Double-Strand Cleavage of pDMAG10. The plasmid pDMAG10[20] was linearized with *Sty*I and labeled at one end with ^{32}P using the Klenow fragment of DNA polymerase I according to standard procedures.[19] In a typical cleavage experiment, the end-labeled DNA (20000 cpm/reaction) was mixed with salts, buffer, spermine, and oligonucleotide-EDTA·Fe. This solution was incubated for 30 min at 25 °C before the cleavage reaction was initiated by the addition of DTT, in a final volume of 40 μL. The reaction was stopped by ethanol precipitation. The pellets were rinsed once with 70% cold ethanol (50 μL), briefly dried in vacuo, and redissolved in TE buffer. Electrophoretic separation of the cleavage products was achieved on a 0.9% agarose gel/TAE buffer. The gel was dried and the cleavage products were visualized by autoradiography.

(20) Mendel, D.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 910.

Double-Strand Cleavage of HIV-CAT DNA. pHIV-CAT[21] was digested with *Bam*HI and the linearized DNA was labeled with α-(^{32}P)-dGTP using the Klenow fragment of DNA polymerase I according to standard procedures.[19] Purification of the labeled DNA and performance of the cleavage experiments were carried out as described for the double-stranded cleavage of pDMAG10 except with sodium ascorbate instead of DTT as the reducing agent.

Acknowledgment. We are grateful to the Office of Naval Research for support of this research. We thank Dr. David Horne for checking synthetic procedures, and careful reading of the manuscript.

(21) We thank A. Frankel for a gift of pHIV-CAT.

Communications to the Editor

Structures of the Beticolins, the Yellow Toxins Produced by *Cercospora beticola*†

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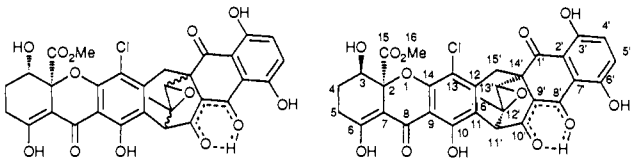
The genus *Cercospora* includes several species responsible for leaf spot diseases in many plants. Among them, *Cercospora beticola* Sacc. is the casual agent of cercosporiose, the most important leaf disease of sugar beet.[1] Two major toxins are produced by several *C. beticola* strains including a red pigment, cercosporin,[2,3] which can initiate the peroxidation of membrane lipids,[4] and a yellow compound successively named "gelben fraktion" (GF)[5] and then "*Cercospora beticola* toxin" (CBT).[1]

In this work, we report the structure of this yellow toxin, which we call beticolin.[6] In fact, there are two isomeric beticolins: beticolin 1 (**1**)[7] and beticolin 2 (**2**).[7] Both compounds exhibit the same biological activities as those already reported for CBT.[8]



1 **2**

On the basis of HREIMS measurments[9] as well as examination

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⊥ Laboratoire de Chimie Structurale, Université P. et M. Curie.

Laboratoire des Médiateurs Chimiques, INRA.

(1) Schlösser, E. *Phytopathol. Mediterr.* **1971**, *10*, 154.

(2) Kuyama, S.; Tamura, T. *J. Am. Chem. Soc.* **1957**, *79*, 5725.

(3) Lousberg, R. J. J. C.; Weiss, U.; Salemink, C. A.; Arnone, A.; Merlini, L.; Nasini, G. *J. Chem. Soc. D* **1971**, 1463.

(4) Macri, F.; Vianello, A. *Plant Cell Environ.* **1979**, *2*, 267.

(5) Schlösser, E. *Phytopathology* **1962**, 295.

(6) *C. beticola* strain CM was grown on diluted V8 medium in Roux flasks. After 10 days, the mycelium was removed from the medium and extracted with ethyl acetate until the filtrate was colorless. The filtrate was washed with water and then evaporated in a vacuum rotary evaporator. The beticolins were separated by flash chromatography using silica gel pretreated with $Ca(H_2PO_4)_2·H_2O$ (Balis, C.; Payne, M. G. *Phytopathology* **1971**, *61*, 1477) and eluted with $CHCl_3$. On TLC (plates pretreated with H_3PO_4 and $Ca(H_2PO_4)_2·H_2O$ and reactivated) with $CHCl_3/CH_3OH/CH_3CO_2H$, 100/2/1, as solvent, the R_f values were 0.54 and 0.33 for beticolin 2 and beticolin 1, respectively. Both were crystallized from hexane/ethyl acetate.

