Pergamon

Tetrahedron Letters, Vol. 35, No. 47, pp. 8797-8800, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01954-1

The yellow toxins produced by *Cercospora Beticola*. Part VIII‡ : Chemical equilibrium between beticolins ; structures of minor compounds : beticolin 6 and beticolin 8.

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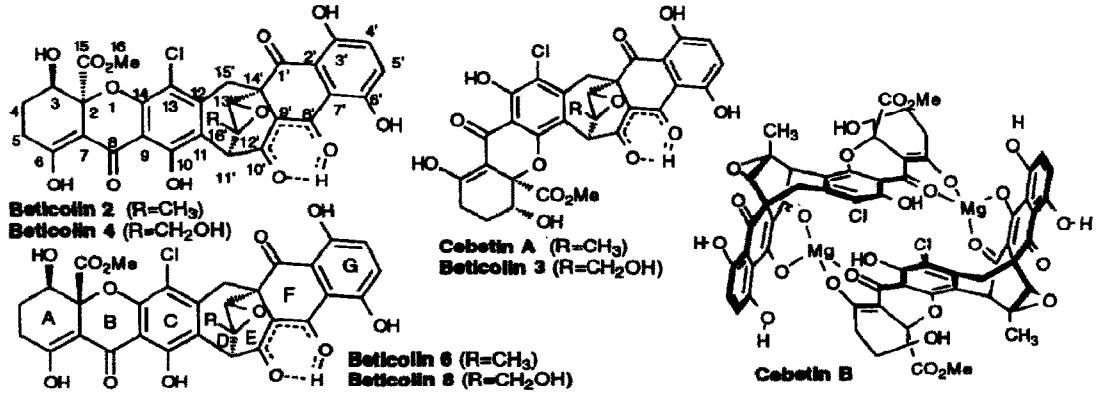
Abstract : *a general chemical transformation of the "linear" beticolin skeleton (beticolin 2 and 4) into a "bent" beticolin skeleton (cebetin A and beticolin 3 respectively) is described. Beticolins 6 and 8, minor components of mycelial extract are also characterized as minor compounds resulting from these transformations.*

The pathogenic fungus, *Cercospora beticola*, which is responsible for leaf spot disease on sugar beet, produces colourful toxins of complex structures : a red one called cercosporin, the structure of which was found to be a hydroxy perylenequinone, and yellow compounds, one of which was known as CBT (*C. beticola* toxin).



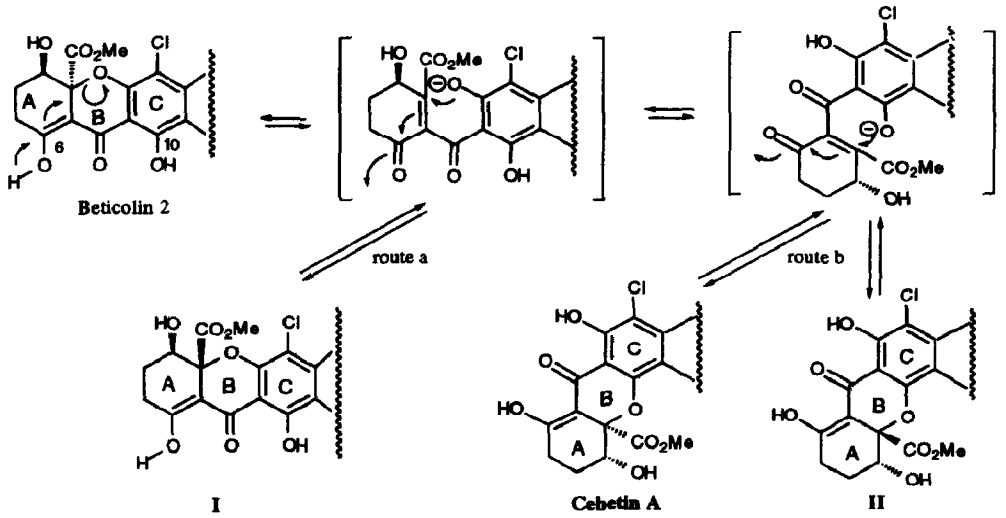
In fact, isolation of several compounds has shown that *C. beticola* produces many "yellow toxins". As part of our ongoing program for the determination of the structures of these toxins, we have already proposed

