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Cercospora Beticola Toxins. Part XI[1]: Isolation and Structure of Beticolin 0.

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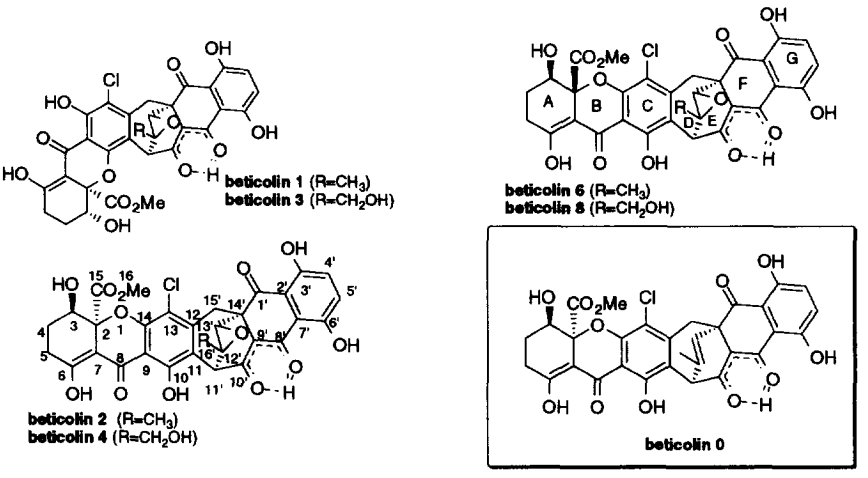
Abstract : *Structure of beticolin 0 has been established through examination of its spectroscopic data including X-Ray diffraction and through its chemical transformation into the previously identified beticolin 2.*

We have isolated from the mycelial extract of a *Cercospora Beticola* strain a number of yellow compounds, one of which was previously known as CBT (*Cercospora Beticola* toxin)[2] . Structure elucidation of some of them showed that they have closely related structures[3-7]. These compounds can be divided in sub-groups according to i) the ring closure of the heterocycle, which can occur with the oxygen being in *para* position of the chlorine atom (*p*-beticolin) or with the oxygen being in the *ortho* position (*o*-beticolin), ii) the relative configuration at C-2 and C-3. Indeed, we observed that in the *o*-beticolin sub-group, substituents on C-2 and C-3 can be either on different sides of the mean plan of the cycle A (*o*-beticolin) or on the same side of this plan (*epi-o*-beticolin). We also showed that the *o*-beticolin skeleton can be transformed under basic conditions in the thermodynamically more stable *p*-beticolin skeleton through the opening of the heterocycle[7], the minor products of this transformation being *epi-o*-beticolins (beticolin **6** and beticolin **8** from beticolin **2** and **4** respectively).

In our previous papers we tried to find spectroscopic and chemical criteria to differentiate *o*- and *p*-beticolins. First, *p*-beticolins (beticolin **1** and **3**) were shown to form complexes with divalent cations such as magnesium to afford stable symetric dimeric compounds with two magnesium atoms[8], each of them being attached to the conjugated keto-enol system of the xanthone of one molecule and to the anthraquinone moeity of the other one[6]. Second, the easy transformation in basic conditions of the *o*-beticolins into the corresponding *p*-beticolins[7], when *p*-beticolins remained unchanged in the same conditions, should allow a rapid discrimination between both skeletons. Third, NOESY experiments allowed observation of characteristic nOe's for both forms[6,7].

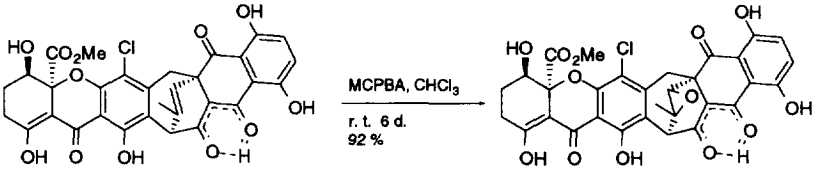


We report here the isolation and the structure of a new compound named beticolin **0**[9], which could be one of the earlier precursor of all the identified beticolins.

DCI-MS performed with NH_3 as reagent gas giving ions at m/z 623 and 640 for MH^+ and $[M + NH_4]^+$ ions respectively indicated a MW of 622. Isotopic ratios and HREI-MS proved clearly a chlorinated structure with $C_{31}H_{23}O_{12}Cl$ as crude formula (measured: 622.0856; calc.: 622.0878 for the ^{35}Cl isotopic contribution). Furthermore, the low energy CAD spectra performed (*eg* MS-MS experiments) on the MH^+ species obtained under the NH_3-DCI source conditions led to assume that the -16 u (one oxygen atom less) relative to the beticolins **1** and **2** concerned the epoxide function of the latter compounds (daughter ions which contained the A-C or F-G rings remaining unchanged).

