Secondary Mould Metabolites. Part 41.[1] Structure and Biosynthesis of *Cercospora beticola* toxin (CBT)

Alberto Arnone,[a] Gianluca Nasini,*[,a] Lucio Merlini,[b] Enzio Ragg[b] and Gemma Assante[c]
[a] *Centro del C.N.R. per le Sostanze Organiche Naturali, Dipartimento di Chimica del Politecnico, Politecnico di Milano, Piazza L. da Vinci 32, I 20133 Milano, Italy*
[b] *Dipartimento di Scienze Molecolari Agroalimentari, Sezione di Chimica, Università di Milano, Via Celoria 2, I 20133 Milano, Italy*
[c] *Istituto di Patologia Vegetale, Università di Milano, Via Celoria 2, I 20133 Milano, Italy*

The structure of *Cercospora beticola* toxin (CBT) **1**, a phytotoxic metabolite, was elucidated on the basis of mass, ^{1}H NMR, ^{13}C NMR and 2D NMR (^{13}C, ^{13}C COSY and ^{1}H, ^{1}H ROESY) spectra and from biosynthetic evidence obtained from incorporation of [1-^{13}C]acetate and [1,2-^{13}C]acetate. The product is a 2:2 complex with Mg of a compound derived from the coupling of two octaketide-derived reduced anthraquinone and xanthone moieties.

Many species of the fungal genus *Cercospora*, responsible for leaf spot diseases in many crops, produce secondary metabolites, some of them deemed to be phytotoxins. Particularly widespread is cercosporin, a red pigment with a perylenequinone structure and photodynamic activity.[2]

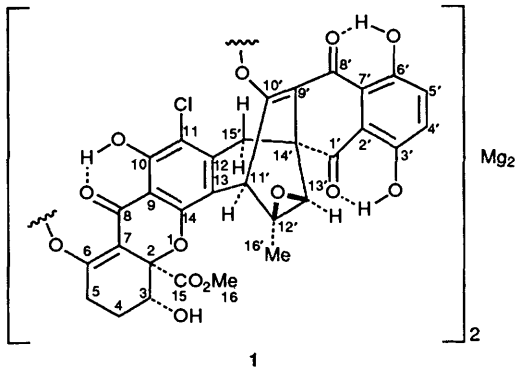
However, Frandsen[3] and later Schlösser[4,5] observed that some strains of *Cercospora beticola* produce, instead of cercosporin, a yellow substance. Schlösser reported antibiotic and phytotoxic activity for this substance, which he named '*Cercospora beticola* toxin' (CBT).[6] The presence of this compound, alone or together with cercosporin, was observed in cultures of other strains of *Cercospora*, and some data on its constitution were reported.[7]

We grew Schlösser's strain of *C. beticola* on different culture media to obtain some batches of the yellow substance. Samples of this compound were distributed for biological activity testing and were found to be active on plant-cell-transport phenomena, including K^+ uptake and H^+ extrusion, depolarization of transmembrane electric potential, and a specific inhibition of a plasmalemma K^+–Mg^{2+}-dependent ATPase *in vitro*.[8,9] A direct effect of our compound on vanadate-sensitive ATPase presumably associated with plasma membrane was also observed.[10] A report[11] that CBT is capable of competing with tritium-labelled dihydrofusicoccin in binding to fusicoccin receptors was not confirmed by more recent studies on a more purified sample of CBT.[12] CBT shows photodynamic activity,[13] measured *in vitro* as the amount of hydroperoxide produced by methyl oleate peroxidation.[14]

In an effort to obtain enough pure CBT for structural studies, a strain of *Cercospora beticola* (IPV-F573), derived from Schlösser's original strain, was grown on glucose-yeast extract in beet filtrate for 2 weeks (see Experimental section). Extraction of the dry mycelium with methanol gave, after purification, CBT **1**. It was obtained as yellow crystals (from acetone–hexane) of m.p. > 300 °C (decomp.), $[\alpha]_D$ +310 × 10^{-1} deg cm^2 g^{-1} (*c* 0.05, MeOH) and appeared identical with a crude sample previously isolated by Schlösser.

A recent report[15] by Ducrot and co-workers on the structure of beticolins **2** and **3**, two stereoisomeric compounds deemed to constitute the toxin, prompts us to publish our own results, which led us to attribute to CBT **1** the structure of a 2:2 complex, with magnesium, of two molecules of a compound which is isomeric with the beticolins, due to different ring junctions and a different position of the chlorine atom. No formation of beticolins was observed in our cultures.

The ^{1}H NMR spectrum of CBT **1** showed the presence of 21 protons (Table 1). They were assigned on the basis of chemical-shift considerations and selective ^{1}H–{^{1}H} decoupling experiments to two *ortho*-coupled aromatic protons (4′- and 5′-H; ^3J 9.1 Hz), a C(5)H$_2$–C(4)H$_2$–C(3)HOH grouping in which the 3-hydroxy proton exhibits a vicinal coupling of 3.7 Hz with 3-H, an isolated methylene group (15′-H$_2$), two weakly coupled methine protons (11′- and 13′-H; ^4J 1.4 Hz), an OMe and a tertiary methyl group (16- and 16′-H$_3$) and three hydrogen-bonded aromatic hydroxy protons (10-, 3′- and 6′-OH).