(7) Beticolin 1 (**1**): mp = 250 °C dec; $[\alpha]_D$ = +950.4° (*c* = 0.043, CH_2Cl_2); ^1H NMR (CD_3COCD_3; 400 MHz δ (ppm) 15.25 (s, 1 H), 13.62 (s, 1 H), 12.39 (s, 1 H), 11.87 (s, 1 H), 11.38 (s, 1 H), 7.31, 7.39 (2 H, *J* = 13.4 Hz, H-4′, H-5′), 5.72 (d, 1 H (exchangeable with D_2O), *J* = 4.5 Hz), 4.57 (dt, 1 H, *J* = 11.4, 4.5 Hz, H-3), 2.91 (ddd, 1 H, *J* = 18, 11.4, 5.2 Hz, H-5β), 2.64 (ddd, 1 H, *J* = 18, 5.7, 0.8 Hz, H-5α), 2.33 (qd, 1 H, *J* = 11.4, 5.2 Hz, H-4α), 2.11 (tdd, 1 H *J* = 11.4, 4.5, 0.8 Hz, H-4β), 3.74 (s, 3 H, H-16), 4.95 (d, 1 H, H-11′, *J* = 1.37 Hz), 4.04 (d, 1 H, H-13′, *J* = 1.3 Hz), 3.31 (s, 2 H, H-15′), 1.7 (s, 3 H, H16′); ^{13}C NMR (100.57 MHz) δ (ppm) 86.9 (C-2), 71.1 (C-3), 25.4 (C-4), 28.1 (C-5), 181.05 (C-6), 101.05 (C-7), 187.1 (C-8), 106.3 (C-9), 157.5 (C-10), 116.4 (C-11), 114.7 (C-12), 144.5 (C-13), 155.6 (C-14), 169.94 (C-15), 53.1 (C-16), 183.4 (C-1′), 114.2 (C-2′), 155.0 (C-3′), 130.9 (C-4′), 127.7 (C-5′), 158.1 (C-6′), 112.4 (C-7′), 186.6 (C-8′), 103.2 (C-9′), 202.0 (C-10′), 44.2 (C-11′), 58.85 (C-12′), 60.04 (C-13′), 49.1 (C-14′), 40.05 (C-15′), 19.4 (C-16′). Beticolin 2 (**2**): mp = 225 °C; $[\alpha]_D$ = +443.3° (*c* = 0.042, CH_2Cl_2); ^1H NMR (CD_3COCD_3; 400 MHz) δ (ppm) 15.25 (s, 1 H), 14.0 (s, 1 H), 12.5 (s, 1 H), 12.15 (s, 1 H), 11.5 (s, 1 H), 7.45, 7.4 (2 H, *J* = 9.8 Hz, H-4′, H-5′), 5.67 (s, 1 H (exchangeable with D_2O)), 4.15 (t, 1 H, *J* = 4 Hz, H-3), 2.92 (ddd, 1 H, *J* = 13, 10, 5 Hz, H-5β), 2.51 (dd, 1 H, *J* = 13, 4 Hz, H-5α), 2.1 (m, 2 H, H-4α, H-4β), 3.67 (s, 3 H, H-16), 4.68 (s, 1 H, H-11′), 4.05 (s, 1 H, H-13′), 3.5 (AB system, 2 H, H-15′, *J* = 12 Hz), 1.55 (s, 3 H, H16′); ^{13}C NMR (100.57 MHz) δ (ppm) 84.5 (C-2), 65.0 (C-3), 23.4 (C-4), 23.2 (C-5), 180.7 (C-6), 99.2 (C-7), 185.6 (C-8), 104.3 (C-9), 155.7 (C-10), 114.6 (C-11), 113.05 (C-12), 143.25 (C-13), 153.2 (C-14), 169.2 (C-15), 52.5 (C-16), 182.2 (C-1′), 111.4 (C-2′), 151.3 (C-3′), 129.2 (C-4′), 125.7 (C-5′), 156.7 (C-6′), 100.5 (C-7′), 182.3 (C-8′), 101.5 (C-9′), 200.1 (C-10′), 42.6 (C-11′), 53.0 (C-12′), 59.8 (C-13′), 47.7 (C-14′), 38.1 (C-15′), 16.2 (C-16′). Assignment of the nonprotonated carbons was made on the basis of the long-range ^1H–^{13}C coupling; one-bond CH correlations were used to assign protonated carbons.

(8) Blein, J.-P.; Bourdil, I.; Rossignol, M.; Scalla, R. *Plant. Physiol.* **1988**, *88*, 429.

