sulfonamide was removed by filtration, and the residue was chromatographed on a silica gel chromatography column with a 10% ethyl acetate–hexane mixture as the eluent. The major fraction isolated from the column contained 600 mg (52%) of *N*,*N*-bis(2-bromo-2-propenyl)benzenesulfonamide (**33**) as a colorless oil: IR (neat) 3080, 2920, 1630, 1480, 1450, 1350, 1150, 1090, 1060, 910, 780, 750, and 690 cm$^{-1}$; NMR ($CDCl_2$, 90 MHz) $\delta$ 4.13 (s, 4 H), 5.57 (br s, 2 H), 5.78 (br s, 2 H), and 7.4–7.9 (m, 5 H). Anal. Calcd for $C_{12}H_{1B}r_2NO_2S$: C, 36.47; H, 3.32; N, 3.55. Found: C, 36.47; H, 3.36; N, 3.53.

A mixture containing 790 mg of **33**, 1.28 g of tri-*n*-butyltin hydride, and 0.6 g of AIBN in 100 mL of benzene was heated at reflux for 10 h. The solvent was removed under reduced pressure, and the residue was subjected to silica gel chromatography with a 20% ethyl acetate–hexane mixture as the eluent. The major fraction contained 322 mg (67%) of a crystalline solid, mp 115–116 °C, whose structure was assigned as *N*-(phenylsulfonyl)-3,4-dimethyl-3-pyrrolidene (**35**) on the basis of its spectral properties: IR (KBr), 2960, 2920, 2840, 1590, 1480, 1450, 1350, 1310, 1250, 1170, 1110, 1080, 850, 770, 745, 700, 610, and 570 cm$^{-1}$; NMR (benzene-$d_6$, 360 MHz) $\delta$ 1.02 (s, 6 H), 3.80 (s, 4 H), 6.92–7.06 (m, 3 H), and 7.82–7.95 (m, 2 H). Anal. Calcd for $C_{12}H_{15}NO_2S$: C, 60.72; H, 6.38; N, 5.90. Found: C, 60.62; H, 6.41; N, 5.85.

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# Ring-Extended Products from the Reaction of Epoxy Carbonyl Compounds and Nucleic Acid Bases

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Purine and pyrimidine bases react with epoxy carbonyl compounds in aqueous solution to yield ring-extended adducts. These products include etheno-modified bases as well as adducts in which the modification involves the formation of an additional six-membered ring. The latter examples are among the first known cases of this type of modification of pyrimidine bases. Plausible mechanisms for the formation of these adducts are discussed.

Epoxides occur widely in nature and have been identified in compounds from microorganisms and plants.[1-6] They are produced also in mammalian systems in the oxidation of polyunsaturated lipids.[7-9] The deleterious effects of some epoxy compounds are well documented. For example, aflatoxin $B_1$, sterigmatocystin, and the polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene are known to be toxic and carcinogenic. Their detrimental effects are thought to be mediated by their conversion in vivo to their epoxides and subsequent modification of nucleic acid bases by these epoxides.[10-18] Simpler monofunctional epoxides have been known to modify nucleic acid bases.[19,20] In addition, the mode of formation and the detailed structures of adducts between carbonyl compounds and nucleic acid bases have been of considerable interest in studies of the constitution and mechanism of action of nucleic acids. Our interest in the modification of nucleic acid bases by malonaldehyde and related systems,[21,22] and in the synthesis of compounds related to the "Y" bases,[23] led us to examine such reactions with epoxy carbonyl compounds, the results of which are reported in this paper.

## Results and Discussion

Very few studies have been undertaken to determine the detailed structures of adducts arising from the reaction of