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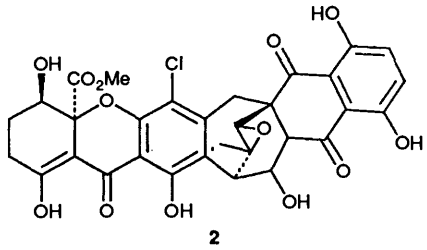

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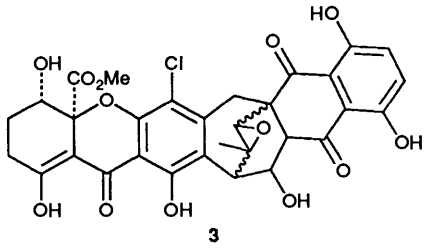

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The broad-band ^{1}H-decoupled ^{13}C NMR spectrum revealed the presence of 31 signals (Table 2), ten of them attributable on the basis of DEPT experiments to five CH, three CH$_2$ and two

CH_3 groups, while the analysis carried out on the fully ^1H-coupled spectrum, as corroborated by selective $^{13}C-\{^1H\}$ decoupling experiments, permitted us to obtain the C,H coupling constants reported in Table 2.

The above NMR evidence indicated that CBT **1** contains at least 13 oxygen atoms. Five of them belong to carbonyl or enolic groups, as they are linked to carbons resonating between δ_C 174.97 and 205.23 (C-6, -8, -1′, -8′, -10′); two belong to a CO_2Me group because the values of 148 Hz for the one-bond C,H coupling[16] of the OMe group and of 4 Hz for the three-bond coupling between the C-15 ester carbon and the 16-methyl protons, as well as the chemical shift of δ_C 172.58 exhibited by C-15, are characteristic of a methoxycarbonyl group; one is part of an oxirane ring since C-13′ at δ_C 61.75 exhibits a typical one-bond C,H coupling[17] of 186 Hz; one belongs to an ethereal-like moiety because the chemical-shift value of C-2 (δ_C 87.28) is indicative of the presence of an oxygen function at this carbon; and four are due to hydroxy groups.

Table 1 ^1H NMR chemical shifts (δ) and coupling constants (Hz) for compounds **1** and **4** in [$^2H_6$]acetone

Proton	1	4	J	1	4
3	4.26	5.68	3,4α	11.7	9.5
4α	2.25	2.74	3,4β	5.2	6.7
4β	1.99	2.85	3,3-OH	3.7	
5α	2.39	5.79	4α,4β	13.0	17.7
5β	2.61		4α,5α	6.5	3.0
16	3.57	3.77	4α,5β	12.0	
4′	7.08	7.47[a]	4β,5α	1.8	6.0
5′	7.18	7.56[a]	4β,5β	6.9	
11′	4.50	4.64	5α,5β	17.9	
13′	3.94	4.30	11′,13′	1.4	
15′α	3.15	3.29	15′α,15′β	17.8	
15′β	3.02	3.44	4′,5′	9.1	8.8
16′	1.42	1.60			
3-OR	5.60	2.20			
8-OAc		2.22[b]			
10-OR	12.79	2.26[b]			
3′-OR	12.39	2.36[c]			
6′-OR	13.61	2.32[c]			

[a,b,c] Assignments may be interchanged.

Measurements of the mass spectra with different techniques indicated a relative molecular mass above 1000 daltons, which was confirmed by a vaporimetric measure, which gave a value of ~1170 daltons. The FAB-MS spectrum shown in Fig. 1 finally indicated a relative molecular mass of 1322 daltons. Elemental analyses gave an average formula of $C_{30-32}H_{21-22}O_{12-14}$, and the absence of both nitrogen and sulfur. As the sum of C, H and O percentages was less than 100, a systematic search for other elements was carried out, and it was found that the compound contains chlorine (5.1%). Taking into account the NMR results, which indicated the presence of 31 C, 21 H, and at least 13 O atoms (which was consistent with the elemental composition), it appeared that CBT had to be a symmetrical dimer formed by two moieties of 661 mass units, of partial composition $C_{31}H_{21}ClO_{13}$, which amounts to 637 daltons. What remains to achieve a partial mass 661 is 24 mass units, which would indicate a magnesium atom. The presence of magnesium was confirmed by elemental analysis, so that the formula $C_{62}H_{42}Cl_2Mg_2O_{26}$ could be attributed to CBT **1**.

As the evidence obtained so far was not enough, also due to the large number of quaternary carbons, for us to obtain a structure for CBT **1**, cultures of *Cercospora beticola* were grown in the presence of either $^{13}CH_3CO_2Na$ or $^{13}CH_3{}^{13}CO_2Na$.