‡ Part VII : Mikes, V.; Milat, M.-L.; Pugin, A.; Blein, J.-P. *Biophys. Biochem. Acta* **1994** accepted for publication.

structures for four compounds, beticolins 2[1], 3, 4[2] and cebetin A[3], the latter one being simultaneously described by Jalal *et al.*[4] accompagnied by its dimer complexing two atoms of magnesium, called cebetin B[4,5].

These four compounds rely on the same skeleton constructed around a chlorinated tetrahydro-xanthone and a partially hydrogenated anthraquinone moieties. However, an important difference between these compounds lies in the cyclization of the heterocycle of the xanthone unit which can occur with the oxygen atom either in *para* or in *ortho* position relative to the chlorine atom of ring C, defining a "bent" and a "linear" skeleton respectively ; as representatives of these two types of skeleton are cebetin A and beticolin 2 respectively, structures of which have been elucidated by X-ray spectroscopy[1,4].

The unambiguous presence of both types of cyclization prompted us to reexamine the spectroscopic data and some of the chemical properties of beticolins. We first made the hypothesis that under basic conditions, beticolin 2 could be transformed into cebetin A, through opening of ring B, promoted by the fragmentation of the enolate formed at C-6, followed by ring closure through a Michael addition on the α–β unsaturated ester (Scheme 1, route b). This assumption was supported by the fact that treatment of beticolin 2 by $MgCO_3$ afforded in a poor but significant yield the dimeric cebetin B[3]. Better results were obtained when beticolin 2 was subjected to the action of K_2CO_3 in acetone (Scheme 2). After neutralization (HCl 1N) a mixture of two products was obtained with cebetin A as major compound.

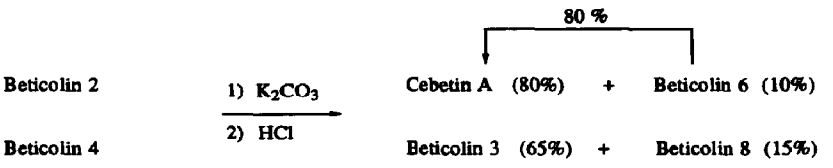


Scheme 1

In fact, the formation of four compounds could be expected in the course of the reaction : beside beticolin 2 and cebetin A, compounds I and II with a linear and a bent skeleton respectively could be formed in the absence of diastereoselectivity in the ring closure step (Scheme 1). Another crucial difference between compounds I and II lies in the relative stereochemistry of the carbomethoxy and the hydroxy group on ring A. It happens that this relative stereochemistry is easily inferred from the ^1H NMR spectrum due to the fact that the peculiar structure of the A/B bicyclic system compels the carbomethoxy group to be axial in a pseudo chair conformation of ring A, so that the relative stereochemistry of the hydroxy group is deduced from the value of the coupling constants of H-3 with vicinal protons H-4 (Scheme 3). The minor product of the treatment of beticolin 2 by K_2CO_3 proved to have a cis relationship between the carbomethoxy and the hydroxy group, and therefore should have the structure of

compound I with a linear skeleton.

Examination of the minor components also isolated from the crude mycelial extract led to the isolation of compound I that we called beticolin 6[6]. Treatment of beticolin 6 in the same conditions (K_2CO_3, CH_3COCH_3 then HCl 1N) resulted in a mixture of starting beticolin 6 (20 %) and cebetin A (80 %) (proportions are determined by ^1H NMR of the crude mixture). It is interesting to note that we did not observe the formation of neither beticolin 2 nor compound II in the transformation of beticolin 6. A possible explanation for this selectivity would be that the presence of the hydroxy group at C-3 might control the diastereoselectivity of the Michael addition in this case, so that the formation of compounds having a cis relationship between the hydroxy and the carbomethoxy group would be the kineticly favoured.

