NEW ANTIBIOTICS FROM THE FUNGUS *EPICOCCUM NIGRUM*

II. EPICORAZINE A: STRUCTURE ELUCIDATION AND ABSOLUTE CONFIGURATION

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An antibiotic, epicorazine A, isolated from a particular strain of the fungus *Epicoccum nigrum*, was shown to be a new epidithiodiketopiperazine from its UV, IR, mass, NMR and CD spectra. X-Ray diffraction measures confirmed this structure and its absolute configuration.

During a search for new antibiotic substances, we selected a strain of *Epicoccum nigrum* whose culture broth was active against *Staphylococcus aureus*;[1] isolation by preparative chromatography of a new antibiotic metabolite, epicorazine A, was described in the precedent paper.[1]

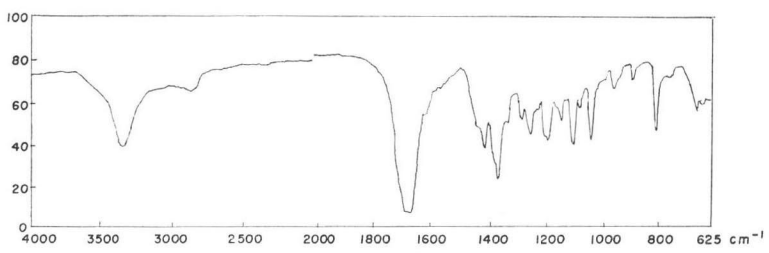
The mass spectrum of epicorazine A shows an unstable molecular ion at *m/e* 420.045 corresponding to the empirical formula $C_{18}H_{16}N_2O_6S_2$ and another ion at *m/e* 356 ($C_{18}H_{16}N_2O_6$) indicating the loss of S_2. Moreover, this spectrum contains typical ions at *m/e* 64; 96; 128; . . . 256 corresponding to S_2, S_3, S_4, . . . S_8. Elemental analysis confirmed this formula:

Calcd. for $C_{18}H_{16}N_2O_6S_2$: C, 51.40; H, 3.81; N, 6.67; S, 15.21
Found: C, 51.12; H, 3.88; N, 6.34; S, 15.55

Such a fragmentation was described by several authors[2,3,4] for epidithiodiketopiperazines. Furthermore, epicorazine A can be detected by spraying TLC plates with iodine-azide reagent[5] which is characteristic of disulfide compounds. The dithiodiketopiperazine structure was also suggested by isolation from the same culture broth of 3,6-dibenzyl-2,5-diketopiperazine[6].

The IR spectrum (Fig. 1) shows characteristic stretching vibration absorptions at 1690 cm^{-1} (amide carbonyl group of a N-substituted diketopiperazine), 3360 cm^{-1} (OH) and 1670 cm^{-1} (α,β-unsaturated carbonyl).

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



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The ^{13}C-NMR spectrum indicates that epicorazine A has a symmetrical structure, the 18 carbon atoms giving only 9 signals; the following data refer to a moiety of the molecule. The spectrum shows four deshielded signals corresponding respectively to an α,β-unsaturated carbonyl, an amidic and two ethylenic carbons (Table 1).

Table 1. ^{13}C-NMR spectrum of epicorazine A in dioxane solution

δ ppm	Assignment
194.5	C-1
165.2	C-9
151.3	C-3
129.5	C-2
76.6	C-8
71.5	C-4
69.9	C-5
49.2	C-6
31.0	C-7

After shaking with D_2O, the doublet at δ 5.95 in the PMR spectrum (Fig. 2) disappeared, confirming the presence of an hydroxyl group; its multiplicity is in favour of a secondary alcoholic function.

Spin decoupling experiments reveal that the four deshielded protons are coupled together as shown by Fig. 2. The coupling constant between the protons at δ 6.91 and δ 6.09 ($J_{2,3}=10.4$ Hz) is in agreement with an α,β-unsaturated ketone included in a six-membered ring. Between 2 and 4 ppm, an ABMX pattern is observed (–CH_2–CH–CH–).

