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ARANOTIN AND RELATED METABOLITES. II.[1] ISOLATION, CHARACTERIZATION, AND STRUCTURES OF TWO NEW METABOLITES

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In the course of our studies of antiviral activity[2] produced by a fungus provisionally designated as Arachniotus aureus (Eidam) Schroeter, we have observed the presence of two new substances in addition to recently reported metabolites.[1]

Silica gel chromatography of the ethyl acetate extract of the fermentation broth using toluene-ethyl acetate (3:1) afforded the following compounds listed in order of their appearance in eluates in Table I.

TABLE I

Metabolites from Arachniotus aureus

	Name	m.p. (dec.)	$[\alpha]_D^{26}$ $CHCl_3$	Empirical Formula*
1.	Aranotin (I)[1]	198-200°	----†	$C_{20}H_{18}O_7N_2S_2$
2.	Apoaranotin (IV)	200-205°	-492°	$C_{20}H_{18}O_6N_2S_2$
3.	Acetylaranotin (II)[1]	210-215°	-549.7°	$C_{22}H_{20}O_8N_2S_2$
4.	Bisdethio-di(methylthio)-acetylapoaranotin "BDAA" (V)	105-107°	-175°	$C_{24}H_{26}O_7N_2S_2$
5.	Bisdethio-di(methylthio)-acetylaranotin "BDA"[1] (III)	213-217°	-282°	$C_{24}H_{26}O_8N_2S_2$

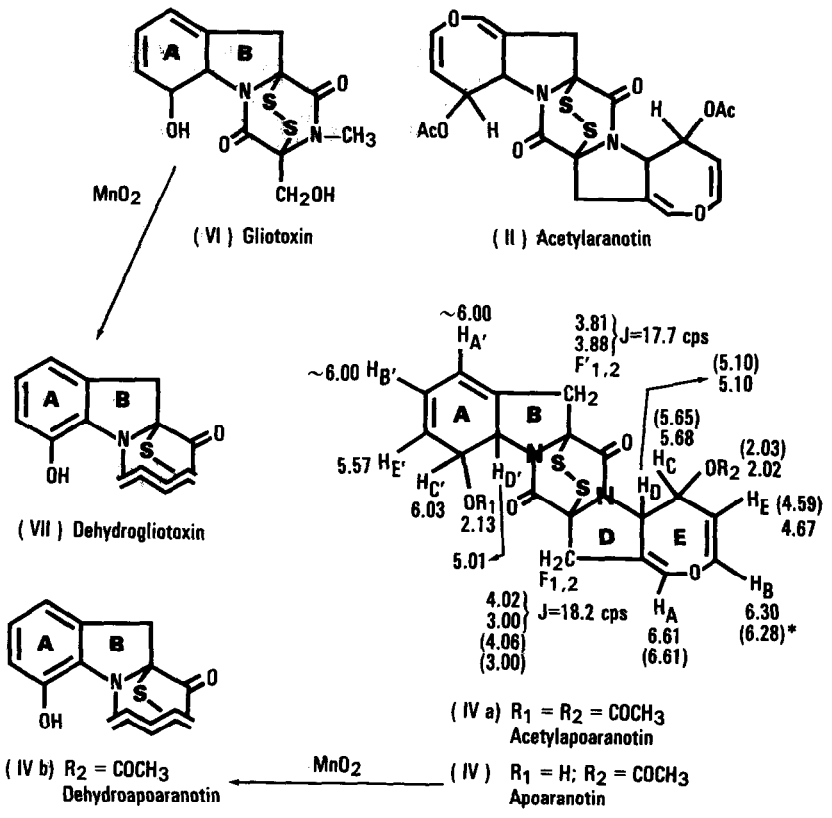
*Satisfactory analyses and high resolution mass spectra were obtained on all compounds with empirical formulae.

†Rotation was not recorded due to insufficient solubility.