Table 2 Selected ^{13}C NMR data for CBT **1** in [$^2H_6$]acetone

Carbon atom	δ_C[a]	^1J(CH)/Hz	$^{>1}J$(CH)/Hz
2	87.28 S br ddd		4 (3-H), 2 (4-H$^\alpha$), 7.5 (4-H$^\beta$)
3	73.53 D m	145	
4	26.76 T m	129	
5	35.84 T m	126.5	
6	191.99 S ddt		8 and 2 (5-H_2), 7 (4-H_2)
7	98.95 S br s		
8	176.64 S s		
9	105.70 S d		5.5 (10-OH)
10	156.63 S d		4.5 (10-OH)
11	115.48 S br dt		6 (10-OH), 3.5 (15′-H_2)
12	140.55 S dt		6 (11′-H), 6.5 (15′-H_2)
13	113.33 S dt		5.5 (11′-H), 3.5 (15′-H_2)
14	154.10 S d		4 (11′-H)
15	172.58 S dq		6 (3-H), 4 (16-H_3)
16	52.40 Q s	148	
1′	205.23 S br s		
2′	113.22 S dd		5.5 (4′-H), 5 (3′-OH)
3′	157.06 S ddd		2.5 (4′-H), 10 (5′-H), 5.5 (3′-OH)
4′	123.95 D d	164	7 (3′-OH)
5′	129.98 D d	162	7 (6′-OH)
6′	155.70 S ddd		2.5 (5′-H), 9.5 (4′-H), 5.5 (6′-OH)
7′	117.59 S dd		5 (5′-H), 5 (6′-OH)
8′	174.97 S s		
9′	102.71 S m		
10′	193.55 S d		7 (11′-H)
11′	50.41 D br q	139	4 (16′-H_3)
12′	59.55 S ddq		5.5 (11′-H), 2 (13′-H), 5.5 (16′-H_3)
13′	61.75 D m	186	
14′	51.22 S dt		5 (13′-H), 3.5 (15′-H_2)
15′	42.93 T br s	132.5	
16′	19.44 Q dd	128	2.5 (11′-H), 2.5 (13′-H)

[a] Capital letters refer to the pattern resulting from directly bonded (CH) couplings and small letters to that from (CH) couplings over more than one-bond. S or s = singlet, D or d = doublet, T or t = triplet, Q or q = quartet, m = multiplet and br = broad (no fine structure but the line is noticeably broadened, indicating unresolved coupling).

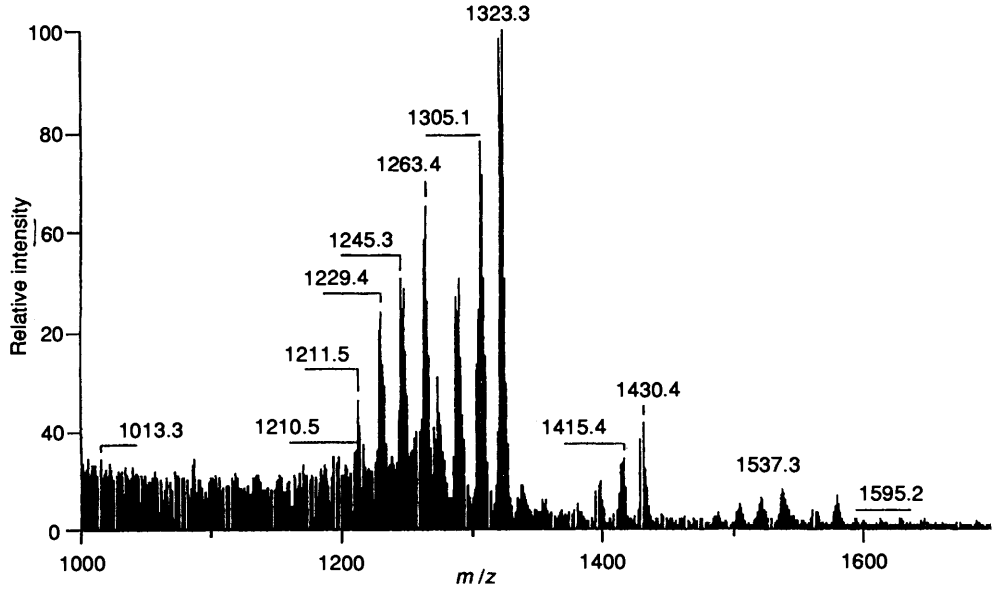


Fig. 1 FAB-MS spectrum of CBT 1

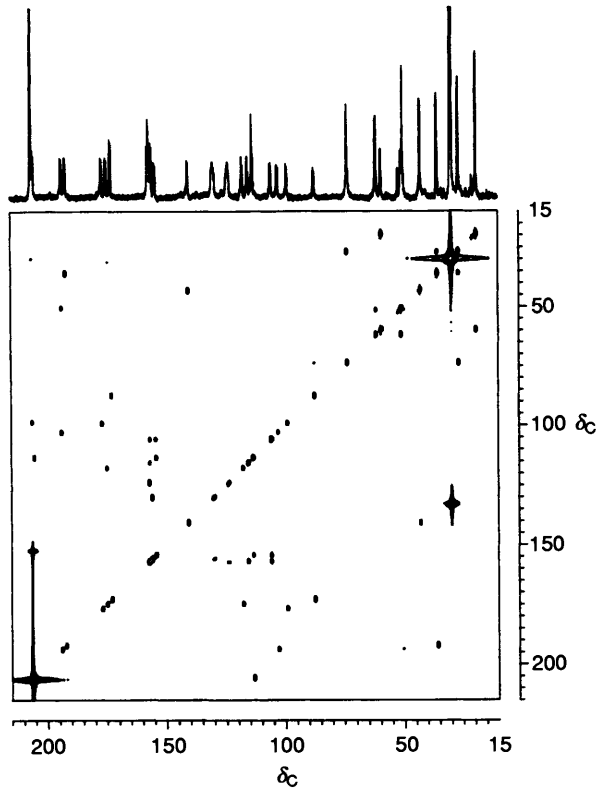


Fig. 2 $^{13}C,^{13}C$ COSY spectrum of CBT 1

Incorporation of the labelled acetate units into CBT was enough to give biosynthetic information (see later) and, in the latter case, to allow us to submit the labelled compound to a $^{13}C,^{13}C$ COSY experiment (Fig. 2), from which some pairs of bonded carbons were identified. Thus, the ^{1}H-decoupled ^{13}C NMR spectrum of CBT derived from [2-^{13}C]acetate showed the enhancement of 16 carbon signals, *viz.* C-3, -5, -7, -9, -11, -13, -15, -1′, -3′, -5′, -7′, -9′, -11′, -13′, -15′ and -16′, while the analysis of the ^{1}H-decoupled ^{13}C NMR and $^{13}C,^{13}C$ COSY spectra of CBT derived from [1,2-^{13}C]acetate revealed one-bond C,C couplings for the resonances of the carbon atoms arising from