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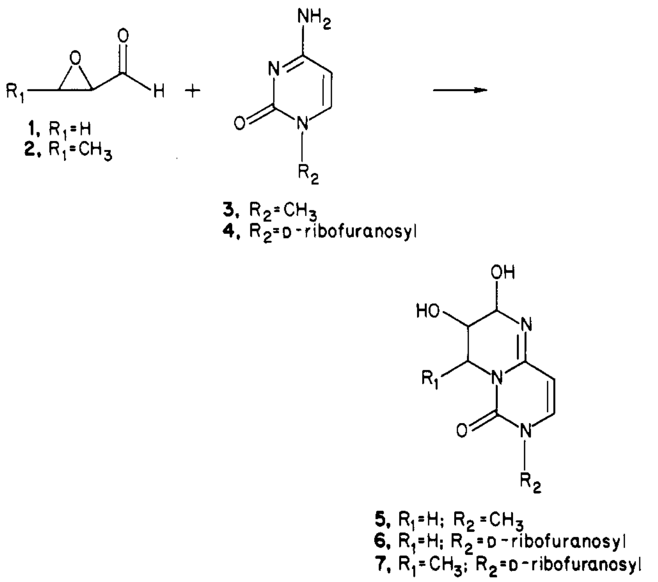
0022-3263/85/1950-5627$01.50/0

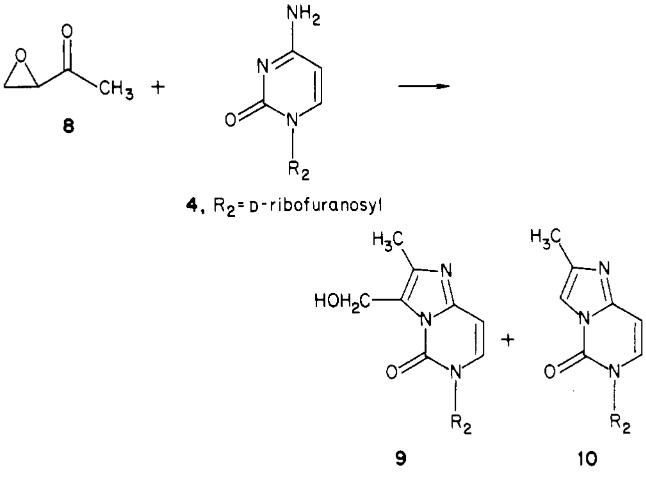
**Scheme I**



epoxides with nucleic acid bases. Almost all of the reported work in this area deals with polycyclic aromatic epoxides or the aflatoxins,[11,13,15,18] where modification involves a monofunctional epoxide moiety. Little, however, is known about the reactivity of multifunctional epoxides toward nucleic acid bases. We have found that epoxy carbonyl compounds readily modify pyrimidine and purine bases to give interesting ring-extended adducts. These modifications were carried out by treating the appropriate nucleoside or alkylated base in aqueous media at defined pHs with the epoxy carbonyl compounds. The latter were prepared from the corresponding enals by a modification of the method of White and co-workers.[24] The modifying reactions were monitored by UV spectral methods and terminated when product formation had maximized. The ribosyl-containing adducts were purified by reversed-phase HPLC on Amberlite XAD-4 resin, while the corresponding alkylated bases were separated on preparative silica gel plates.

The reaction of glycidaldehyde (**1**) with cytidine (**4**) at pH 10 yielded a white crystalline adduct (mp 111–113 °C) in 41% yield. To facilitate interpretation of the spectral data and assignment of structure for this product, the related adduct from 1-methylcytosine (**3**) was also prepared. The mass spectrum of the latter showed a molecular ion at $m/z$ 197 and a more intense peak at $m/z$ 179, indicating facile loss of $H_2O$ from the adduct. The UV spectrum exhibited absorbance maxima at 223 ($\epsilon$ 10 400) and 286 nm ($\epsilon$ 9700), suggesting the absence of extended conjugation. The 360-MHz $^1H$ NMR spectrum in $Me_2SO-d_6$ showed doublets at $\delta$ 7.26 and 5.65 integrating for one proton each and with coupling constant of 7.8 Hz (cytosine moiety). Two singlets at $\delta$ 4.83 and 5.70 which underwent exchange with $D_2O$ were attributed to the presence of hydroxyl groups. A doublet at $\delta$ 5.27 (1 H, $J$ = 2.7 Hz) and multiplets at $\delta$ 3.72 (1 H) and 3.62 (2 H) were assigned as the remaining protons of a newly formed ring. The methyl group appeared as a singlet at $\delta$ 3.17. In the $^{13}C$ NMR spectrum (in $Me_2SO-d_6$), three additional carbon resonances, apart from those of the cytosine ring and the methyl group, occurred at $\delta$ 58.4, 65.4, and 90.7 and were indicative of the presence of a saturated three-carbon moiety. Taken collectively, the data suggested that the new compound was 7-methyl-3,4-dihydro-2,3-dihydroxy-2*H*-pyrimido[1,6-*a*]pyrimidin-6(7*H*)-one (**5**) (Scheme I). The spectral data for the cytidine adduct **6** were more complex because of the presence of the D-ribofuranosyl moiety; however, excellent correlation was clearly evident between **5** and **6** for the modified base moiety. In both neutral and acidic (pH 5) media, cytidine was converted to adduct **6** in 38% and 40% yields, respectively. No ethenocytidine derivative was isolated in any of these cases.[22] It should be mentioned that formation of six-membered rings in the modification of pyrimidine nucleosides is rare.

