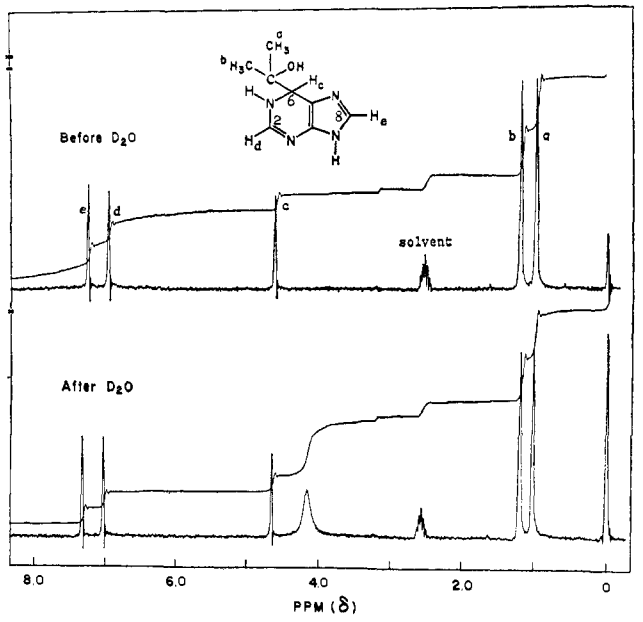

Figure 2. Nmr spectra (Varian A60-A) of 2-propanol–purine photoadduct (10 wt %) in DMSO-d_6 before (upper) and after (lower) addition of D_2O; chemical shifts (δ) referred to internal TMS standard. Very broad underlying band in the 6–8-ppm region is established by the integrated absorption curve. Recrystallizing solvent band (methanol) at 3.2 ppm has been deleted for clarity.

under high resolution) unambiguously require addition of the carbinol carbon of the alcohol to a purine ring carbon.

The site of addition on the ring was established by experiments with purine substituted by deuterium at the 6 and 8 positions, respectively.[2] In the nmr spectrum of the methanol photoadduct of 8-deuteriopurine, the singlet at 7.2 ppm is absent but the spectrum is otherwise unchanged. On the other hand, in the spectrum of the 6-deuteriopurine–methanol adduct, the 6.9- and 7.2-ppm peaks are unchanged, but the 4.89-ppm band (corresponding to the 4.62-ppm line in Figure 2) is absent. The splitting pattern of the two CH alcohol protons further upfield is simplified accordingly. We conclude that the site of methanol addition is the 6 position of purine, and that the 6.9- and 7.2-ppm singlets correspond respectively to the C-2 and C-8 purine protons. Similar experiments with the ethanol adducts show that addition occurs again at the 6 position in *both* isomers. The two compounds must therefore be diastereomers. We assume that 2-propanol also adds at the same site. The assignment of the hydrogen positions among the four possible tautomeric structures is still uncertain. The interesting nonequivalence of the a and b positions implies a preferred conformation around the C_6–C_α bond. This may involve either an intramolecular hydrogen bond or specific intermolecular interactions.

Ferrioxalate actinometry[3] gave $\varphi = 0.24 \pm 0.03$ for the room temperature photoreaction (2537 Å) of purine in all three neat alcohols, at concentrations near 10^{-4} *M* and intensities around 10^{-8} einstein/(cm^2 min). The quantum yield of the methanol reaction decreases sharply to about 0.18 on addition of cyclohexene, up to about 10^{-2} *M*, but remains unchanged on further additions up to 0.1 *M*. Two pathways seem to be involved in this reaction.

Absorption spectrum changes generally similar to those seen in the purine reaction have been observed after irradiation of deoxygenated ethanol solutions of many heterocyclic molecules, including pyridine, pyrazine, pyrimidine, benzimidazole, benzoxazole, quinoline, isoquinoline, quinoxaline, 1,4,5-triazanaphthalene, and phenazine. It would appear that the photoaddition of alcohols to heteroaromatic molecules may be a quite general reaction. We note that photolysis of acridine in anaerobic methanol or ethanol has been shown to give the respective 9-α-hydroxyalkyl-9,10-dihydroacridines.[4]

A detailed account of this work will be given elsewhere.[5] Further studies on the mechanism and generality of the reaction are in progress.