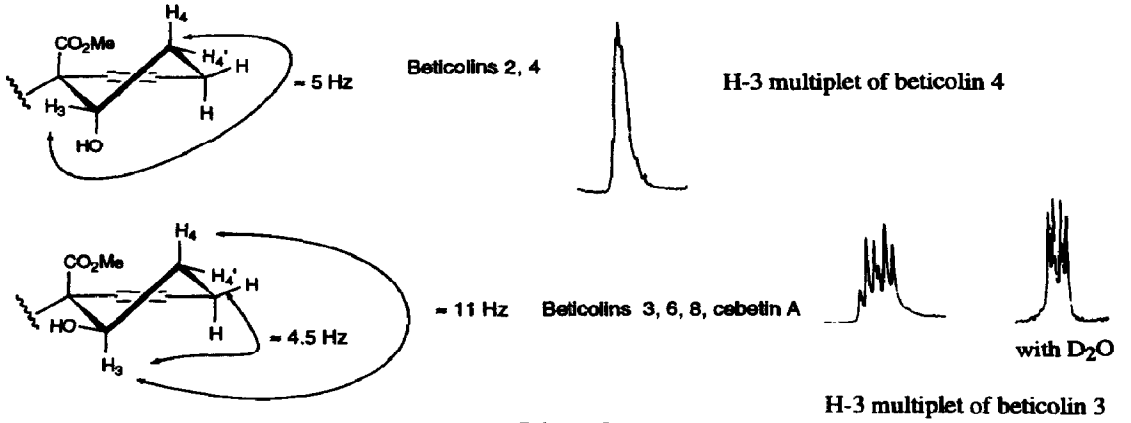


Scheme 2

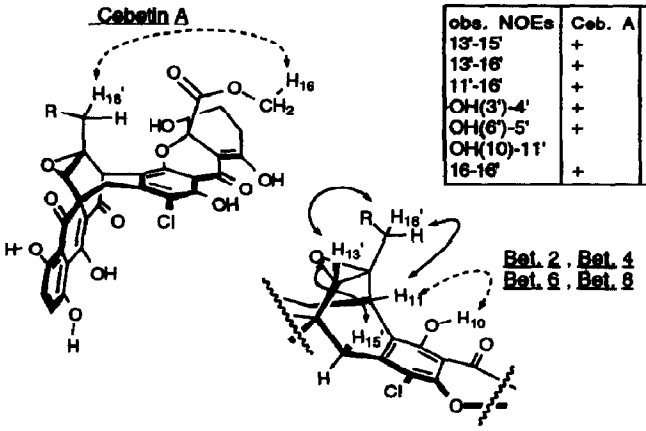
Confirmation of the structure of beticolin 6 was achieved by 2D ^1H-^1H phase sensitive NOESY[7] experiments (400 MHz). Beticolin 6 showed as beticolin 2 a nOe between the benzylic proton at C-11' and this of the phenolic hydroxy group at C-10 which is characteristic of the linear skeleton[3]. On the other hand, beticolin 1 exhibited a weak nOe between the methyl protons (C-16') and the protons of the methyl ester (C-16)[3](Scheme 4).

A similar chemical transformation was observed between beticolins 3 and 4 : treatment of beticolin 4 with potassium carbonate under sonication (2 h.) yielded a mixture of two main products, beticolin 3 being the major one ; the minor product of the reaction, which proved to be the hydroxylated analog of beticolin 6, was also characterized as a minor component of the mycelial extract, and was called beticolin 8[8]. Spectroscopic analysis of beticolin 8 gave the same evidence as beticolin 6 for a linear skeleton with a cis relationship between the hydroxy group at C-3 and carbomethoxy group at C-2. (Scheme 4)

It is interesting to note that all these compounds are present in the mycelial extract but in different amounts. They all inhibit plasma membrane H^+-ATPase. The structure/activity relationship as well as the kinetics of their biosynthesis are under investigation.