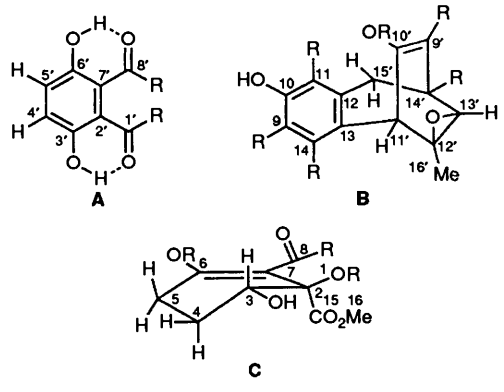
Table 3 Values for ^{1}J(C,C) observed in the ^{13}C NMR spectrum of [1,2-^{13}C]acetate-derived CBT 1

$^{1}J^{a}$	Hz	^{1}J	Hz
2, 15	63.6	13, 14	71.8
3, 4	37.7	1′, 2′	53.9
5, 6	41.8	3′, 4′	65.5
7, 8	66.5	5′, 6′	65.2
9, 10	65.2	7′, 8′	55.0
9, 14	67.5	9′, 10′	61.0
10, 11	77.5	12′, 16′	47.6
12, 15′	44.9	13′, 14′	42.2

[a] Together with the cross-peaks corresponding to the above reported ^{1}J(C,C)-values, the $^{13}C,^{13}C$ COSY spectrum of CBT 1 showed weaker cross-peaks arising from ^{1}J(C,C) between carbons of adjacent acetate units, *i.e.* C(4)–C(5), C(11)–C(12), C(12)–C(13), C(1′)–C(14′), C(4′)–C(5′), C(6′)–C(7′) and C(10′)–C(11′).

intact acetate units and additional one-bond C,C couplings for the carbons arising from adjacent acetate units (Table 3).

The above reported evidence, together with the results described below, permitted us to construct the structural fragments **A**–**C**.



Fragment **A**. This fragment was characterized by the presence of a tetrasubstituted aromatic ring whose carbons resonate between δ_C 113.22 and 157.06. Two hydrogen-bonded hydroxy groups were located at C-3′ and C-6′ because their protons at δ_H

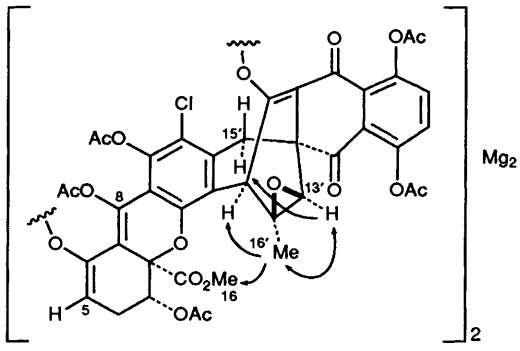
12.39 and 13.61 were vicinally coupled[18] to the *ortho*-disposed C-4′ and C-5′ carbons ($^3J_{C,H}$ 7 Hz). Additional vicinal and geminal couplings were also observed between the hydroxy protons and C-2′ and C-3′, and C-7′ and C-6′ respectively ($^3J_{C,H}$ 5 and $^2J_{C,H}$ 5.5 Hz). As expected, the *ortho*-disposed 4′- and 5′-methine protons presented two-bond couplings with C-3′ and C-6′, ($^2J_{C,H}$ 2.5 Hz) and three-bond couplings with C-2′ and C-6′, and C-3′ and C-7′ respectively ($^3J_{C,H}$ 5.5, 9.5; and 10, 5 Hz). Finally, the one-bond couplings observed in the $^{13}C,^{13}C$ COSY spectrum between C-1′ and C-2′ and between C-7′ and C-8′ allowed us to complete part structure **A**.

Fragment **B**. The presence of a phenolic moiety having a methine and a methylene group *para* and *meta*-positioned with respect to the hydroxy group followed from the chemical-shift values ranging from δ_C 105.70 to 156.63 exhibited by the aromatic carbons and from the C,H couplings observed between the 10-hydroxy proton and C-9, -10 and -11 ($J_{C,H}$ 4.5–6 Hz), between the 11′-methine proton and C-12, -13 and -14 ($J_{C,H}$ 4–6 Hz), and between the 15′-methylene protons and C-11, -12 and -13 ($J_{C,H}$ 3.5–6.5 Hz). Other carbons of this fragment, *i.e.* C-9′, -10′, -12′, -13′, -14′ and -16′, exhibited additional C,H couplings with 11′-H and/or 15-H_2. One-bond couplings were observed between C-9′ and C-10′, whose chemical shifts suggest that they are part of an enolic moiety, between C-12′ and C-16′, and between C-13′ and C-14′. The two couplings of 2.5 Hz observed between the C-16′ methyl carbon and 11′-H and the C-13′ oxirane proton, together with the fact that the C-16′ methyl protons presented three-bond couplings only with C-11′ and C-13′ ($J_{C,H}$ 4 and ~3 Hz) imply not only that C-12′ is linked to C-11′ and C-13′, but also that C-12′, resonating at δ_C 59.55, is part of the oxirane ring. Finally, the observation that the multiplet due to C-9′ simplified upon selective irradiation of 11′-H, 13′-H and 15′-H_2 and that the doublet due to C-10′ presented a C,H coupling of 7.5 Hz with 11′-H permitted us to complete the fragment by joining C-9′ and C-15′ with C-14′, and C-10′ with C-11′.