Acknowledgment. It is a pleasure to thank Mr. Michael Z. Blumberg and Dr. Gwendolyn Sherman for their assistance in some aspects of this work.

(2) The deuteriopurines were prepared according to the procedures of M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **86**, 696 (1964). For further validation of the assignments of the deuterium positions, see also: S. Matsuura and T. Goto, *Tetrahedron Letters* 1499 (1963); *J. Chem. Soc.*, 623 (1965); F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, **29**, 1988 (1964); W. C. Coburn, Jr., M. C. Thorpe, J. A. Montgomery, and K. Hewson, *J. Org. Chem.*, **30**, 1110 (1965).

(3) C. G. Hatchard and C. A. Parker, *Proc. Roy. Soc.* (London), **A235**, 518 (1956).

(4) H. Göth, P. Cerutti, and H. Schmid, *Helv. Chim. Acta*, **48**, 1395 (1965).

(5) J. S. Connolly, Ph.D. Thesis, Brandeis University, 1968, and manuscript submitted for publication.

(6) National Institutes of Health Predoctoral Fellow, 1965–1967.

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Aranotin and Related Metabolites from *Arachniotus Aureus*. I. Determination of Structure

Sir:

The antiviral activity[1] produced by a fungus provisionally designated as *Arachniotus aureus* (Eidam) Schroeter prompted a detailed investigation of its metabolites. These metabolites[2] belong to the class of sulfur-containing diketopiperazines such as gliotoxin[3] and sporidesmins.[4]

One of the active[1] metabolites is aranotin (**3a**), $C_{20}H_{18}O_7N_2S_2$, mp 198–200° dec. Acetylation of aranotin afforded another metabolite, acetylaranotin (**3**), $C_{22}H_{20}O_8N_2S_2$; mp 201–215° dec; mass spectra, *m/e* 440, accompanied by ions at *m/e* 64 due to loss of S_2;[5] ir (Nujol) 1740, 1230 (acetyl), and 1665 cm^{-1} (amide); uv (C_2H_5OH) end absorption with shoulders at 270 (ϵ 1800) and 222 mμ (ϵ 10,200); CD (CH_3OH), two Cotton effects, a positive at 267 and a negative at 230 mμ. The major metabolite is bisdethiodi(methylthio)acetylaranotin (BDA) (**4**), $C_{24}H_{26}O_8N_2S_2$; mp 213–217° dec; nmr τ 4.24

(1) The detailed study of biological properties will be described in detail elsewhere by D. C. DeLong, *et al.*

(2) Fermentation conditions will be described elsewhere by M. Stark and coworkers.

(3) M. R. Bell, J. R. Johnson, B. S. Wildi, and R. B. Woodward, *J. Am. Chem. Soc.*, **80**, 1001 (1958).

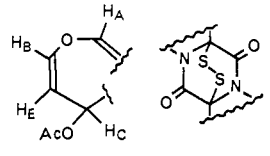
(4) (a) G. Lowe, A. Taylor, and L. C. Vining, *J. Chem. Soc., Sect. C*, 1799 (1966); (b) R. Rahman and A. Taylor, *Chem. Commun.*, 1032 (1967).

(5) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day, Inc., San Francisco, Calif., 1964, p 249.

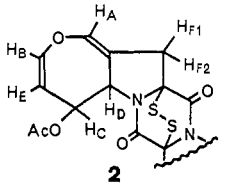
(m, C*H*OAc), 7.75 (s, SCH_3), and 7.94 (s, OCOH_3), 1:3:3. The low-field region (τ 3.0–5.5) of the nmr spectra of BDA (**4**) was similar to that of acetylaranotin (**3**) (Figure 1). Alkaline hydrolysis of **4** afforded the diol **4a**, $C_{20}H_{22}O_6N_2S_2$; mp 218–222° dec; nmr τ 5.3 (m, C*H*OH) and 7.8 (s, SCH_3), 1:3.