^1H and ^{13}C NMR spectra of beticolin **0** (CD_3COCD_3, 400 MHz) presented some similitudes with those obtained for beticolin **2** : the dihydroxy naphtoquinone moeity and the bicycle A/B remained unchanged, the only difference was observed on the bridged bicycle D/E : the resonance of carbons C-13' and C-12' (δ=59.8 and 53.0 ppm respectively in beticolin **2**) were replaced by a CH resonance at δ= 125.62 ppm and a quaternary carbon at δ=140.4 ppm ; moreover, resonance of the protons of the methyl group (H-16') was shifted from δ= 1.55 ppm to δ= 1.95 ppm and resonance of H-13' from 4.05 to 6.37 ppm. This observation is in agreement with the replacement of the 12'-13' epoxy by a trisubstituted 12'-13' double bond.

Confirmation of beticolin **0** structure was achieved by its chemical transformation into beticolin **2** through epoxidation of the double bond of the bridged D/E bicycle ($CHCl_3$,MCPBA, rt, 6 days) which was found to occur with complete regio- and stereoselectivity.



Furthermore, evidence for a *o*-beticolin form was given by the observation of the nOe's obtained in a NOESY experiment ($CDCl_3$, 400 MHz, Fig. 1, all observed nOe's are positive) : for beticolin **0**, the same nOe as for beticolin **2** is observed between the phenolic proton H-10 and the benzylic proton H-11'.

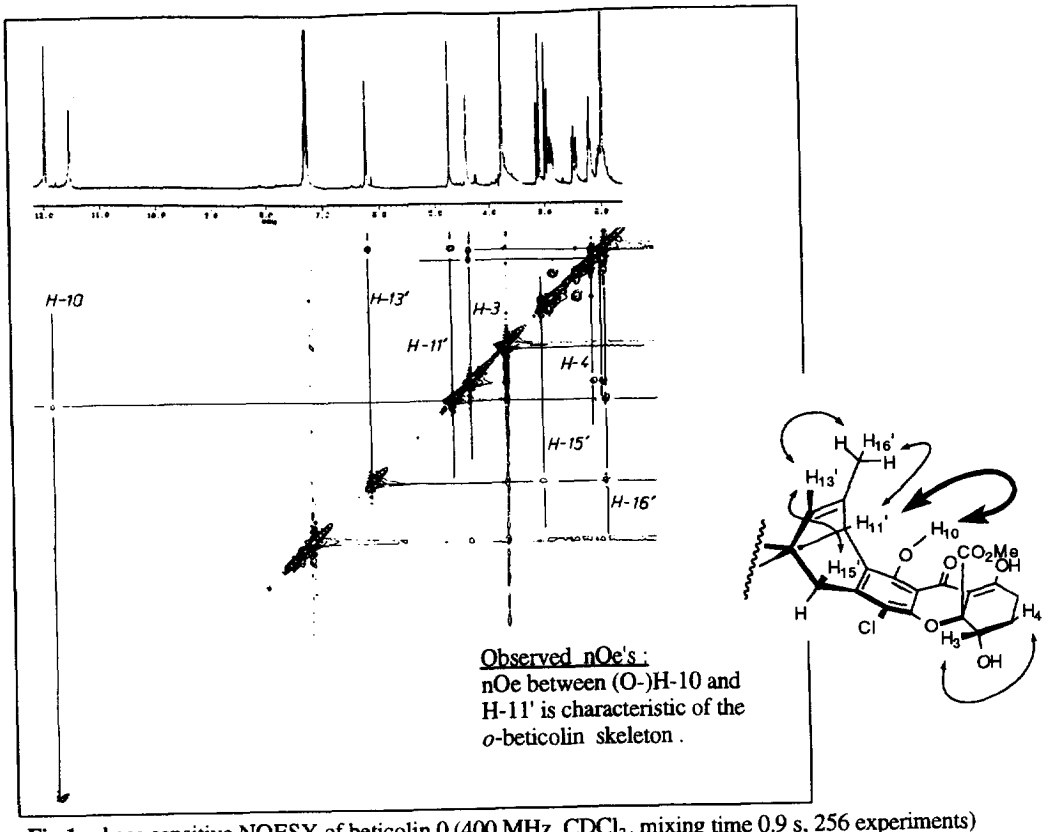


Fig 1: phase sensitive NOESY of beticolin 0 (400 MHz, $CDCl_3$, mixing time 0.9 s, 256 experiments)

Finally, monocrystals were obtained and X-ray diffraction analysis confirmed the structure of beticolin **0** as shown in Fig. 2[10].

