The Yellow Toxins produced by *Cercospora beticola*.† Revised Structures of Beticolin 1 and Beticolin 3

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The revised structures of beticolins **1** and **3** are described, based on chemical and spectroscopic evidence.

The pathogenic fungus *Cercospora beticola* is responsible for leaf spot disease on sugar beet. It produces colourful toxins with complex structures: a red one called cercosporin,[1,2] and also some yellow compounds, one of which is known as CBT (*Cercospora beticola* toxin).[3,6]

We isolated ten yellow compounds from a mycelial extract of a *C. beticola* strain and purified them by flash chromatography and successive crystallizations. The structures of two of them, beticolins **2** and **4**, have been determined by X-ray diffraction analysis.[7–9] The structures of beticolins **1** and **3** shown in Scheme 1 were proposed as the most reasonable on the basis of the comparisons of their MS, ^{1}H and ^{13}C NMR data with those of beticolin **2** and **4**, respectively.[8,10]

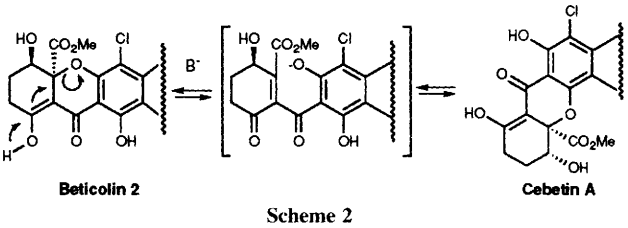
More recently, Jalal *et al.* reported the structure, and also obtained by X-ray diffraction analysis, of a compound named cebetin B[11,12] (Scheme 1). This compound was isolated as a dimeric magnesium chelate. The monomeric form, cebetin A, was present as a minor component of the extract and its structure was deduced by comparing NMR data of the two forms. Beticolin **2** and cebetin A show many similarities. They can be distinguished at two points; (i) the cyclization of the heterocycle occurs, for beticolin **2**, with the hydroxy group being in the *ortho* position relative to the chlorine atom, whereas it occurs at the *para* position relative to the chlorine atom for cebetin A; and (ii) the relative configuration of the secondary alcohol (cycle A) and the carboxymethyl group.

These results incited us to reinvestigate the spectroscopic data of beticolins. We also submitted them to chemical modifications in order to find additional criteria to discriminate the two types of cyclization. The results of these experiments led us to propose a revised structure for beticolins **1** and **3** (Scheme 1).

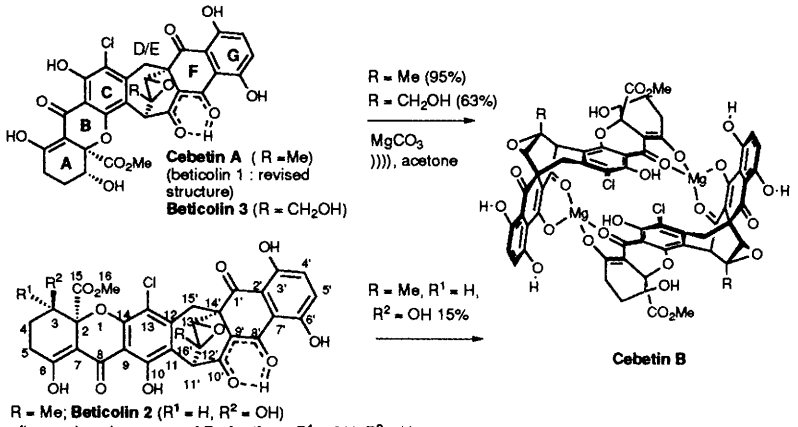
We first tried to obtain dimeric Mg^{2+} complexes of beticolins **1** and **2**. Treatment of beticolin **1** with one equivalent of magnesium carbonate in acetone under sonication resulted, almost quantitatively (95% after recrystallization), in a compound with spectroscopic data‡ identical to those reported for cebetin B[11,12] (Scheme 1). The same reaction performed with beticolin **2** also yielded cebetin B but only with a 15% yield (based on ^{1}H NMR spectrum of the crude mixture). Furthermore, after acidification of this reaction mixture, beticolin **2** was recovered (85%) with beticolin **1** (15%). These results were inconsistent with our previously reported structure for beticolin **1** and led us to reassign for this compound the structure of cebetin A.

The surprising formation of cebetin B from beticolin **2** might be explained by a reto-Michael process allowing the ring opening of the oxygenated heterocycle (ring B) and a reclosure through a nucleophilic addition of the hydroxy group, being in the *para* position relative to the chlorine atom, on the unsaturated system formed during the first step of the process (Scheme 2).