**Scheme II**



The formation of six-membered rings was also seen in the reaction of 2,3-epoxybutanal (**2**). For example, in aqueous solutions at pH 10, cytidine (**4**) was transformed into **7** in 30% yield, while in neutral and acidic (pH 5) media, conversion to **7** occurred in 45% and 36% yields, respectively.

When the less reactive epoxy carbonyl compound, 3,4-epoxybutanone (**8**), was employed, modification of cytidine was still observed but the transformation occurred in low yield (about 13%) under neutral, acidic, or basic conditions. The products of these reactions were the ethenocytidine derivatives **9** and **10** (Scheme II). These products were identified by their mass spectral, UV, and NMR data.[22]

A plausible and generalized mechanism for the formation of these adducts is shown in Scheme III. Attack by the amino group of cytidine on the carbonyl carbon of the epoxy carbonyl compound produces the amino alcohol intermediate **11**. Ring opening of the epoxide moiety in **11** may occur in two ways. Direct nucleophilic attack by N-3 on the terminal position of the epoxide results in the formation of the six-membered ring products **6** and **7**, which are observed for the epoxy aldehydes **1** and **2**. In the case of the epoxy ketone **8**, the initially formed intermediate **11** has a tertiary alcohol group which will dehydrate rapidly to form the imine **12**. If ring opening in **12** involves the internal carbon of the epoxide (i.e., the allylic and now more electrophilic carbon) intermediate **13** is generated. This species can eliminate a proton to give **9** or it can eliminate $H_2C{=}O$ to give **10** (Scheme III). Differentiation between the two pathways therefore resides on the ability of intermediate **11** to eliminate water to produce an $\alpha,\beta$-unsaturated epoxide.

Glycidaldehyde has been reported to show high specificity toward guanine components in its modification of nucleic acids.[25] Despite its importance as a probe in the