All these mass, IR and NMR data are consistent with the structure shown in Fig. 3.

This structure was confirmed by X-ray determination of a crystal of epicorazine A, which was described elsewhere[7]. Bond lengths and interatomic distances show the existence of two symmetrical intramolecular chelates between the hydroxyl and amide carbonyl. Such a chelation could be anticipated from the unusually large chemical shift of H-4α (5.95 ppm) and the very small value of $J_{4,4\alpha}$ with respect to a secondary alcohol function. The dihedral angle H-4 C O H-4α calculated from atom

Fig. 2. PMR spectra of epicorazine A in CD_3COCD_3
1: Spectrum of epicorazine A
2: Spectrum of epicorazine A after irradiation at δ 4.8
3: Spectrum of epicorazine A after irradiation at δ 6.9

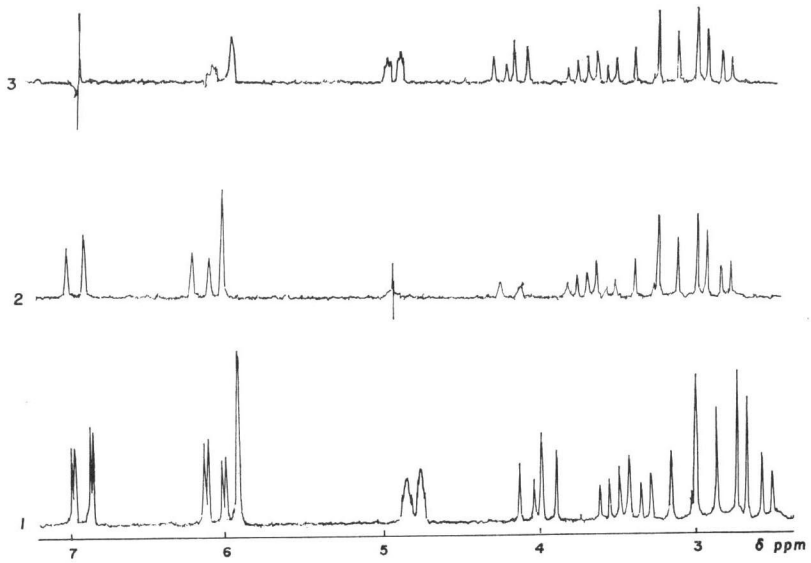


Fig. 3. Formula of epicorazine A

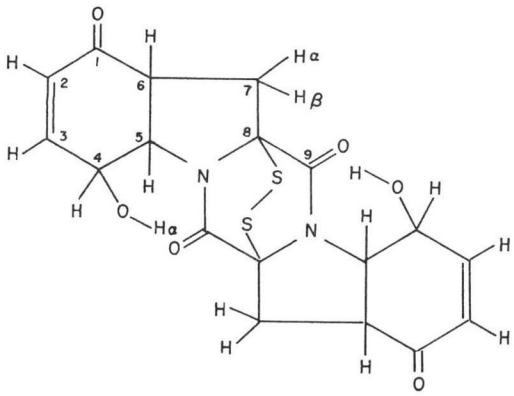
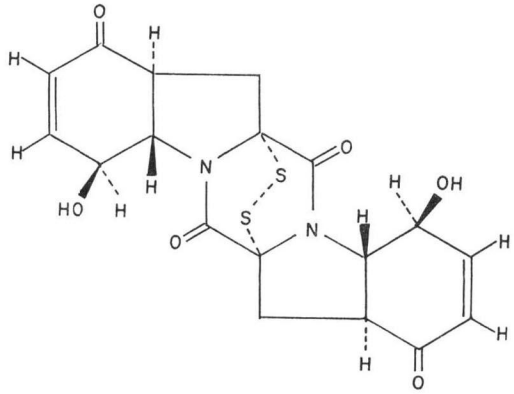
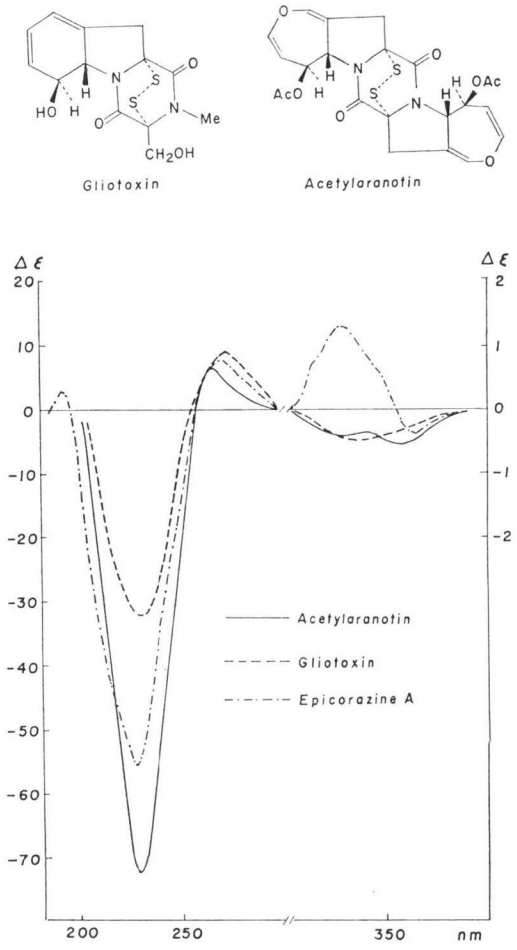


Fig. 4. Absolute configuration of epicorazine A



positions is 107.18°; application of KARPLUS relation[8] gives $J_{4,4\alpha} \sim 1$ Hz, which is consistent with the measured $J_{4,4\alpha} = 0.8$ Hz.

Fig. 5. CD spectra of epicorazine A and related compounds



Moreover, as shown by Fig. 4, H-4 and H-5 on the one hand, H-5 and H-6 on the other hand, are in *trans* position, as it could be expected from the values $J_{4,5} = 8.5$ Hz and $J_{5,6} = 12.3$ Hz.