Fragment **C**. The C,H interactions observed in decoupling experiments between C-2 and 3-H and 4-H_2, and between C-7 and 5-H_2, together with the one-bond C,C, couplings observed between C-2 and C-15; C-5 and C-6; and C-7 and C-8, allowed us to extend the above mentioned C(3)–C(5) connection. Furthermore, the H,H couplings presented by 3-H, 4-H_2 and 5-H_2 suggested that C-2 and C-7 are linked together to form a cyclohexene ring in which 3-H, assumed to be β, 4-H^α and 5-H^β are disposed *trans* diaxially ($^3J_{H,H}$ 11.7 and 12.0 Hz), and that C-6 and C-7, resonating at δ_C 191.99 and 98.95 respectively, belong to an enolic moiety.

CBT appeared to be rather sensitive to many reagents, giving rise to complex mixtures, so that it was not possible to obtain structural evidence from chemical degradation. However, acylation of CBT **1** with acetic anhydride and pyridine at 0 °C afforded the decaacetate **4**. Four of each molecular half's acetyl groups derive from the alcoholic and from three phenolic hydroxy groups, while the fifth is due to the acetylation of the 8-OH group arising from the enolization of the C-8 α,β-unsaturated carbonyl group. This appears clearly from the ^1H NMR spectrum of derivative **4**, which showed the presence of an olefinic proton at δ_H 5.79 and the absence of those resonances which were assigned to 5-H_2 in the ^1H NMR spectrum of CBT **1** (Table 1).

Nuclear Overhauser experiments (NOEs) carried out on this compound as well as on CBT **1** gave additional stereochemical information and evidence of the connection between fragments B and C. In fact, the cross-peaks observed in a 2D ROESY (rotating-frame Overhauser enhancement spectroscopy) spectrum of CBT **1** between the C-16 methyl protons of the methoxycarbonyl group and 4-H^α, but not with 4-H^β, and between the same methyl protons and 16′-H_3 (3% in the acetate



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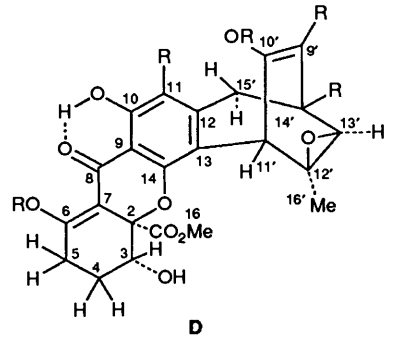
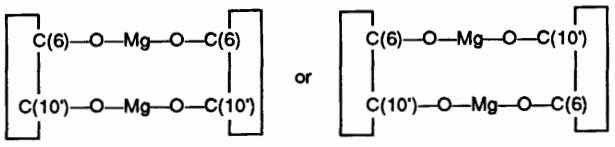
4) imply that these protons are spatially close and hence on the same, α side of the molecule. The former NOE allowed us to assign the *cis* relative configuration to the CO_2Me and the 3-OH groups, 3-H being β-disposed, while the latter evidence, as corroborated by the chemical shift of δ_C 154.10 by C-14 in CBT **1**, which is indicative of an oxygen-bearing carbon, suggests that fragments **B** and **C** are linked together *via* a C(2)–O–C(14) bridge and a C(8)–C(9) bond to give a xanthone moiety **D** where the C-8 carbonyl group gives rise to an intramolecular hydrogen bond with the 10-OH proton at δ_H 12.79. The randomization of ^{13}C-labelling in the sequence from C-11 to C-13 in fragment **B** is consistent with a standard biosynthetic pathway for fungal xanthones (see later). Additional NOEs observed between 13′-H and 16′-H_3 (14% in **4**) and between 13′-H and the C-15′ methylene proton at δ_H 3.15 in **1** and at δ_H 3.29 in **4** (3%) indicate that these protons are α-disposed too.

To assemble the structure of CBT **1** we must connect fragment **D** *via* five linkages to one Cl atom, one Mg atom and to fragment **A**. The presence in the $^{13}C,^{13}C$ COSY spectrum of a weak cross-peak between C-1′ and C-14′ indicates that these carbons, which derive from two adjacent [1,2-^{13}C]acetate units, are linked together. Inspection of a Dreiding model of fragment **D** clearly shows that the remaining C(8′)–C(9′) linkage can be formed in only one way to give a dihydroanthraquinone moiety. The large value of 77.5 Hz observed in CBT **1** for the one-bond C,C coupling between the sp^2-hybridized aromatic carbons C-10 and C-11 (Table 3) suggests that the chlorine atom is located at C-11, since it is known that the value of such C,C couplings increases with the electronegativity of the substituent.[19] Finally, the Mg atom can be linked to the O-6 or O-10′ oxygen atoms.