Scheme 3



obs. NOEs	Ceb. A	Bet. 2	Bet. 6	Bet.3	Bet.4	Bet.8
13'-15'	+	+	+	+	+	+
13'-16'	+	+	+	+	+	+
11'-16'	+	+	+	+	+	+
OH(3')-4'	+	+	+	+	+	+
OH(6')-5'	+	+	+	+	+	+
OH(10)-11'		+	+		+	+
16-16'	+					

Scheme 4

Acknowledgments : This work is supported by I.N.R.A. and a grant from the Conseil Régional de Bourgogne. The authors wish to thank C. Descoins for his encouragement.

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6- After extraction with ethyl acetate, 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 6: Rf : 0.33 (system 1), 0.24 (system 2) , mp = 200-202°C ,
$[\alpha]_D$ = +1350° (c = 0.01, CH_2Cl_2).
^1H NMR δ (ppm) CD_3COCD_3 (400 MHz) : 15.35 (s, 1H), 14.95 (s, 1H), 12.61 (s, 1H), 12.23 (s, 1H), 11.65 (s, 1H), 7.58, 7.55 (2H J=12 Hz, H-4', H-5'), 4.65 (dd, 1H,J=12 , 5 Hz, H-3), 3.1 (ddd, 1H, J=17, 12, 5.2 Hz, H-5b), 2.64 (ddd, 1H, J=17, 5.7, 1 Hz, H-5a), 2.33 (qd, 1H, J= 12, 5.2 Hz, H-4a), 2.11 (tdd, 1H J=12, 5, 1 Hz, H-4b), 3.82 (s, 3H,H-16), 4.72 (d, 1H, H-11', J=1.4 Hz), 4.22 (d,1H, H-13', J=1.4 Hz), 3.57 (s, 2H, H-15'),1.68 (s,3H, H16').
^{13}C NMR δ (ppm) (100.57 MHz): 85.9 (C-2), 70.7 (C-3), 24.9 (C-4), 28.3 (C-5), 180.6 (C-6), 101.3 (C-7), 186.4 (C-8), 105.6 (C-9), 157.1 (C-10), 115.3 (C-11), 143.7 (C-12), 113.4 (C-13), 154.8 (C-14), 169.0 (C-15), 52.3 (C-16), 201.5 (C-1'), 111.9 (C-2'), 154.4 (C-3'), 129.6 (C-4'), 127.1 (C-5'), 157.3 (C-6'), 107.6 (C-7'), 183.3 (C-8'), 102.6 (C-9'), 184.5 (C-10'), 43.3 (C-11'), 52.9 (C-12'), 59.1 (C-13'), 48.7 (C-14'), 39.4 (C-15'), 17.0 (C-16')

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8- **Beticolin 8**: Rf : 0.15 (system 1), 0.15 (system 2) , mp = >220°C (dec)
^1H NMR δ (ppm) CD_3COCD_3 (400 MHz) : 15.40 (s, 1H), 13.85 (s, 1H), 12.50 (s, 1H), 12.15 (s, 1H), 11.60 (s, 1H), 7.50 7.45 (2H, H-4', H-5'), 5.28 (d, 1H exchangeable with D_2O, J= 5Hz, OH-3), 4.55 (dt, 1H,J=12 , 5 Hz, H-3), 3.05 (m, 1H, H-5b), 2.80 (ddd, 1H, J=16, 6, 1 Hz, H-5a), 2.41 (m 1H, H-4a), 2.15 (m, 1H, H-4b), 3.75 (s, 3H,H-16), 4.95 (d, 1H, H-11', J=1.3 Hz), 4.30 (d,1H, H-13', J=1.3 Hz), 3.35-3.55 (system AB, J=17 Hz. 2H, H-15'),3.88, 4.05 (2H, H16').

(*Received in France* 14 *September* 1994; *accepted* 28 *September* 1994)