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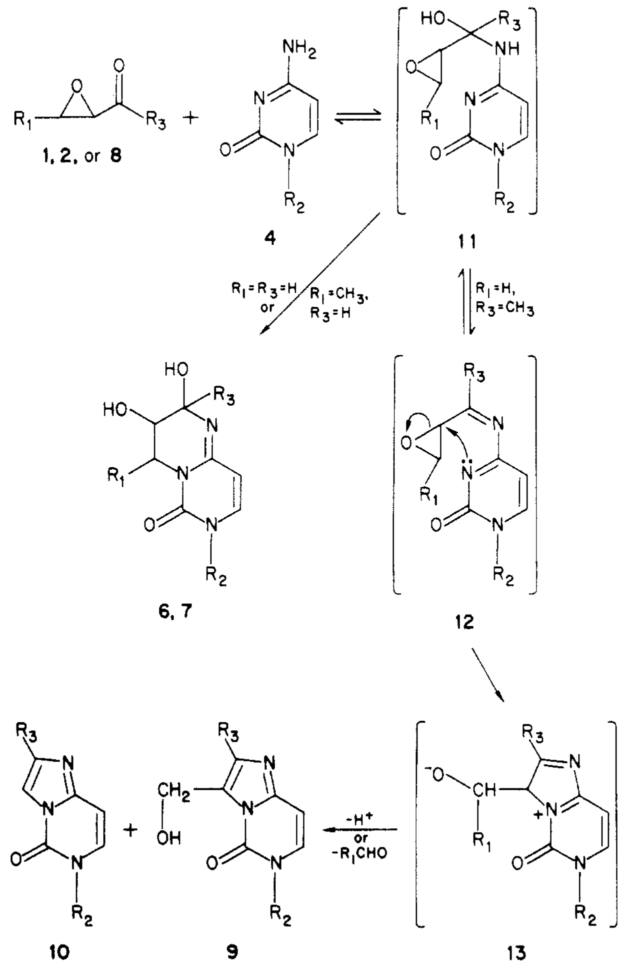
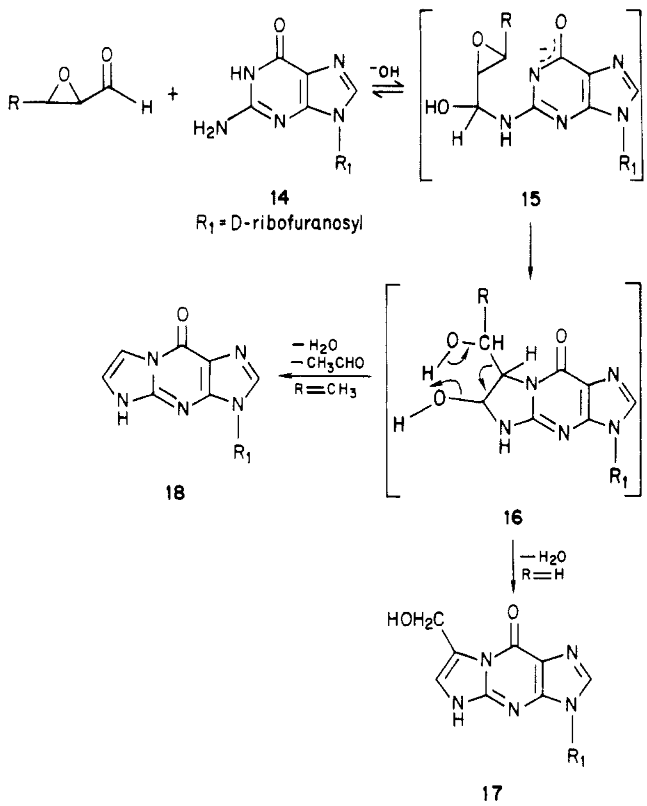
**Scheme III**



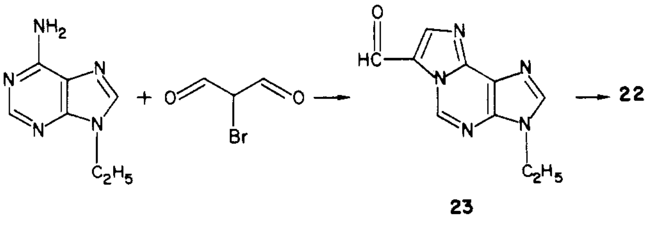
mode of action of alkylating carcinogens,[26] the structural details of its modification of guanine residues had not been fully determined.[27] Since publication of our original communication on the structure of the adduct from the reaction of guanosine and glycidaldehyde at pH 10,[28,29] we have performed extensive delayed decoupling and NOE experiments which unequivocally established the correct structure as the regioisomer **17**. However, adduct **17** was not isolated when the reaction was conducted under neutral or acidic conditions. 2,3-Epoxybutanal also failed to provide adducts with guanosine in neutral or acidic media. In basic media, however, 1,*N*$^6$-ethenoguanosine (**18**) was isolated in 41% yield. The formation of adducts **17** and **18** can best be appreciated by considering the regiochemistry and a plausible mechanism for the transformations. Attack of the exocyclic amino group on the carbonyl carbon of the epoxy aldehyde would generate intermediate **15**. Ring opening involving the internal carbon of the epoxide by the N-1 anion of guanosine (p$K_a$ = 9.2), under the basic conditions (pH 10), would result in the formation of **16**. Intermediate **16** can eliminate water to form **17** or it can undergo a double elimination through a cyclic transition state as shown to give **18**. Both pathways are observed depending on the structure of the epoxide (Scheme IV). The synthesis of 1,*N*$^6$-ethenoguanosine (**18**) has been reported previously[30] from the reaction of chloroacetaldehyde

**Scheme IV**



and guanosine in about an 8% yield. The procedure using the epoxybutanal is a more efficient way to produce this compound.