Epicorazine A has optical rotation properties: $[\alpha]_D^{22} -293°$ (c 1.7 mg/ml, $CHCl_3$). Comparison of its CD curve with those of other known dithiodiketopiperazines (gliotoxin, acetylaranotin)[9] (Fig. 5) showed that all these fungal metabolites have the absolute configuration shown by Fig. 4. Applying the octant rule[10] to this absolute configuration gave a result which is consistent with the positive COTTON effect observed at 326 nm (n-π^* transition of the carbonyl). An ultimate confirmation of CD data interpretation was afforded by measuring the FRIEDEL's pairs by X-ray diffraction of a crystal of epicorazine A.

The chirality of the asymmetric centers which bring the disulfide bridge are R; consequently, the helicity of the disulfide bond is left-handed.

Experimental

General methods

Mass spectra were recorded on a AEI MS 9 spectrometer at 70 eV, using direct insertion probe.

PMR spectra were measured on a 90 MHz Bruker-Spectrospin; chemical shifts are reported in ppm downfield from internal TMS. ^{13}C-NMR spectra were determined on a ^{13}C Bruker spectrometer. IR spectra were recorded on a Perkin-Elmer 157G spectrometer. UV spectra were measured on a Jobin-Yvon Duospac 203 spectrophotometer. Optical rotations were determined on a Jobin-Yvon digital polarimeter. CD spectra were measured at 25°C on a Jobin-Yvon Dicrograph Mark III-S. X-ray measures were obtained on a four-circle automated Nonius diffractometer, using copper radiation.