Raney nickel desulfurization of both acetylaranotin (**3**) and BDA (**4**) afforded the same bisdethioacetylaranotin (**5b**), $C_{22}H_{22}O_8N_2$; mp 201–202°; ir (Nujol) 1730, 1232 (acetyl), and 1660 cm^{-1} (amide); CD (CH_3OH), negative Cotton effect at 226 mμ. Therefore, **3** and **4** have the same carbon skeleton and differ only in the mode of functionalization of sulfur. The presence of amide bands in the ir spectra in conjunction with the negative Cotton effects at 230 mμ in **3** and **4** showed the presence of a diketopiperazine moiety.[6,7] The positive Cotton effect at 267 mμ in acetylaranotin was assigned to the disulfide chromophore,[8] and the facile loss[5] of a unit of mass 64 in the mass spectrum of **3** confirmed its presence.

The chemical shifts of H_A at τ 3.37 and 3.41, in **3** (Figure 1) and **4**, respectively, and H_B at τ 3.68 and 3.69 are in good agreement for α protons on an enol ether.[9] The chemical shifts of H_E at τ 5.37 and 5.30 in **3** and **4** correspond closely to a β proton in an enol ether.[9] The coupling constant, J_{BE}, of 7.5 and 8.0 Hz in **3** and **4** is in excellent agreement with a seven-membered cyclic enol ether group[10] but inconsistent with a smaller cyclic enol ether ring.[12] In BDA (**4**) H_C at τ 4.17 moved upfield to τ 5.3 upon deacetylation. Therefore, H_C is on the same carbon atom that bears the acetyl group. The couplings J_{CE} = 1.5 and J_{BC} = 2.1 Hz in **3** are in agreement with vinylic and allylic relationships.[13]



1



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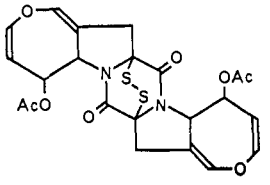
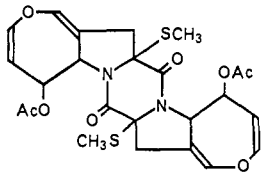


3



4

(6) D. Balasubramanian and D. B. Wetlaufer, *J. Am. Chem. Soc.*, **88**, 3449 (1966).

(7) H. Herrmann, R. Hodges, and A. Taylor, *J. Chem. Soc.*, 4315 (1964).

(8) (a) H. Ziffer, U. Weiss, and E. Charney, *Tetrahedron*, **23**, 3881 (1967); (b) M. Carmack and L. A. Neubert, *J. Am. Chem. Soc.*, **89**, 7134 (1967).

(9) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Inc., New York, N. Y., 1959, p 61.

(10) D. R. Larkin, personal communication. 4,5,6,7-Tetrahydrooxepin[11] showed $J_{1,2}$ = 7.5 Hz. We thank Dr. D. R. Larkin (Celanese Chemical Co., Corpus Christi, Texas) for sending us a copy of the nmr spectrum.

(11) D. R. Larkin, *J. Org. Chem.*, **30**, 335 (1965).

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(13) E. W. Garbisch, Jr., *J. Am. Chem. Soc.*, **86**, 5561 (1964).

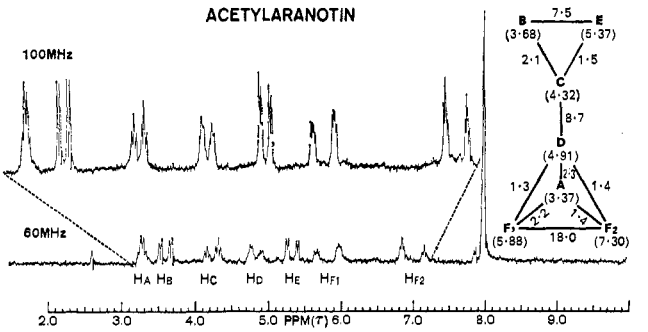


Figure 1.