Adenine bases have also been found to be modified by functionalized epoxides. For example, glycidaldehyde and 2,3-epoxybutanal convert adenosine to the ethenoadenosine derivatives **20** and **21** in low yields under acidic conditions. Unambiguous proof of the structure of these



**20**, R=H; $R_1$=D-ribofuranosyl
**21**, R=CH$_3$; $R_1$=D-ribofuranosyl
**22**, R=H; $R_1$=C$_2$H$_5$

adducts came from an alternate synthesis of the ethyl analogue **22** from the reduction of 9-ethyl-1,*N*$^6$-ethenoadenine-10-carboxaldehyde prepared by the reaction of 9-ethyladenine and bromomalonaldehyde.[22]

In summary, functionalized epoxides are ubiquitous in nature, but few studies have been reported on the reactivity of such epoxides with nucleic acid bases. We have found that epoxy carbonyl compounds are able to modify

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both purine and pyrimidine bases to give extended ring systems some of which have been rarely encountered.

## Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a JEOL FX90Q pulse Fourier transform NMR spectrometer or on a Bruker WM 360 pulse Fourier transform NMR spectrometer. Tetramethylsilane was the internal reference. Mass spectra at 30 eV were obtained on a Hewlett-Packard 5985 GC/MS system. The ultraviolet data were taken with a Cary Model 219 ultraviolet–visible spectrophotometer. HPLC separations were done at low pressure utilizing a column of Amberlite XAD-4 resin (230–400 mesh). Preparative layer chromatography was done on E. Merck silica gel PF-254.

**Preparation of 1-Methylcytosine (3).** This compound was prepared by the method of Hosmane and Leonard[31] in a 63% yield.

**Preparation of Epoxy Carbonyl Compounds.** The epoxy carbonyl compounds were prepared by the method of White and co-workers.[24] Glycidaldehyde (1) was obtained in a 22% yield: bp 48–52 °C (75 torr) [lit.[32] bp 57–58 °C (100 torr)]; IR (neat) 1700 (C=O), 1200, 970, and 820 cm$^{-1}$ (epoxide ring); $^{1}$H NMR (CDCl$_3$) $\delta$ 3.43–2.05 (m, 3 H), 8.96 (d, 1 H, $J$ = 6.4 Hz). 2,3-Epoxybutanal (2) was obtained in a 61% yield: bp 51–56 °C (40 torr); IR (neat) 1730 (C=O), 1270 and 970 cm$^{-1}$ (epoxide ring); $^{1}$H NMR (CDCl$_3$) $\delta$ 1.50 (d, 3 H, $J$ = 5.1 Hz), 3.08–3.14 (m, 1 H), 3.29–3.35 (m, 1 H), 9.01 (d, 1 H, $J$ = 5.9 Hz). 3,4-Epoxybutanone (8) was obtained in a 60% yield: bp 66–80 °C (60 torr) [lit.[24] bp 60–80 °C (60 torr)]; IR (neat 1700 (C=O), 1240 and 825 cm$^{-1}$ (epoxide ring); $^{1}$H NMR (CDCl$_3$) $\delta$ 2.06 (s, 3 H), 2.83–3.07 (m, 2 H), 3.36–3.42 (m, 1 H).

**General Procedure for the Reaction of Epoxy Carbonyl Compounds with Nucleosides or Alkylated Nucleic Acid Bases.** One of three buffers was employed: (i) basic medium (pH 10), NaOH/NaHCO$_3$; (ii) acidic medium (pH 5), acetic acid/sodium acetate; (iii) neutral medium (pH 7.4), potassium phosphate.