Confirmation of this new reassigned structure for beticolin **1** was achieved by comparison of the correlations present in the 2D ^{1}H–^{1}H phase sensitive NOESY[13] experiments (400 MHz) of beticolin **2** and cebetin A. The former showed a NOE between the benzylic proton at C-11′ and the phenolic hydroxy group at C-10. This correlation was missing in the spectrum of cebetin A. In this case, a weak NOE between the methyl protons at C-16′ and the protons of the methyl ester (C-16) was observed which is only consistent with the structure of cebetin A. Some other NOEs characteristic of the bridged seven membered ring (D/E) and of the *para* diphenolic moiety (ring G) are observed for both compounds (Table 1).§,¶ The presence of a NOE between the benzylic proton at C-11′ and the proton of the phenolic hydroxy group at C-10 was



Scheme 2



Scheme 1

Table 1 Observed NOE differences

Obs. NOEs	Bet. 1	Bet. 2	Cebetin B	Bet. 3	Bet. 4
13–15′	+	+	+	+	+
13′–16′	+	+	+	+	+
11′–16′	+	+	+	+	+
OH(3′)–4′	+	+	+	+	+
OH(6′)–5′	+	+	+	+	+
OH(10)–11′		+			+
16–16′	+		+		
OH(6′)–16			+		

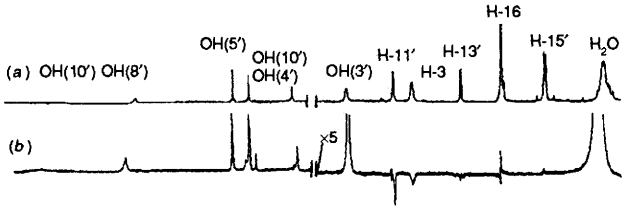


Fig. 1 NOE difference spectra of beticolin **2** (300 MHz; CD_3COCD_3). (*a*) Normal spectrum (δ 11.2–15.3 and 2.8–5.7). (*b*) NOE difference with irradiation of OH(10).

unexpected because of the possible chelation of the latter by the neighbouring carbonyl groups, compelling the hydroxy group to point in the 'wrong' direction; however, the chemical shifts of this phenolic proton (δ 12.15) indicates that it is not so strongly chelated as the proton of the enolic hydroxy group at C-6 (δ 14.0), so that detection of this critical NOE become possible; nevertheless, to confirm the presence of this NOE, we measured 1D NOE differences, to have some indication of the size of the effect (Fig. 1) which proved to be about 4 to 6%. However, the low solubility of these compounds and the difficulty in obtaining good results in NOE difference experiments due to the problems related to chemical exchange prompted us to advocate NOESY experiment as a more sensitive method to achieve a rapid discrimination between both types of cyclization.

^{2}D NOESY experiments performed on cebetin B showed the same NOE between the two methyl groups as observed for cebetin A (Table 1). The dimeric structure of cebetin B in solution is also confirmed by the observation of a strong NOE between the protons of the methyl ester at C-16 and the proton of the phenolic hydroxy group at C-6′,¶ which is only consistent with an interaction between the two units of the dimer.

Similar experiments were performed with beticolins **3** and **4**. When treated with magnesium carbonate, beticolin **3** was transformed with a high yield (63%) into the hydroxylated derivative of cebetin B,‖ its structure has been determined by analogy with cebetinB. Under the same conditions, beticolin **4** gave only trace amount of this dimeric compound; therefore, we can infer that the correct structure of beticolin **3** corresponds to a similar heterocyclic ring closure as for cebetin A. NOESY experiments performed on beticolin **3** and **4** confirm the beticolin type skeleton for beticolin **4**.

Thanks to the present combined approaches, chemical transformations and NMR studies, we are able to point out the differences that allow a rapid discrimination between the two types of cyclization. The major difficulty in the structure elucidation of this family of compounds could be overcome. Furthermore, we can assume that the compounds having a 'linear' skeleton (beticolins **2** and **4**) can undergo, in basic media, spontaneous transformation to the corresponding compounds with a 'bent' skeleton (cebetin A and beticolin **3**). This could explain why Jalal *et al.* and Arnone *et al.* did not find beticolin **2** in their extracts. The absence of a Mg-complex in our case could be due to the different extraction conditions.

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Footnotes

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‡ Beticolin **1** (2.73 mg, 4.28 μmol) and $MgCO_3$ (2.83 mg, 1 equiv.) were dissolved in acetone (0.25 ml) and sonicated for 8 h and the reaction mixture was left at room temperature without stirring for 3 days. The solid residue was then removed by filtration and washed with acetone (2 ml). The organic phase was concentrated under reduced pressure and cebetin B (2.65 mg, 95%) crystallised from hexane–ethyl acetate. Mp = >300 °C. $[\alpha]_D$ = +220° (*c*. 0.048, CH_2Cl_2). ^{1}H NMR δ (CD_3COCD_3; 400 MHz *J*/Hz): 13.60 (s, 1H, OH-6′), 12.80 (s, 1H, OH-10), 12.38 (s, 1H, OH-3′), 7.1, 7.21 (2H, *J* 9.5, H-4′, H-5′). 5.65 [d, 1H (exchangeable with D_2O) *J* 4.2], 4.3 (dt, 1H, *J* 11.4, 4.5, H-3), 2.62 (ddd, 1H, *J* 18, 11.4, 5.2, H-5β), 2.37 (ddd, 1H, *J* 18, 5.7, 0.8, H-5α), 2.25 (qd, 1H, *J* 11.4, 5.2, H-4α), 2.01 (tdd, 1H *J* 11.4, 4.5, 0.8, H-4β), 3.55 (s, 3H, H-16), 4.52 (d, 1H, H-11′, *J* 1.4), 3.92 (d, 1H, H-13′, *J* 1.4), 3.15, 3.05 (2H, *J* 17.5, H-15′), 1.7 (s, 3H, H16′). These spectroscopic data are in agreement with those given in the literature[11] and were found to be identical to those obtained with an authentic sample given by G. Nasini.[12]