From the above data, the partial structure **1** can be written for acetylaranotin. The chemical shift of H_D at τ 4.91 and 4.81 in **3** and **4** indicated that H_D was (a) on a carbon atom adjacent to an oxygen or nitrogen atom or (b) olefinic.[14] Hydrogenation data ruled out the latter. The chemical shifts of H_{F1} and H_{F2} at τ 5.88 and 7.30 in **3** and τ 6.95 in **4**, together with the geminal coupling $J_{F1,F2}$ = 18.0 Hz in **3**, showed that H_{F1} and H_{F2} belong to a methylene group. Finally, the small couplings in acetylaranotin of J_{AD} = 2.3, $J_{A,F1}$ = 2.2, and $J_{A,F2}$ = 1.4 Hz suggested these to be allylic.[13] The partial structure of acetylaranotin can now be expanded to **2**.

Desulfurization of BDA (**4**) with amalgamated aluminum[15] afforded partially desulfurized bisdethiomethylthioacetylaranotin (**5a**), $C_{23}H_{24}O_8N_2S$; mp 221–223°; ir (Nujol) 1740, 1230 (acetyl), and 1665 cm^{-1} (amide). The nmr spectrum of **5a** was more complex than that of **4**. However, the singlets at τ 7.85, 7.90, and 7.96 could be assigned to one SCH_3 and two $OCOCH_3$. A comparison of the nmr spectra of **3**, **4**, and **5a** in conjunction with their mass spectra and elemental analyses reveals that, while in **3** and **4** the two cooperating α-thio-α-amino acids of the anhydropeptide[3] are identical, in **5a** they are not. Further, on Raney nickel desulfurization, **3** and **4** afforded the same product, **5b**. Consequently we can now assign complete structures **3** and **4** for acetylaranotin and BDA, respectively.[16] Clearly, the structure of the partially desulfurized product obtained from BDA, **4** is **5a**, and, being an unsymmetrical anhydropeptide, even the acetyl singlets have different chemical shifts.

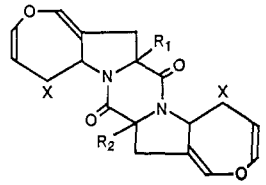
In the nmr spectrum of bisdethioacetylaranotin (**5b**) the quartet at τ 5.64 was assigned to H_G, introduced during desulfurization. Double irradiation showed that H_{F1} and H_{F2} were centered at τ 7.025 and 7.205, respectively. On irradiation at τ 7.06 the quartet at τ 5.64 collapsed to a singlet. Deacetylation of **5b** afforded the diol **5c**, $C_{18}H_{18}O_6N_2$; mp 248–250°; ir (Nujol) 3200–3125 (OH), 1670, 1635, 1615 (amide), and 1277 cm^{-1} (enol ether); CD (H_2O), negative Cotton effect at 220 mμ. Oxidation of **5c** with manganese dioxide afforded the α,β-unsaturated ketone **5d**, $C_{18}H_{14}O_6N_2$; mp 250° dec; uv max (95% C_2H_5OH) 255 (ϵ 7800)

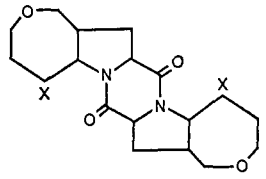
(14) D. G. I. Kingston, Lord Todd, and D. H. Williams, *J. Chem. Soc., Sect. C*, 1669 (1966).

(15) J. R. Johnson and J. B. Buchanan, *J. Am. Chem. Soc.*, **75**, 2103 (1953).