The nucleoside or alkylated base was dissolved in the appropriate buffer and stirred to allow dissolution. The epoxy carbonyl compound was added, and the pH was adjusted accordingly. The reaction mixture was then stoppered and allowed to stir at room temperature. In studies with epoxybutanone, the reaction mixtures were heated to facilitate conversions. In all cases, when product formation had reached a maximum as evidenced by UV spectroscopy or TLC, the reaction mixtures were neutralized and the solvent removed in vacuo. The methylated adducts were purified on silica gel preparative layer plates using 5–20% MeOH/CHCl$_3$ as the eluent, while the ribosyl adducts were separated by HPLC on a column of Amberlite XAD-4 resin (230–400 mesh) using 2–20% EtOH/H$_2$O as the eluent.

**Reaction of 1-Methylcytosine (3) with Glycidaldehyde (1) at pH 10.** 1-Methylcytosine (3) (0.138 g, 1.1 mmol) and glycidaldehyde (1) (0.108 g, 1.5 mmol) were allowed to react for 6 h at room temperature at pH 10. Separation yielded 0.074 g (0.37 mmol, 34%) of 5 in the band with $R_f$ 0.30. Product 5 crystallized from MeOH/ether as white crystals: mp 159–162 °C; UV (H$_2$O) $\lambda_{\max}$ 223 ($\epsilon$ 1.04 × 10$^4$), 286 nm (9.7 × 10$^3$); mass spectrum, $m/z$ (relative intensity) 197 (M$^+$, 5.1), 179 (M$^+$ – H$_2$O, 74.0), 162 (M$^+$ – H$_2$O – OH, 100), 150 (94.0); $^{1}$H NMR (Me$_2$SO-$d_6$) $\delta$ 3.17 (s, 3 H), 3.62 (m, 2 H), 3.72 (m, 1 H), 4.83 (brs, 1 H), 5.27 (d, 1 H, $J$ = 2.7 Hz), 5.70 (brs, 1 H), 5.65 (d, 1 H, $J$ = 7.8 Hz), 7.26 (d, 1 H, $J$ = 7.8 Hz); $^{13}$C NMR (Me$_2$SO-$d_6$) $\delta$ 35.0, 58.4, 65.4, 90.7, 94.8, 143.5, 148.7, 155.1.

Anal. Calcd for C$_8$H$_{11}$N$_3$O$_3$·$^1/_2$H$_2$O: C, 46.60; H, 5.87; N, 20.38. Found: C, 46.47; H, 5.53; N, 20.87.

**Reaction of Cytidine (4) with Glycidaldehyde (1) at pH 10.** Cytidine (4) (0.244 g, 1.0 mmol) and glycidaldehyde (1) (0.097 g, 1.3 mmol) were allowed to react for 6 h at room temperature in basic medium. After purification the product was crystallized from MeOH, giving 0.130 g (0.41 mmol, 41%) of 6 as white needles: mp 111–113 °C; UV (H$_2$O) $\lambda_{\max}$ 223 nm ($\epsilon$ 9.4 × 10$^3$), 279 (7.9 × 10$^3$); mass spectrum, $m/z$ (relative intensity) 297 (M$^+$ – H$_2$O, 3.9), 165 ("base" + H – H$_2$O, 75.5), 135 (100.0); $^{1}$H NMR (Me$_2$SO-$d_6$) $\delta$ 3.51–3.67 (m, 4 H), 3.75–3.81 (m, 2 H), 3.94–4.04 (m, 2 H), 4.84–5.19 (m, 4 H), 5.29 (br s, 1 H), 5.74–5.81 (m, 3 H), 7.50 (d, 1 H, $J$ = 8.1 Hz); $^{13}$C NMR (Me$_2$SO-$d_6$) $\delta$ 58.2, 61.1, 65.1, 70.0, 72.9, 84.6, 87.2, 90.9, 96.4, 137.2, 148.1, 153.7.

Anal. Calcd for C$_{12}$H$_{17}$N$_3$O$_7$: C, 45.71; H, 5.43; N, 13.33. Found: C, 45.71; H, 5.53; N, 13.59.

**Reaction of Cytidine (4) with Glycidaldehyde (1) at pH 7.4.** Compound 6 was obtained in 38% yield from this reaction after 3.5 h.

**Reaction of Cytidine (4) with Glycidaldehyde (1) at pH 5.** A 40% yield of 6 was obtained from the reaction of cytidine (4) with glycidaldehyde (1) at pH 5 for 6 h.