Epicorazine A

IR (KBr, ν cm^{-1}) (Fig. 1): 3360, 1690, 1670, 1410, 1380, 1365, 1250, 1195, 1185, 1140, 1095, 1030, 810. UV λ_{nm}^{MeOH} (ε): max, 215 (24,400); shoulder, 260 (4,000). PMR (CD_3COCD_3): Fig. 2 and Table 2. ^{13}CMR (dioxane): Table 1. CD spectrum (CH_3CN, Fig. 5) shows five COTTON effects: nm ($\Delta\varepsilon$): 185 (+2.2), 218 (−56), 265 (+9.3), 326 (+1.5), 369 (−0.35).

Table 2. Parameters and assignment of PMR spectrum of epicorazine A in CD_3COCD_3 solution.

Shift δ ppm	Assignment	Coupling constants J Hz
6.91	H-3	$J_{2,3}$ =10.4
6.09	H-2	$J_{3,4}$ = 1.8
5.95	OH-4α	$J_{2,4}$ = 2.1
4.81	H-4	$J_{4,4\alpha}$ = 0.8
4.02	H-5	$J_{4,5}$ = 8.5
3.46	H-6	$J_{5,6}$ =12.3
3.02	H-7α + H-7β	$J_{6,7\alpha}$ =12.3 $J_{6,7\beta}$ = 5.8 $J_{7\alpha,7\beta}$ =14

Crystal data are: space group $P_{2_12_12_1}$; Z=4; a=11.35, b=12.71, c=13.03 Å. Atomic positions were published elsewere[7].

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References

1) BAUTE, M. A.; G. DEFFIEUX, R. BAUTE & A. NEVEU: New antibiotics from the fungus *Epicoccum nigrum*. I. Fermentation, isolation and antibacterial properties. J. Antibiotics 31: 1099~1101, 1978
2) MICHEL, K. H.; M. O. CHANEY, N. D. JONES, M. M. HOEHN & R. NAGARAJAN: Epipolythiopiperazinedione antibiotics from *Penicillium turbatum*. J. Antibiotics 27: 57~64, 1974
3) SHANNON, J. S.: Studies in mass spectrometry: Sporidesmin and sporidesmin B. Tetrahed. Lett. 1963: 801~806, 1963
4) BERG D. H.; R. P. MASSING, M. M. HOEHN, L. D. BOECK & R. L. HAMILL: A30641, a new epidithiodiketopiperazine with antifungal activity. J. Antibiotics 29: 394~397, 1976
5) KREBS, J. G.; D. HEUSSER & H. WIMMER: "Spray reagents" in thin-layer chromatography. 2nd *Ed.* by E. STAHL, p. 882, Springer-Verlag, New York, 1969
6) BAUTE M. A.; R. BAUTE, G. BOURGEOIS & G. DEFFIEUX: Isolement de dibenzyl-3, 6 dioxo-2, 5 pipérazine (bis-anhydrophénylalanine) à partir des cultures d'une souche du champignon *Epicoccum nigrum* LINK (Adélomycètes). Bull. Soc. Pharm. Bordeaux 112: 169~172, 1973
7) DEFFIEUX, G.; M. GADRET & J. M. LEGER: Structure cristalline d'un métabolite fongique original du groupe des épidithio-3, 6 dioxo-2, 5 pipérazines: l'épicorazine A. Acta Cryst. B33: 1474~1478, 1977
8) KARPLUS, M.: Contact electron-spin coupling of nuclear magnetic moments. J. Chem. Phys. 30: 11~15, 1959
9) NAGARAJAN, R. & R. W. WOODY: The circular dichroism of gliotoxin and related epidithiopiperazinediones. J. Am. Chem. Soc. 95: 7212~7222, 1973
10) MOFFITT, W.; R. B. WOODWARD, A. MOSCOWITZ, W. KLYNE & C. DJERASSI: Structure and the optical rotatory dispersion of satured ketones. J. Am. Chem. Soc. 83: 4013~4018, 1961