D

The two units forming CBT **1** can be now coupled as depicted in Scheme 1, *via* C(6)–O–Mg–O–C(6) and C(10′)–O–Mg–O–C(10′) linkages, or *via* two C(6)–O–Mg–O–C(10′) linkages, both couplings giving rise to a C_2 symmetry axis for the whole molecule. This arrangement explains the half-signals in the ^1H and ^{13}C NMR spectra. The choice in favour of the head-to-tail coupling could be made on the basis of NOE connectivities arising from the above mentioned 2D ROESY spectrum



Scheme 1 Possible coupling modes of the two units to give CBT **1**

performed on CBT **1**. Specifically, in the cross-section (a) depicted in Fig. 3, 3′-OH showed NOEs with the aromatic protons, 13′-H and 15′-H_2, which are well accounted for by the through-space interactions between protons *ca.* 4 Å apart belonging to the same moiety, and in cross-section (b) 10-OH showed NOEs with 6′-OH and 15′-H^{β} with intensities that are of the same magnitude as those found for 3′-OH. This fact suggests that the interatomic distances between the protons involved in the latter experiment are in the region of 4 Å too.

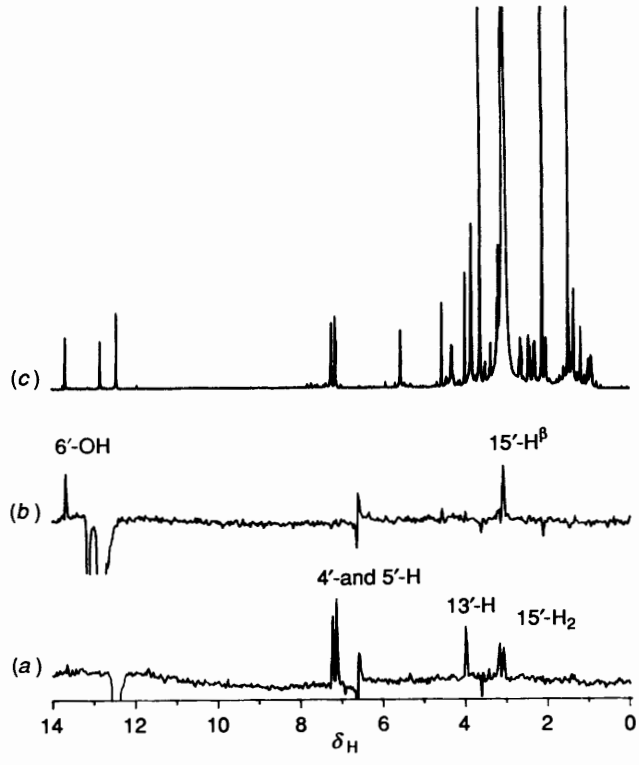


Fig. 3 Cross-sections of the 2D ROESY experiment performed on CBT **1** at ^{1}H chemical shift of 3′-OH (*a*) and 10-OH (*b*), and control spectrum (*c*)

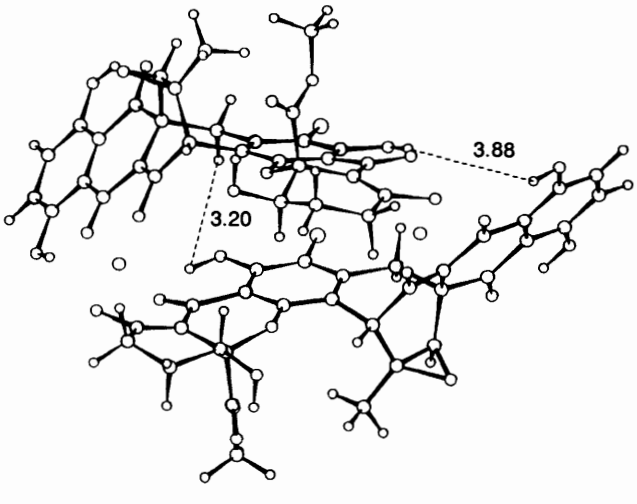
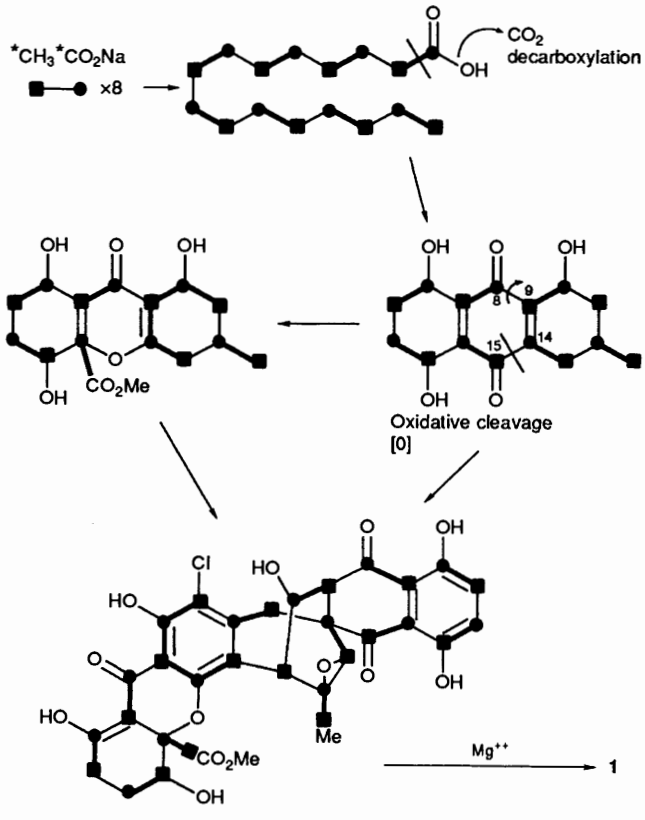