**Reaction of Cytidine (4) with 2,3-Epoxybutanal (2) at pH 10.** Cytidine (4) (0.277 g, 1.1 mmol) was stirred with 2,3-epoxybutanal (2) (0.123 g, 1.4 mmol) at room temperature for 18 h in basic medium. Purification yielded 0.110 g (0.33 mmol, 30%) of 7 as hygroscopic white crystals: mp 119–121 °C; UV (H$_2$O) $\lambda_{\max}$ 222 nm ($\epsilon$ 7.8 × 10$^3$), 280 (6.3 × 10$^3$); mass spectrum, $m/z$ (relative intensity) 179 ("base" – OH, 12.1), 164 (19.4), 135 (49.0); $^{1}$H NMR (Me$_2$SO-$d_6$) $\delta$ 1.10 (d, 3 H, $J$ = 6.5 Hz), 3.44–3.60 (m, 3 H), 3.80 (m, 1 H), 4.04–4.32 (m, 2 H), 4.32–4.34 (m, 1 H), 5.03–5.21 (m, 3 H), 5.36 (brs, 1 H), 5.75–5.90 (m, 3 H), 7.50 (d, 1 H, $J$ = 8.1 Hz); $^{13}$C NMR (Me$_2$SO-$d_6$) $\delta$ 18.6, 61.3, 62.4, 69.3, 70.2, 73.3, 84.7, 87.1, 88.7, 96.6, 137.2, 148.4, 153.8.

Anal. Calcd for C$_{13}$H$_{19}$N$_3$O$_7$·$^1/_2$H$_2$O: C, 46.15; H, 5.96; N, 12.42. Found: C, 45.79; H, 6.39; N, 12.17.

**Reaction of Cytidine (4) with 2,3-Epoxybutanal (2) at pH 5.** A 36% yield of adduct 7 was obtained when cytidine (4) was allowed to react with 2,3-epoxybutanal (2) for 16 h in acidic medium.

**Reaction of Cytidine (4) with 2,3-Epoxybutanal (2) at pH 7.4.** The reaction of cytidine (4) with 2,3-epoxybutanal (2) provided a 45% yield of 7 when the reaction was allowed to proceed for 23 h at pH 7.4.

**Reaction of Cytidine (4) with 3,4-Epoxybutanone (8) at pH 10.** Cytidine (4) (0.245 g, 1.0 mmol) and 3,4-epoxybutanone (8) (0.122 g, 1.4 mmol) were allowed to react at 50 °C for 48 h in basic medium. Separation of reaction mixture yielded two products. The first product was identified as 2-methyl-3-(hydroxymethyl)-6-$\beta$-D-ribofuranosylimidazo[1,2-*c*]pyrimidin-5-(6*H*)-one (9) and was isolated in 5% yield, 7% conversion (0.015 g, 0.05 mmol) as white needles after crystallization from MeOH/ether: mp 189–191 °C; UV (H$_2$O) $\lambda_{\max}$ 283 nm ($\epsilon$ 1.2 × 10$^4$); mass spectrum, $m/z$ (relative intensity) 311 (M$^+$, 7.0), 179 ("base" + H, 100.0), 162 (57.6); $^{1}$H NMR (Me$_2$SO-$d_6$) $\delta$ 2.23 (s, 3 H), 3.70–5.50 (m, 11 H), 6.05 (d, 1 H, $J$ = 4.4 Hz), 6.57 (d, 1 H, $J$ = 7.8 Hz), 7.71 (d, 1 H, $J$ = 7.8 Hz).

Anal. Calcd for C$_{13}$H$_{17}$N$_3$O$_6$·$^1/_2$H$_2$O: C, 48.74; H, 5.66; N, 13.12. Found: C, 48.72; H, 5.12; N, 12.67.