The new metabolites, apoaranotin (IV) and "BDAA" (V) showed a large negative Cotton effect (cd, CH_3OH) at 229 (-49.0) and 225 mμ ($\Delta\varepsilon$ -52.5), respectively. These values, in conjunction with an amide band in the ir spectrum (1660 cm^{-1}, nujol), indicated the presence of a diketopiperazine ring.[1,3]

The spectral characteristics of apoaranotin (IV) showed its similarity to gliotoxin[4] (VI) and to acetylaranotin[1] (II). Direct inlet high resolution mass spectrometry of apoaranotin gave the highest molecular weight fragment at m/e 382, accompanied by ions at m/e 64 due to the loss of sulfur.[1] Calcd. for $C_{20}H_{18}O_6N_2$: 382.11376; found: 382.11649. Apoaranotin: UV, λ_{max}^{EtOH} 265mμ (ε3,880); cd (CH_3OH), 347 (-0.72), 320 (-0.42), 270 (+5.3), and 229 mμ ($\Delta\varepsilon$ -49.0). Gliotoxin: UV, λ_{max}^{EtOH} 267 mμ (ε 4,360); cd (CH_3OH), 338 (-0.5), 322 (-0.46), 272 (+7.4), and 233 mμ ($\Delta\varepsilon$ -34.1). The ir spectrum in nujol revealed, in addition to the amide band at 1660, the presence of hydroxyl (3350), acetate (1725, 1245), and C-O (1145 cm^{-1}). Acetylation of apoaranotin (Ac_2O, Py) gave after silica gel chromatography a crystalline acetate, IVa, m.p. 177-180° (dec.), $C_{22}H_{20}O_7N_2S_2$, m/e 424 (M^+-S_2). Calcd. for $C_{22}H_{20}O_7N_2$: 424.12392; found: 424.12705. The identity of one-half of this molecule with that of acetylaranotin (II) was obvious from the splittings and chemical shifts[1] of protons A, B, C, D, E, and $F_{1,2}$ (Rings D and E).* Spin decoupling permitted the assignment of protons A', B', C', D', E', and $F'_{1,2}$ (Rings A and B). The structure consonant with these assignments was further corroborated by conversion of the acetate (MnO_2 in $CHCl_3$) IVa to dehydroapoaranotin (IVb). This phenol, m.p. 183-186° (dec.), $C_{20}H_{18}O_6N_2S_2$, m/e 380 (M^+-S_2) had a very similar UV spectrum [shoulders λ_{max}^{EtOH} 215 (21,000), 260 (4,900), 305 mμ (ε 3,550)] to that of dehydrogliotoxin[5] (VII) [shoulders λ_{max}^{EtOH} 215 (21,000), 253 (4,700), and 305 mμ (ε 3,550)]. The low field protons in IVb (δ = 6.72, 6.80, 6.88, 7.02, and 7.10 ppm) were also in good agreement with the aromatic protons in the spectrum of dehydrogliotoxin[5] (δ = 6.70, 6.87, 6.95, 7.10, and 7.18 ppm). Therefore, apoaranotin must have structure IV.

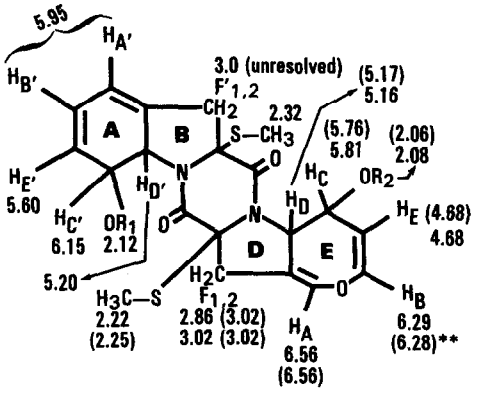
*Chemical shifts are given in δ ppm; nmr spectra were recorded in $CDCl_3$ using Varian 100 MHz spectrometer with TMS as internal standard. The values in parentheses refer to acetylaranotin[1] (II).