Fig. 4 Molecular model of CBT **1**, with 10-OH – – – – 15′-H$^{\beta}$ (3.20 Å) and 10-OH – – – – 6′-OH (3.88 Å) distances shown. The Mg ions (not included in the calculations) are inserted for sake of clarity

Since the distance between 10-OH and 6′-OH within the same moiety is 10.7 Å, as measured from an energy-minimized molecular model (see Experimental section), the interaction found for these two protons must necessarily arise from hydrogens located in the two symmetry-related moieties. The same applies for the interaction between 10-OH and 15′-H$^{\beta}$, which are 5.9 Å distant. It is possible to build a model with a head-to-tail arrangement where 10-OH is close in space to 6′-OH and 15′-H$^{\beta}$, at a distance of 3.88 and 3.20 Å respecively (Fig. 4). This arrangement is similar to that observed in the solid state for a compound called cebetin B, also isolated from *Cercospora beticola*, whose X-ray structure, recently disclosed by Hossain *et al.* in a communication at a meeting,[20] appears to be the same as that of CBT. As no data have been published so far on cebetin B, we can only suppose that CBT and cebetin B are one and the same compound.

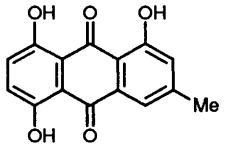
Structure **1** is consistent with biogenetic considerations. Inspection of the ^{13}C-labelling resulting from incorporation of acetate (Scheme 2) indicate that each of the two identical



Scheme 2

moieties of CBT are formed by coupling of two octaketide-derived units, both of 15 carbon atoms due to decarboxylation. One moiety is a reduced anthraquinone, and the other is a reduced xanthone, this latter being formed from an anthraquinone *via* cleavage of the C(14)–C(15) bond, and free rotation around the C(8)–C(9) bond, followed by addition–elimination cyclization. The cleavage is similar to that occurring in other fungal xanthones, such as α- and β-diversonolic esters.[21] Consistent with this hypothesis is the randomization of labelling in the chlorine-bearing aromatic ring, which requires an axis of symmetry in the intermediate benzophenone[22] and therefore indicates that chlorine must be introduced *after* the anthraquinone–xanthone rearrangement.

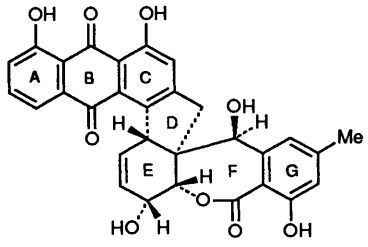
The substitution patterns on both moieties indicate that they



5

derive from the same precursor, which, on aromatization, should give elminthosporin **5**. As a matter of fact, elminthosporin was occasionally found in our cultures of *Cercospora beticola*.

On the other hand, the type of coupling between the two units is quite unusual, the only other example known to us being that of rubellins A (**6**) and B–D, four metabolites produced by a strain of the closely related fungus, *Mycosphaerella rubella*, whose structures were elucidated by some of us.[23]



6

Experimental

M.p.s were measured on a Kofler apparatus and are uncorrected. UV and IR spectra were recorded with a Jasco Uvidec-510 spectrophotometer and a Perkin-Elmer 177 instrument, respectively; optical rotations were taken on a Jasco DIP-181 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}; and mass spectra on a Finnigan MATTSQ70 spectrometer. NMR spectra were acquired at room temperature on Bruker AC-250 and AMX-600 spectrometers. The vaporimetric measure was performed by Mikroanalytisches Labor Pascher, Remagen, Germany, and the microanalyses by Redox, Cologno Monzese, Italy.

Isolation and Purification of CBT **1**.— A strain of *Cercospora beticola*, named IPV-F573 (Institute of Plant Pathology, University of Milan), originally obtained from Professor Schlösser in 1972, was maintained on MPGA [malt, peptone, glucose, agar (20:2:20:15 g dm^{-3})] slants and sub-cultured for 2 weeks at 24 °C in 25 stationary Erlenmeyer flasks (300 cm^3) containing 30 cm^3 of a liquid medium of the composition: glucose (20 g dm^{-3}), yeast extract (2 g dm^{-3}) in beet filtrate, obtained by boiling frozen beet leaves (300 g dm^{-3}) in deionized water. For the ^{13}C incorporation studies, 99% sodium [1,2-^{13}C]acetate or 99% sodium [1-^{13}C]acetate (Aldrich) (1 mg cm^{-3}) was added to the liquid culture 4 days after the inoculation. The mycelium was separated from the culture filtrate, dried in a ventilated oven at 40 °C, and extracted in a Soxhlet apparatus with hexane, then with methanol. The MeOH extract was evaporated, water (150 cm^3) was added, and the mixture was extracted with AcOEt. The organic solvent was dried (Na_2SO_4) and evaporated to give crude product (0.8 g). This was dissolved in a mixture of CH_2Cl_2 (5 cm^3) and MeOH (1 cm^3) and the solution was stirred while hexane (200 cm^3) was added. The precipitate was collected and chromatographed on a column of Merck silica gel with CH_2Cl_2–MeOH (15:1) as eluent. The fractions with a typical yellow-green fluorescence were collected and evaporated to yield pure *CBT* **1** (80 mg); R_f 0.2 in CH_2Cl_2–MeOH (9:1); m.p. >300 °C (decomp.); $[\alpha]_D$ +310 (*c* 0.05, MeOH) [Found: C, 57.4; H, 3.3; Cl, 5.1; Mg, 3.3. $(C_{31}H_{21}O_{13}ClMg)_2$ requires C, 56.30; H, 3.20; Cl, 5.36; Mg 3.68]; *m/z* (FAB, thioglycerol) 1323 $[MH]^+$; λ_{max}(EtOH)/nm 227, 275sh, 338, 420 and 437 (ε/dm^3 mol^{-1} cm^{-1} 65 900, 31 750, 51 750, 27 050 and 27 350; ν_{max}(KBr)/cm^{-1} 3440 (OH), 1720 and 1600 (CO).