0002-7863/92/1514-1478$03.00/0

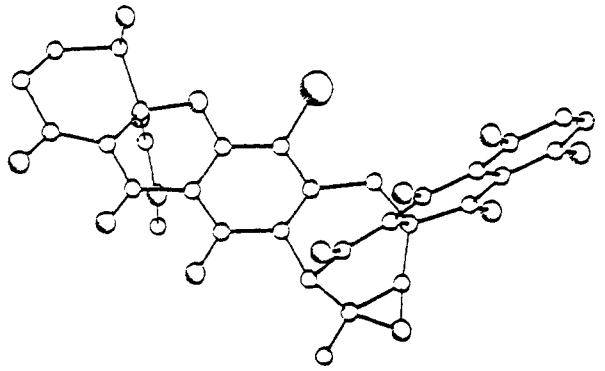
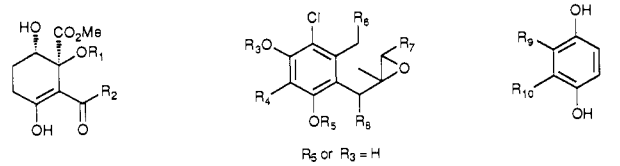


Figure 1. ORTEP structure of becticolin 2 (2) (relative configuration).

of the relative abundances of the isotopic peaks[10] of the molecular ion, the formula $C_{31}H_{23}ClO_{13}$ was deduced for both **1** and **2**. The ^1H NMR spectrum (CD_3COCD_3) of **1**[7] exhibits resonances for five phenolic protons, two aromatic protons, two methyl groups, an isolated benzylic methylene unit, and two isolated spin systems. These latter were identified as a system of six coupled protons corresponding to a CH_2CH_2CHOH substructure and a second spin system of two weakly coupled protons (δ = 4.04 ppm, 4.95 ppm, ^4J = 1.4 Hz). The ^{13}C spectrum of **1** exhibits signals for three quaternary sp^3, 18 nonprotonated sp^2, five methine, three methylene, and two methyl carbons.

Determination of the beticolin skeleton was partially achieved by analysis of a long-range ^1H–^{13}C 2D NMR chemical shift correlation experiment.[11] This experiment permitted assignment of partial structures A, B, and C, but no further connectivity could be surmised from these data, and the substitution of the aromatic ring of B was not clearly established. Analysis of data for **2** indicated the presence of the same structural features.



A B C

The structures of these compounds were ultimately obtained through X-ray analysis of **2**,[12] which was the only one to form suitable crystals (Figure 1). The phenol/quinone oxygen atoms were distinguished on the basis of bond lengths and on location of the hydrogen atoms, which were all observed in the last Fourier difference maps (except the resonant hydrogen atom of the planar β-keto/phenol system[13] C-8′, C-9′, C12′). All of the phenol hydrogens are intramolecularly H bonded to the phenol/quinone oxygen of the adjacent ring.

Because of the very similar spectroscopic data of both compounds, the diastereoisomeric structure **1** was assigned for beticoiin 1 on the basis of NMR data, which show that the hydroxy group of partial structure A is axial in **2** and equatorial in **1**.[14] The presence of cis stereochemistry at positions 2 and 3 of beticoiin 1 was supported by mass spectrometric determination of the proton affinity order (PA) of these compounds. Proton transfer was performed in a collision cell[15] between the MH^+ ion (generated in the source) and NMe_3 as nucleophilic reagent (PA value 942 kJ mol^{-1}),[16] giving rise to the $[Me_3NH]^+$ ion. The $MH^+/[Me_3NH]^+$ ratio is higher for **1** than for **2** (2.79 vs 0.94), indicating a higher PA value for **1**. This result could be explained by hydrogen bonding, which is only possible in a cis isomer. However, the relative stereochemistry between the C-11′–C-12′–C-13′–C-14′ substructure and the C-15 ester is not clearly inferred since no significant NOE effects were detected in either **1** or **2**.

Further attempts to obtain suitable crystals of **1** which would allow X-ray investigations in order to confirm the proposed structure are in progress.

Acknowledgment. We thank S. Wiedemann for the gift of *C. beticola* strains, P. H. Lambert, Institut de recherches Servier, for HREIMS measurements, and C. Descoins for initiation and coordination of this work.

Registry No. 1, 137516-40-6; **2**, 137622-91-4.

Supplementary Material Available: Listings of crystallographic data, atomic coordinates, bond lengths, bond angles, anisotropic thermal factors, and hydrogen coordinates for **2** (5 pages); tables of observed and calculated structure factors for **2** (13 pages). Ordering information is given on any current masthead page.