§ ^{1}H-^{1}H NOESY experiments were performed on a BRUKER AM 400 (400 MHz) using standard programs and were interpreted prior to symetrisation. Mixing times are usually chosen to be about the largest relaxation time of the protons of the molecule; *i.e.* beticolin **2**: 2.3 s; cebetin B: 1.2 s; beticolin **3**: 1.7 s; beticolin **4**: 1.9 s; due to the wide range of relaxation times of the different protons in cebetin A (0.2 to 3.3 s) two different experiments have been needed using 2.4 and 0.9 s as mixing times.

¶ Attribution of the phenolic protons of beticolins **2**, **3**, **4** and of cebetin B was made by analogy with cebetin A[8,11] for which observation of long range (^2J and ^3J) ^{1}H–^{13}C correlations between the phenolic protons and the nearest aromatic carbons allowed an unambiguous assignment.

‖ ^{1}H NMR δ (CD_3COCD_3; 400 MHz *J*/Hz): 13.65 (s, 1H, OH-6′), 12.80 (s, 1H, OH-10), 12.40 (s, 1H, OH-3′), 7.1, 7.25 (2H, *J* 9.5, H-4′, H-5′), 6.23 [d, 1H (exchangeable with D_2O) *J* 4.5], 4.22 (dt, 1H, *J* 11, 4.5, H-3), 2.6 (ddd, 1H, *J* 18, 11.4, 5.2, H-5β), 2.4 (ddd, 1H, *J* 18, 5.7, 0.8, H-5α), 2.3 (qd, 1H, *J* 11.4, 5.2, H-4α), 2.05 (tdd, 1H *J* 11.4, 4.5, 0.8, H-4β), 3.6 (s, 3H, H-16), 4.81 (bs, 1H, H-11′), 4.05 (bs, 1H, H-13′), 3.13, 3.01 (2H, *J* 17.5, H-15′), 3.8 [bt, 1H (exchangeable with D_2O) *J* 5], 3.45–3.6 (m, 2H, H16′).

References

1 R. J. J. C. Lousberg, U. Weiss, C. A. Salemink, A. Arnone, G. Nasini and L. Merlini, *J. Chem. Soc., Chem. Commun.*, 1971, 1463.
2 M. E. Daub and M. Ehrenshaft, *Plant. Physiol.*, 1993, **89**, 227.
3 J. P. Blein, I. Bourdil, M. Rossignol and R. Scalla, *Plant. Physiol.*, 1988, **88**, 429 and ref. therein.
4 E. Schlösser, *Phytopathol. Mediterr.*, 1971, **10**, 154.
5 G. Assante, R. Locci, L. Camarda, L. Merlini and G. Nasini, *Phytochemistry*, 1977, **16**, 243.
6 G. Assante, R. Locci and G. Nasini, *Riv. Patal. Veg. S. IV*, 1986, **22**, 41.
7 M.-L. Milat, T. Prangé, P.-H. Ducrot and J.-P. Blein, *Supp. Plant Physiol.*, 1991, **96**, 140.
8 M.-L. Milat, T. Prangé, P.-H. Ducrot, J.-C. Tabet, J. Einhorn, J.-P. Blein and J.-Y. Lallemand, *J. Am. Chem. Soc.*, 1992, **114**, 1478.
9 T. Prangé, N. Neuman, M.-L. Milat and J.-P. Blein, *Acta Crystallogr. Sect B*, 1994, accepted for publication.
10 M.-L. Milat, T. Prangé, P.-H. Ducrot, J.-C. Tabet, J. Einhorn, J.-P. Blein and J.-Y. Lallemand, *Tetrahedron Lett.*, 1993, **34**, 1483.
11 M. A. F. Jalal, M. B. Hossain, D. J. Robeson and D. Van der Helm, *J. Am. Chem. Soc.*, 1992, **114**, 5967.
12 Arnone *et al.* have proposed a structure for CBT which differs from their previous results (ref. 5 and 6) but confirms the structure of Jalal's cebetin B. (A. Arnone, G. Nasini, L. Merlini, E. Ragg and G. Assante, *J. Chem. Soc. C.*, 1993, 145).
13 G. Bodenhausen, H. Kogler and R. R. Ernst, *J. Magn. Reson.*, 1984, **58**, 370.