NEW ANTIBIOTICS FROM THE FUNGUS *EPICOCCUM NIGRUM*

III. EPICORAZINE B: STRUCTURE ELUCIDATION AND ABSOLUTE CONFIGURATION

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Comparison of UV, IR, PMR and CD spectra of epicorazine B with those of epicorazine A, a previously isolated metabolite of *Epicoccum nigrum*, showed that they were isomers with the same epidithiodiketopiperazine skeleton. X-Ray determination of epicorazine B indicated that the difference is related to a *cis-trans* configuration.

A chromatographic study of the chloroformic extract of the culture broth of *Epicoccum nigrum* (strain 751–5) indicated the presence of at least two antibiotic compounds, which were named epicorazine A and epicorazine B.[1] Structure elucidation of the former was related in a previous paper.[2]

Epicorazine B was isolated by preparative thick-layer chromatography as described previously.[1] The yield was about one tenth of epicorazine A.

Several results suggested that these compounds were isomers; mass spectra of both metabolites showed the same molecular ion at *m/e* 420 corresponding to the formula $C_{18}H_{16}N_2O_6S_2$, as also the $(M-S_2)^+$ ion at *m/e* 356 and the series of ions corresponding to S_2, S_3, ... S_8; elemental analysis of epicorazine B confirmed the same empirical formula

Found: C 51.02, H 3.94, N 6.20, O 23.15, S 15.48.
Calcd. for $C_{18}H_{16}N_2O_6S_2$: C 51.40, H 3.81, N 6.67, O 22.80, S 15.21.

On the contrary, the IR spectrum of epicorazine B (Fig. 1) showed two bands at 3360 and 3450 cm^{-1}, whereas only the latter was present in the spectrum of epicorazine A.

The PMR spectrum of the purified material (Fig. 2) showed superposition of two spectra, one of

Fig. 1. IR spectrum of epicorazine B (KBr)

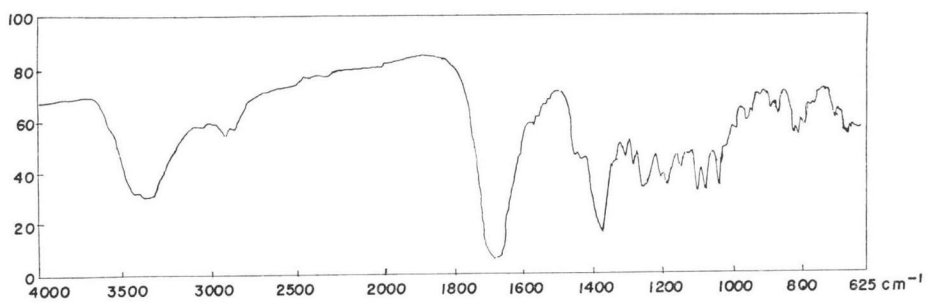

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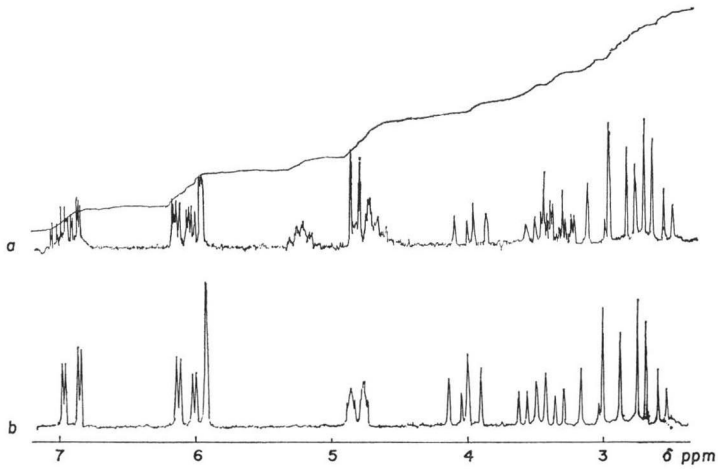
Fig. 2. PMR spectra of epicorazine B (a) and epicorazine A (b)



them corresponding to epicorazine A. This was in accord with the existence of the same skeleton in both metabolites, which on the other hand would differ in their proton distribution. The ethylenic protons were almost unchanged, but the coupling constants were different, showing that configurations of six-membered rings were not identical. On shaking with D_2O, two doublets at δ 5.9 and δ 4.78 disappeared; they could be assigned to hydroxylic protons H-4α and H-4'α. One of them (at δ 5.9) was present in epicorazine A, but the other (at δ 4.78) was different by its shift and its coupling constant; this finding, already revealed by IR spectrum, suggested that the secondary alcohol functions of both metabolites were not similar according to their environment. At δ 5.2, a multiplet was assigned to H-4' of a secondary alcohol. At δ 4.75, in addition to the doublet of H-4'α, a multiplet was present which could be assigned to two protons H-4 and H-5'. A multiplet at δ 3.3 included two under-spectra assigned to H-6 and H-6' with different coupling constants. Finally, at δ 2.8, this signal already observed for epicorazine A, was assigned to the four protons H-7, H-8, H-7' and H-8'. This spectroscopic interpretation is summarized in Table 1. According to this assignment, $J_{5,6}$= 12.3 Hz is consistent with a *trans* vicinal coupling, whereas $J_{5',6'}$=4 Hz shows a *cis* coupling. This difference involves the asymmetry of the molecule and explains the unlike conformation for the hexagonal rings. Consequently, the protons H-4α and H-4'α would have different positions, with respect to the amide carbonyl groups.