(16) Since **3** and **4** are symmetrical, the chemical environment of any particular proton in one-half of the molecule is exactly identical with the corresponding proton in the other half, and, therefore, each signal in the nmr spectra of **3** and **4** represents two protons.

and 310 mμ (ε 2200); nmr[17] τ 2.92 (d, 2, J = 7.5, OC*H*=CHCO) and 4.02 (d, 2, J = 7.5 Hz, OCH=C*H*CO).





5a R_1=H, R_2=SCH_3, X=<H, OAc

5b R_1=R_2=H_G, X=<H, OAc

5c R_1=R_2=H, X=<H, OH

5d R_1=R_2=H, X=O

6a X=<H_C, OAc

6b X=<H, OH

6c X=O

Four moles of hydrogen was consumed when bisdethioacetylaranotin was hydrogenated; the reaction product was octahydrobisdethioacetylaranotin[18] (**6a**); mass spectrum, M^+ 450; nmr τ 4.63 (sextet, 2, C*H*OAc), between 5.5 and 6.5 (m, 12, four C*H*$_2$O and four C*H*NCO groups), and 7.95 (s, 6, two OCOC*H*$_3$). Spin decoupling showed that H_C was coupled to one proton at τ 5.65 and two protons at τ 7.74 and 8.09, consistent with structure **6a**. Deacetylation of **6a** afforded the diol **6b**, $C_{18}H_{26}O_6N_2$; mp 230–232°; ir (Nujol) 3350, 3200 (OH) and 1635 cm^{-1} (amide); CD (H_2O), negative Cotton effect at 222 mμ. Oxidation of **6b** with acetic anhydride and dimethyl sulfoxide[19] afforded the ketone **6c**, $C_{18}H_{22}O_6N_2$; mp 229–230°; ir (Nujol) 1720 (ketone) and 1655 cm^{-1} (amide); nmr τ 5.07 (d, 2, CONC*H*CH).

Acknowledgments. We thank Mr. W. Jankowski of Varian Associates for 100-MHz and double-irradiation experiments.

(17) The nmr spectrum was taken in CF_3COOH. All other nmr spectra were taken in $CDCl_3$ with TMS as internal standard.

(18) This compound was chromatographically homogeneous, but resisted crystallization. Upon deacetylation it afforded crystalline diol **6b**. All compounds gave satisfactory elemental analyses except for **6a**. All compounds gave the expected molecular ions in the mass spectra.

(19) J. D. Albright and L. Goldman, *J. Am. Chem. Soc.*, **87**, 4214 (1965).

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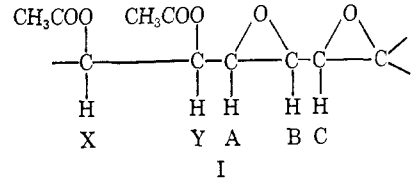
Received February 3, 1968

Crotepoxide, a Novel Cyclohexane Diepoxide Tumor Inhibitor from *Croton macrostachys*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of the fruits of *Croton macrostachys* Hochst. *ex* A. Rich. (Euphorbiaceae)[3] showed significant inhibitory activity in Lewis lung carcinoma in mice (LL).[4] We report herein the isolation and structural elucidation of crotepoxide, a novel tumor-inhibitory cyclohexane diepoxide derivative from *C. macrostachys*.