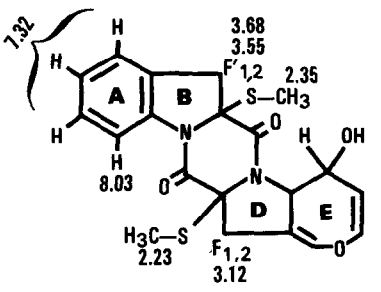
Bis-dethio-di(methylthio)acetylapoaranotin "BDAA" (V) is a colorless $C_{24}H_{26}N_2O_7S_2$ compound. M^+ 518. Its UV spectrum was similar to that of gliotoxin (vide supra). The identity of one-half of the molecule with that of "BDA" was apparent from the splittings and chemical shifts of protons A, B, C, D, E, and $F_{1,2}$. The structure of the other half of the molecule could be deduced from the spin decoupling experiment and spectral data of dideacetoxy "BDAA" (Va) and its dehydration product (Vb).

Hydrolysis of V (5 minutes, 0.2 N NaOH, R.T.) gave rise to dideacetoxy compound Va (nmr spectrum complex with an unresolved envelope of four methylene protons at δ = 3.02 ppm). Further treatment of this derivative with alkali (12 hrs) afforded a good yield of anhydro "BDAA" (Vb), m.p. 184-186° (methanol), $C_{20}H_{20}O_4N_2S_2$. Calcd.: 416.08485; found: 416.08646. The striking similarity of its UV spectrum λ_{max}^{EtOH} 252 (10,675), 276 (7,000),

286 mμ (ε 6,425)], and the aromatic portion of the nmr spectrum [δ = 8.03 and 7.32 ppm (1:3)] with the corresponding spectra of strychnine [λ_{max}^{EtOH} 254 (12,600), 278 (4,275), and 288 mμ (ε 3,400)], [δ = 8.0 and 7.2 ppm (1:3)] left no doubt as to the nature of the A ring in Vb. Aromatization of this ring also resulted in the change of methylene protons in ring B from a multiplet in Va to an AB quartet at δ = 3.55 and 3.68 ppm, J = 17 cps, and to a narrow unresolved multiplet in ring D at 3.12 ppm.



(V) BDAA $R_1 = R_2 = COCH_3$



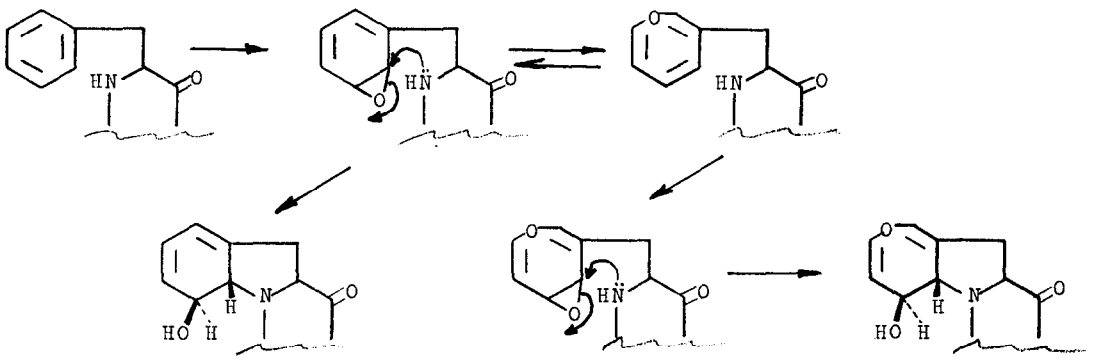
(V b) Anhydro"BDAA"

**The values in parentheses refer to "BDA"[1] (III).

The structures and stereochemistry[6] of these metabolites appear to represent a novel biogenetic transformation of a benzene ring, and seem to be best accommodated by the intervention of a benzene oxide-oxepine,[7] or its biochemical equivalent, which could also explain the formation and stereochemistry of gliotoxin. A similar intermediate would also satisfactorily rationalize the 1,2-hydrogen shifts (NIH shift) observed by Witkop[8] in aromatic hydroxylations.

Biosynthetic origin of metabolites from Arachniotus aureus is at present under study in these laboratories.

e.g.



Gliotoxin Type Aranotin Type

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