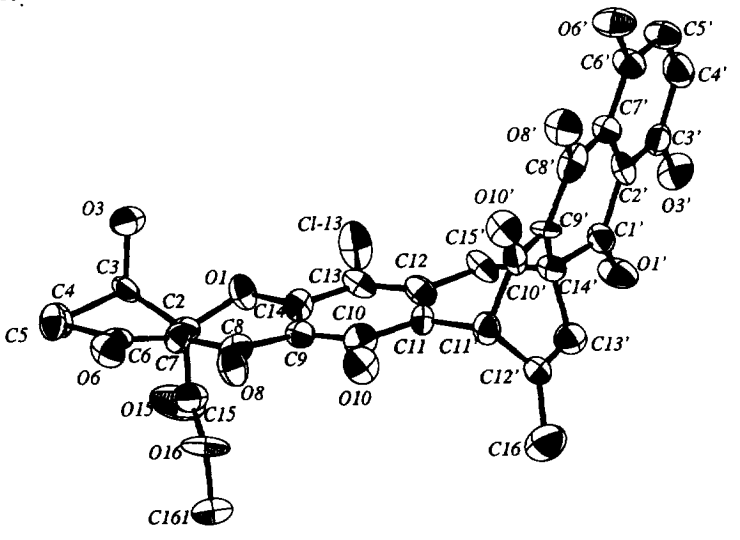


Fig.2 : ORTEP view of beticolin **0** (ellipsoids are drawn at the 50% probability level)

These last results demonstrated the validity of the chemical and spectoscopic criteria which were developed in our previous papers to differentiate *p*- from *o*-beticolins. Furthermore the *o*-beticolins seem to give mono crystals more easily than the corresponding *p*-beticolins[3,5]. These results will help us in structure elucidation of the minor compounds present in crude mycelial extracts.

References and notes

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9- After extraction with ethyl acetate from *C. beticola* mycelium, beticolins were separated by flash chromatography using silica gel pretreated with $Ca(H_2PO_4)_2,H_2O$ and H_3PO_4 (Balis, C.; Payne, M. G. *Phytopathology* **1971**, *61*, 1477) and eluted with $CHCl_3$. TLC analysis was performed using two systems : 1) $CHCl_3$/MeOH/CH_3COOH 100/2/1 and 2) Hexane/ethyl acetate 1/1. Crystallization from ethyl acetate/hexane afforded **Beticolin 0**: Rf : 0.49 (system1), 0.38 (system 2), mp = 222-228°C, $[\alpha]_D$ =+528 (c = 0.9, CH_2Cl_2).

^1H NMR δ (ppm) CD_3COCD_3 (400 MHz) : 14.9 (s, 1H), 13.95 (s, 1H), 12.55 (s, 1H), 12.0 (s, 1H), 11.58 (s, 1H), 7.47, 7.50 (2H J=10 Hz, H-4', H-5'), 6.37 (bs,1H, H-13'), 4.83 (bs, 1H, H-11'), 4.56 (dd, 1H,J=4 , 2.5 Hz, H-3), 3.81 (s, 3H,H-16), 3.12, 3.32 (2H, J=18 Hz H-15'), 2.95 (ddd, 1H, J=17, 11, 7 Hz, H-5b), 2.59 (ddd, 1H, J=19, 7, 1 Hz, H-5a), 2.15-2.23 (m, 2H, H-4), 1.95 (s,3H, H16'). Attribution of the phenolic protons is achieved through examination of the long range ^1H-^{13}C coupling constants.

^{13}C NMR δ (ppm) (100.57 MHz): 83.83 (C-2), 64.7 (C-3), 22.26 (C-4), 23.15 (C-5), 179.77 (C-6), 99.48 (C-7), 186.08 (C-8), 105.76 (C-9), 155.05 (C-10), 115.24 (C-11), 142.27 (C-12), 113.04 (C-13), 153.40 (C-14), 169.24 (C-15), 51.6 (C-16), 200.68 (C-1'), 112.66 (C-2'), 151.5 (C-3'), 123.99 (C-4'), 127.7 (C-5'), 155.79 (C-6'), 111.19 (C-7'), 180.8 (C-8'), 103.91 (C-9'), 185.4 (C-10'), 43.3 (C-11'), 140.4 (C-12'), 125.62 (C-13'), 41.51 (C-14'), 37.86 (C-15'), 18.40 (C-16')

10- Bright yellow monocrystals were obtained by slow evaporation at room temp. of a 2:1 methanol/anisole solution. The crystal parameters are as follows : Space group $P2_12_12_1$, (Z=4) with a=20.185(6) , b=19.471(5) and c=8.236(3) Å. The structure was solved by direct methods and anisotropically refined to R = 7.9% (using 2351 observed structure factors). Details of the beticolin **0** and the uncomplexed beticolin **1** structures will be published elsewhere.

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