CBT Decaacetate **4**.—CBT **1** (50 mg) was dissolved in dry pyridine (1 cm^3) and treated with Ac_2O (1.5 cm^3) for 2 days at 0 °C. Dilution with water, extraction with CH_2Cl_2, successive washing of the extract with saturated aq. $NaHCO_3$, water, saturated aq. $KHSO_4$, and water, and drying and evaporation of the solvent gave a product, which was flash-chromatographed with hexane–AcOEt (2:1) to give compound **4** (20 mg) as a creamy solid, m.p. 219–221 °C; $[\alpha]_D$ +45.4 (*c* 0.1, MeOH); ν_{max}(KBr)/cm^{-1} 1780 and 1710 (CO). ^{1}H NMR data are reported in Table 1.

^{1}H and ^{13}C NMR data are reported in Tables 1 and 2. Chemical shifts are in δ from $SiMe_4$ as internal standard; *J*-values are given in Hz. ^{13}C Signals were analysed by means of selective ^{1}H decoupling experiments using standard procedures. The ^{1}H,^{13}C connectivities were checked with inverse heteronuclear multiple bond coherence (HMBC) experiments at 600.13 MHz. ^{13}C,^{13}C Connectivities were established by means of double-quantum-filtered COSY experiments on the [1,2-^{13}C]acetate-enriched sample of CBT (150 mg/2.5 cm^3; [$^2H_6$]acetone at 150.92 MHz, using the AMX-600 spectrometer and a 10 mm probe. 512 × 2048 Free-induction decays (fids) were collected by using a standard DQF-COSY pulse program (216 scans, recycling delay 2 s, spectral width 38 500 Hz, composite pulse decoupling during acquisition) and transformed in magnitude mode after zero-filling to 1024 × 1024 real data points and weighting with a 90-deg-shifted sine-bell squared in both dimensions. ^{13}C,^{13}C Coupling constants were measured, after lorentzian-gaussian resolution enhancement, from monodimensional spectra acquired at 150.9 MHz. Percentage enrichments of ^{13}C were measured according to ref. 24, by applying the formula % enrichment = 1.1 × I_s/I_c, where I_s is the sum of the intensities of the ^{13}C doublet and I_c is the intensity of the central line. The average enrichment is 3.1 ± 1.1%, the range going from 0.55% for C-11′ to 7.7% for C-15. No enrichment, as expected, was observed in the methyl group of the ester (C-16).

The 2D-ROESY spectrum of CBT **1** was measured at 600.13 MHz with a continuous spin-lock (duration of 0.4 s, 16 dB attenuation, corresponding to an H_2 field strength of 6500 Hz, pulse program ('roesytp') and the carrier set at 6.6 ppm. The CBT concentration was 20 mg/0.5 cm^3 in [$^2H_6$]acetone. 512 × 1024 Fids were collected (9100 Hz spectral width, recycling time 1.5 s, 64 scans) in TPPI (time proportional phase increment) mode and transformed phase-sensitive, with zero-filling in *f*1, after apodization with a 90-deg-shifted sine-bell squared function. After Fourier transform, the baseline of the 2D spectrum was corrected in both dimensions with a fifth-degree polynomial. Interactions involving OH hydroxy-group protons were checked with selective ROESY experiments (not shown), performed with selective excitation of single protons (40 ms gaussian pulse, 512 data points, 1% truncation error) and 0.4 s spin-lock (pulse program 'Roesy-1D,' 9000 scans, 1.5 s recycling delay, 9100 Hz spectral width, 16K complex points, transmitter set on resonance). The NOE difference experiments carried out on the decaacetate **4** were obtained using standard procedures.

Molecular modelling was performed on a Silicon Graphics 4D35-GT, equipped with 16 Mbyte memory, 1 Gbyte hard disk, running the package InsightII/Discover (BIOSYM Technologies, San Diego, California, version 2.1.0). The atomic potentials and charges were taken from the software library, using the

CVFF force field. Energy minimization was performed using a conjugate gradient algorithm, until the maximum energy derivative was less than 0.1 kcal/Å^2.* No Mg ion was inserted during the energy minimization of the dimer; however, a distance of 2.88 Å, twice the O–Mg bond length, was used as a constraint between oxygens at positions 10′ and 6. Further constraints were defined, using the NOE interactions, by setting the distances between 10- and 6′-OH and between 10-OH and 15′-H$^{\beta}$ at less than 4 Å.

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* 1 cal = 4.184 J.

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