Table 1. Parameters and interpretation of PMR spectrum of epicorazine B

Shift, δ ppm	Assignment	Coupling constants J Hz
		$J_{2,3}=J_{2',3'}=10.0$
6.94	H-3'	$J_{3,4}=1.8$
6.87	H-3	$J_{3',4'}=3.6$
6.05	H-2	$J_{2,4}=2.1$
6.04	H-2'	$J_{2',4'}=4.0$
5.9	H-4α	$J_{4,4\alpha}=0.8$
5.2	H-4'	$J_{4',4'\alpha}=5.7$
4.78	H-4'α	$J_{4',5'}=5.0$
4.75	H-4	$J_{4,5}=8.5$
4.6	H-5'	$J_{5,6}=12.3$
3.95	H-5	$J_{5',6'}=4.0$
3.3	H-6+H-6'	$J_{6,7\alpha}=J_{6',7'\alpha}=12.3$
2.8	H-7α+H-7β	$J_{6,7\beta}=J_{6',7'\beta}=5.8$
	H-7'α+H-7'β	$J_{7\alpha,7\beta}=J_{7'\alpha,7'\beta}=14.0$

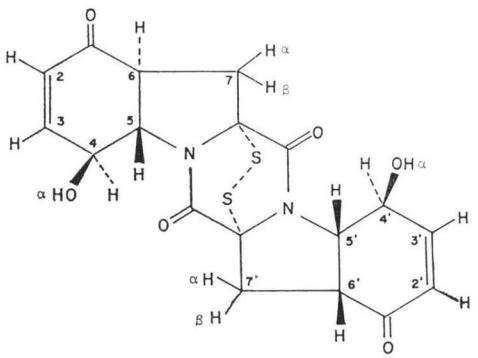
Epicorazine B was crystallized from a mixture, $CHCl_3$ - hexane (3: 1), as amber coloured needles; details on the determination of the crystalline structure will be published elsewhere. The crystal

parameters are given in Table 2 and the configuration of the molecule in the crystal is shown in Fig. 3. This X-ray determination confirms the results of spectroscopic studies. As predicted by the PMR spectrum, H-5 and H-6 are in *trans* position whereas H-5′ and H-6′ are in *cis* position. Moreover, there is only one intramolecular hydrogen bonding in epicorazine B: between the hydroxyl group OH-4 and the proximate carbonyl, whereas the hydroxyl group OH-4′ is linked by an intermolecular bond; this explains the different hydroxyl IR absorptions (3450 and 3360 cm^{-1}). The length of the S-S bond (2.09 Å) is in good agreement with those already found in other epidithiopiperazinediones[3]. Although the absolute configuration of the molecule was not determined by X-ray diffraction, the circular dichroism studies mentioned below support the configuration shown. The chirality of the two asymmetric carbons bonded to sulfur atoms is R and the helical sense of the disulfide bond is left-handed, with a dihedral angle of 10.8°.

Table 2. Crystal parameters of epicorazine B

Empirical formula	$C_{13}O_6N_2S_2H_{16}$, C_2H_5OH
a	15.716 (2) Å
b	8.074 (1) Å
c	16.221 (3) Å
β	109.41 (1)°
Space group	P_{2_1}
Molecules/cell	4
Observed density	1.43
Calcd. density	1.50

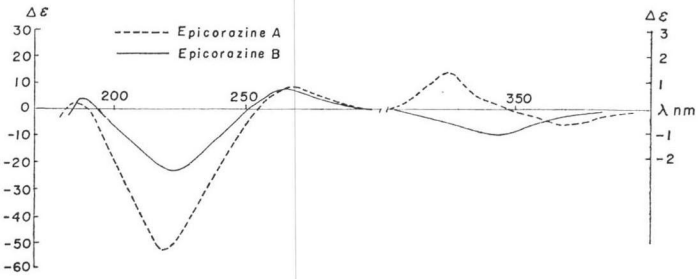
Fig. 3. Absolute configuration of epicorazine B



The CD spectrum of epicorazine B (Fig. 4) is similar to that of epicorazine A: it shows a very important negative effect at 230 nm and a positive one near 270 nm, which is consistent with a diketopiperazine ring bridged by a disulfide bond[4]. This similarity of both CD spectra indicates that epicorazine B possesses the same absolute configuration as epicorazine A[2].

To summarize, epicorazine A and epicorazine B are isomers which differ only by a moiety of their molecules. At first glance, the low production level of the latter could make it appear an artifact arising from epicorazine A during isolation procedures; but such a molecular transformation would be difficult to explain. Distinct biosynthesis of both metabolites by special enzymatic systems seems more likely. In this respect, it may be pointed out that strain 751–5 of *Epicoccum nigrum* proves capable of synthetizing simultaneously two isomeric dithiodiketopiperazines and the related, non-sulfured *bis*-anhydrophenylalanine[5].

Fig. 4. CD spectra of epicorazines A and B



Experimental

Apparatus and operational techniques were published in a precedent paper[2].

Physical properties of epicorazine B: mp. 192°C; $[\alpha]_D^{22}$ −320° (*c* 0.5 mg/ml, MeOH); water insoluble, soluble in $CHCl_3$, MeOH, CH_3CN; UV (MeOH) λ_{max} 220 nm (ε 15,300); IR (KBr) ν_{max} 3450, 3360, 1690, 1670, 1370 cm^{-1}, PMR (CD_3COCD_3): assignment is summarized in Table 1; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$): 188 (+4.8); 222 (−26.7); 264 (+9.0); 342 (−1.1).

Acknowledgements

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