. These m/z values correspond to the metal-complexed dimer of cebetin B and its derivative ions [M^+ of $(C_{31}H_{21}O_{13}Cl)_2Mg_2$ corresponds to m/z 1321 and 1323, $M^+ - H_2O$ to m/z 1303 and 1305, $M^+ - COOCH_3$ to m/z 1262 and 1264, and $M^+ - COOCH_3 - H_2O$ to m/z 1244 and 1246]. The low-energy LDFT mass spectra of cebetin B (negative-ion mode) show two strong ion peaks at m/z 633 (100) and 595 (50) arising from the loss of C=O from the monomer–Mg complex ($C_{31}H_{21}O_{13}ClMg$) ($MH^+ - CO$) and the further loss of CH_3 and the metal ion from the complex ($M^+ - Mg + 2H - CO - CH_3$), respectively. These peaks as well as their related peaks [m/z 632 ($M^+ - CO$), 594 ($MH^+ - Mg - CO - CH_3$)] are also present in the DCI spectra (NH_3, negative ion) of cebetin B.

FAB mass spectra of cebetin A, obtained after repeated attempts, failed to show either the metal-complexed dimer or the monomer molecular ion peaks. The compound breaks down into numerous fragment ions and produces a forest of ions with no distinct peaks above m/z 500. However, the spectra of cebetin A (CI with NH_3 or CH_4, positive-ion mode) show a strong signal at m/z 595 (100); the peaks representing the Mg complex (m/z 632 and 633) are absent. Instead, peaks corresponding to the molecular ion of the metal-free ligand molecule (M = $C_{31}H_{23}O_{13}Cl$) (MH^+) and the products after the loss of C=O and CH_3 (m/z 595, 598) are observed. The presence of a chlorine atom in cebetin A is indicated by a relatively strong (41–64%) abundance of M + 2 ions.[42] The mass spectral data strongly suggest that cebetin A is a metal-free isomer of cebetin B. The effect of the absence of metal is also observed in the ^1H NMR spectra of cebetin A. The protons in the vicinity of the ligand groups, especially in the anthraquinone moiety, show differences in their chemical shifts compared to those of cebetin B (Table I). This is considered to be due to changes in the conformation of the molecule after the disappearance of strain caused by metal binding. The proton at position 2′ in cebetin B shows considerably high deshielding and produces a weakly coupled doublet at 4.51 ppm. A structural model shows that it lies close to the plane of aromatic ring A, which is likely to cause this large deshielding. Mg binding seems to cause considerable strain on the seven-membered ring, as a result of which the torsion angle at C-(2′)–C(4)–C(3)–C(11) is 12.7°, although C(4) and C(3) are part of aromatic ring A. When Mg is not present and molecules are not in dimeric form (as proposed for cebetin A), this torsion angle is likely to approach 0°. A structural model of cebetin A with a 0° torsion angle at this position shows that the 2′ proton aligns itself with the plane of aromatic ring A, which may cause even further deshielding of this proton. A 0.37 ppm downfield shift of this proton has been observed in cebetin A compared to that in cebetin B. The slight decrease in the torsion angle at C-(2′)–C(4)–C(3)–C(11) also causes considerable change in the geometry of the two molecular planes because of its unique position and may be associated with the other observed minor changes in the proton chemical shifts.

Structure and Biological Activity. Cebetins have been known as phytotoxic and antibacterial metabolites of *C. beticola* since their isolation by Frandsen in 1955. The fungus continues to cause damage to sugar beet crops. To determine the effect of cebetins on the host cells, sugar beet cell suspension cultures have been grown in the absence and in the presence of cebetin A and cebetin B. At a concentration of 1 ppm or above, both compounds kill the sugar beet cells in the presence of light. In darkness, however, the compounds are ineffective and most of the sugar beet cells remain viable. This observation strongly indicates that cebetins are photoactivated phytotoxins. *C. beticola* produces another photoactivated phytotoxin called cercosporin,[4-8] which is structurally different from cebetins. Cercosporin and a range of other photoactivated phytotoxins reported from a number of fungi are hydroxyperylenequinone type compounds having a common structural component, which is capable of oxidation to diquinone species (Scheme IA). Cebetins do not have a perylenequinone backbone, but its *p*-hydroxyanthraquinone moiety may oxidize to a diquinone species (Scheme IB) under suitable circumstances. If these transitions from quinone to diquinone and vice versa are involved in the toxicity, then cebetins may behave similarly to hydroxyperylenequinone compounds.

Another important structural feature of cebetins is their ability to chelate Mg^{2+} ions. The two ligand groups in the molecule are contributed by two different structural moieties, a xanthone and an anthraquinone. However, both ligand groups are structurally identical, being (i) α,β-unsaturated β-hydroxy keto groups and (ii) part of a bicyclic system in which one of the two six-membered rings is unsaturated. Other naturally occurring Mg^{2+} ligand groups (such as those present in chlorophylls) are structurally different from the ones present in cebetins. At this time, the comparative stability of the cebetin B–Mg complex is unknown. If the ligand group of **2** is found to be highly specific for Mg^{2+} ions, then cebetins are capable of disrupting cellular activities that involve this essential metal ion. Schlösser, in his preliminary studies,[2,15-18] has shown that the cebetins are capable of binding other biologically important metal ions, such as Mn^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{3+}. The unique structural backbone, the presence of a chlorine atom and an epoxide function, and the ability to form diquinone species and to bind various metal ions may separately or synergistically contribute to various aspects of the biological activities reported for cebetins.

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Supplementary Material Available: For cebetin B, tables of atomic coordinates, anisotropic thermal parameters, bond distances, bond angles, and endocyclic torsion angles (9 pages); a listing of observed and calculated structure factors (31 pages). Ordering information is given on any current masthead page.

(42) DCI spectrum (NH_3, positive ion) of cebetin A show the following relative ion abundances. m/z: 639 (13), 641 (6); 612 (68), 614 (31); 595 (100); 597 (41); 596 (41), 598 (7). Analogous data for cebetin B is unavailable due to poor resolution in the spectra obtained.