Fractionation of the ethanol extract, guided by assay against LL, revealed that an active principle was concentrated, successively, in the methanol layer of a 10% aqueous methanol–Skellysolve B partition and in the 1-butanol layer of a 1-butanol–water partition. Further fractionation involving silicic acid chromatography yielded crotepoxide (II),[5] $C_{18}H_{18}O_8$; mp 150–151°; $[\alpha]^{25}D$ +74° (c 1.70, $CHCl_3$); λ_{max}^{MeOH} 274 mμ (ε 1050) and 281 mμ (ε 860); $\lambda_{max}^{CHCl_3}$ 3.35, 5.71, 5.78, 6.24, 6.31, 6.89, 7.29, 7.87, 8.20, 9.00, 9.60, 10.24, and 11.12 μ; nmr signals (in $CDCl_3$) at τ 2.28 (5 H, m, aromatic), 4.27 (1 H, d, J_{XY} = 9.5 cps, >CHOAc), 5.02 (1 H, d, d, J_{XY} = 9.5 and J_{AY} = 1.5 cps, >CHOAc), 5.42 and 5.75 (2 H, doublets, J = 12.0 cps, CH_2OCOPh), 6.32 (1 H, d, J_{BC} = 2.5 cps), 6.56 (1 H, d, d, J_{BC} = 2.5 and J_{AB} = 4.0 cps), 6.90 (1 H, d, d, J_{AB} = 4.0 and J_{AY} = 1.5 cps), 7.88 (3 H, s, acetate), and 7.95 (3 H, s, acetate).



I

Crotepoxide was converted into several crystalline derivatives. Hydrogenation using platinum oxide catalyst yielded the hexahydro derivative ($C_{18}H_{24}O_8$; mp 121–122°; $[\alpha]^{30}D$ +59° (c 1.35, $CHCl_3$)) which exhibited no signals for aromatic protons in the nmr spectrum. Treatment with aqueous methanolic potassium hydroxide yielded a triol, V ($C_7H_{10}O_5$; mp 101–102°; $[\alpha]^{27}D$ +30° (c 1.06, CH_3OH)), and benzoic acid. Treatment with aqueous methanolic hydrochloric acid for 30 min yielded the monochlorohydrin III ($C_{18}H_{19}ClO_8$; mp 170–171°; $[\alpha]^{29}D$ −4° (c 1.35, $CHCl_3$)), while prolonged treatment yielded the deacetyldichlorohydrin IV ($C_{14}H_{16}Cl_2O_6$; mp 241–242°, $[\alpha]^{25}D$ −10° (c 0.71, CH_3OH)). IV was readily converted to a triacetate, $C_{20}H_{22}Cl_2O_9$, mp 217–218°, and under more drastic conditions to a tetraacetate, $C_{22}H_{24}Cl_2O_{10}$, mp 153–154°. The triacetate was unreactive toward Jones reagent, and its nmr spectrum in acetone or in DMSO showed a sharp singlet for the hydroxyl proton, indicative of the direction of opening of the second epoxide to yield IV.

Treatment of crotepoxide with aqueous methanolic hydriodic acid yielded a monoiodohydrin, VI ($C_{18}H_{19}IO_8$; mp 143–144°; $[\alpha]^{28}D$ −46° (c 1.40, $CHCl_3$)), and an ene diol derivative, VII ($C_{18}H_{20}O_8$; mp 145–146°; $[\alpha]^{28}D$ +127° (c 1.51, $CHCl_3$)). The ene diol was converted into the triacetate ($C_{20}H_{22}O_9$; mp 141–142°; $[\alpha]^{28}D$ +151° (c 0.91, $CHCl_3$)) and, under more drastic conditions, into an oily product, the spectral characteristics of which were in agreement with the tetraacetate structure. Oxidation of the ene diol with Jones

(1) Tumor Inhibitors. XXIX. Part XXVIII: S. M. Kupchan, A. P. Davies, S. J. Barboutis, H. K. Schnoes, and A. L. Burlingame, *J. Am. Chem. Soc.*, **89**, 5718 (1967).

(2) Supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health (PH-43-64-551).

(3) Fruits were gathered in Ethiopia in March 1965. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. Department of Agriculture by the CCNSC.

(4) The *in vivo* inhibitory activity was assayed under the auspices of the Cancer Chemotherapy National Service Center by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(5) Crotepoxide showed significant inhibitory activity against Walker carcinosarcoma 256 in rats at 300 mg/kg and Lewis lung carcinoma in mice at 200 mg/kg.