(9) Exact mass measurements are as follows. **1**: $M^{\bullet+}$ calcd for $C_{31}H_{23}O_{13}{}^{35}Cl$ m/z 638.0827 and for $C_{31}H_{23}O_{13}{}^{37}Cl$ m/z 640.0797, found 638.0775 and 640.0846, respectively; $[M - CO_2CH_3]^+$ calcd for $C_{29}H_{20}O_{11}{}^{35}Cl$ m/z 579.0694 and for $C_{29}H_{20}O_{11}{}^{37}Cl$ m/z 581.0664, found 579.0655 and 581.0682, respectively. **2**: $M^{\bullet+}$ calcd for $C_{31}H_{23}O_{13}{}^{35}Cl$ m/z 638.0827 and for $C_{31}H_{23}O_{13}{}^{37}Cl$ m/z 640.0797, found 638.0820 and 640.0868, respectively; $[M - CO_2CH_3]^+$ calcd for $C_{29}H_{20}O_{11}{}^{35}Cl$ m/z 579.0694 and for $C_{29}H_{20}O_{11}{}^{37}Cl$ m/z 581.0664, found 579.0685 and 581.0690, respectively.

(10) Found and calculated relative isotopic abundances for **2** (found, calcd): m/z 638 (100, 100), 639 (32.3, 35.6), 640 (39.4, 40.7), 641 (12.1, 12.9), 642 (2.8, 2.9), and 643 (0.5, 0.5) for $C_{31}H_{23}O_{13}Cl$.

(11) COLOC pulse sequence for J_{C-H} = 8 Hz; see: Kessler, et al. *J. Magn. Reson.* **1984**, *57*, 331.

(12) X-ray analysis of **2**: Philips PW-1100 automatic four-circle diffractometer, equipped with Cu Kα radiation (λ = 1/5418 Å) and graphite monochromator. The system is orthorhombic, space group $P2_12_12_1$ with a = 20.231 (6) Å, b = 18.921 (5), Å, c = 8.223 (3) Å, and Z = 4. The structure was solved by direct methods and refined with anisotropic thermal factors to $R = \sum w_i||F_o| - |F_c||/\sum w_i|F_o| = 6.0\%$ for 2480 structure factors (unitary weights). No absorption corrections were made.

(13) The least-squares mean plane for the five atoms of the tautomeric system has a c^2 value of 40.5, and maximum deviations out of plane are +0.067(5) and −0.021(5) Å for the C-8′ and C-9′ atoms, respectively.

(14) Any other change relative to structure **2** is unlikely (the collision-activated dissociation spectra of the MH^+ ion from **1** and **2** are qualitatively identical).

(15) (a) Kentamaa, H. I.; Cooks, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 4122. (b) Meyerhoffer, W. J.; Bursey, M. M. *Org. Mass Spectrom.* **1989**, *24*, 169. (c) Cole, R. B.; Prevost, C.; Tabet, J. C. *Adv. Mass Spectrom.* **1989**, *11B*, 1054. (d) Orlando, R.; Murphy, C.; Fenselau, C.; Hansen, G.; Cotter, R. J. *Anal. Chem.* **1990**, *62*, 125.

(16) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. H.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17* (Suppl. 1), 134.

1-Phenylthieno[3,4-*d*]borepine: A New 10 π Electron System Isoelectronic with Azulene

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Much attention has recently been focused on the chemistry of borepines.[1] The interest in this ring system stems from its isoelectronic relationship to the carbon species tropylium ion, since a neutral sp^2-hybridized boron atom is regarded as equivalent to a carbocation.[2] Ashe et al.[1f] and Nakadaira et al.[1g] have claimed

(1) (a) Leusink, A. J.; Drenth, W.; Noltes, J. G.; van der Kerk, G. J. M. *Tetrahedron Lett.* **1967**, 1263–1266. (b) Axelrad, G.; Halpern, D. *J. Chem. Soc. D* **1971**, 291. (c) Eisch, J. J.; Galle, J. E. *J. Am. Chem. Soc.* **1975**, *97*, 4436–4437. (d) van Tamelen, E. E.; Brieger, G.; Untch, K. G. *Tetrahedron Lett.* **1960**, No. 8, 14–15. (e) Jeffries, A. T., III; Gronowitz, S. *Chem. Scr.* **1973**, *4*, 183–187. (f) Ashe, A. J., III; Frederick, J. D. *J. Am. Chem. Soc.* **1987**, *109*, 1897–1880. (g) Nakadaira, Y.; Sato, R.; Sakurai, H. *Chem. Lett.* **1987**, 1451–1452.

0002-7863/92/1514-1479$03.00/0