The second product was identified as 2-methyl-6-$\beta$-D-ribofuranosylimidazo[1,2-c]pyrimidin-5(6*H*)-one (10) and was isolated in a 7% yield, 10% conversion (0.019 g, 0.07 mmol) as off-white blunt crystals: mp 110–112 °C; UV (H$_2$O) $\lambda_{\max}$ 278 nm ($\epsilon$ 8.9 × 10$^3$); mass spectrum, $m/z$ (relative intensity) 281 (M$^+$, 2.1), 150 (15.9), 149 ("base" + H, 100.0); $^{1}$H NMR (Me$_2$SO-$d_6$) $\delta$ 2.24 (s, 3 H), 3.70–5.50 (m, 8 H) 6.05 (d, 1 H, $J$ = 4.9 Hz), 6.62 (d, 1 H, $J$ = 7.8 Hz), 7.51 (s, 1 H), 7.72 (d, 1 H, $J$ = 7.8 Hz).

Anal. Calcd for C$_{12}$H$_{15}$N$_3$O$_5$: C, 51.24; H, 5.38; N, 14.94. Found: C, 50.93; H, 5.56; N, 14.99.

**Reaction of Cytidine (4) with 3,4-Epoxybutanone (8) at pH 5.** Compounds 9 and 10 were obtained in 9% yield (12% conversion) and 4% yield (6% conversion), respectively, when 4 was allowed to react with 8 in acidic medium at 50 °C for 48 h.

**Reaction of Cytidine (4) with 3,4-Epoxybutanone (8) at pH 7.4.** Cytidine (4) was allowed to react with epoxybutanone (8) at 50 °C for 20 h at pH 7.4. Adducts 9 and 10 were produced in 8% and 2% yields, respectively.

**Reaction of Guanosine (14) with 2,3-Epoxybutanal (2).** Guanosine (14) (0.735 g, 2.6 mmol) was added to 180 mL of H$_2$O, and the pH was adjusted to 10. Epoxybutanal (2) (0.300 g, 3.5 mmol) was added, and the reaction was stirred at room tem-

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perature for 3.5 h. The reaction mixture was neutralized and cooled overnight at 5 °C to allow precipitation of product. The product was collected by vacuum filtration and then lyophilized to yield 0.330 g (1.1 mmol, 41%) of ethenoguanosine (**18**). The spectroscopic data of **18** were consistent with the literature values.[30]

**Reaction of Guanosine (14) with Glycidaldehyde (1).** Guanosine (1.942 g, 6.9 mmol) was placed in $H_2O$ (200 mL), and the solution was basified to pH 10 with warming to aid dissolution. Glycidaldehyde (1) 0.57 g (7.9 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized and cooled to allow precipitation of white crystals. This material was suspended in water and lyophilized to give 1.364 g (4.0 mmol) of 5,9-dihydro-7-(hydroxymethyl)-9-oxo-3-$\beta$-D-ribofuranosyl-3*H*-imidazo[1,2-*a*]purine (**17**) as white crystals in 59% yield: mp >300 °C dec; UV (0.1 N HCl) $\lambda_{max}$ 300 nm ($\epsilon$ 7.7 × $10^3$), 276 (9.9 × $10^3$), 226 (2.54 × $10^4$), (pH 7 buffer) 285 (9.9 × $10^3$), 228 (2.63 × $10^4$), (0.1N NaOH) 310 (7.4 × $10^3$), 285 (6.6 × $10^3$), 238 (2.85 × $10^4$); mass spectrum, *m*/*z* (relative intensity) 207 (2.6), 189 (75.9, "base" + 2 H − OH), 188 (36.5), 133 (7.1); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 3.53–3.65 (m, 2 H), 3.91 (m, 1 H), 4.12 (m, 1 H), 4.45 (m, 1 H), 4.84 (d, 2 H, *J* = 6.0 Hz), 4.98 (t, 1 H, *J* = 6.0 Hz), 5.05 (t, 1 H, *J* = 5.3 Hz), 5.16 (d, 1 H, *J* = 4.7 Hz), 5.42 (d, 1 H, *J* = 6.0 Hz), 5.81 (d, 1 H, *J* = 5.9 Hz), 7.23 (s, 1 H), 8.14 (s, 1 H), 12.36 (s, 1 H); $^{13}$C NMR ($Me_2SO$-$d_6$) $\delta$ 55.0, 61.2, 70.2, 73.6, 85.1, 86.8, 113.7, 115.9, 124.6, 137.3, 146.7, 150.2, 153.6.

Anal. Calcd for $C_{13}H_{15}N_5O_6{\cdot}H_2O$: C, 43.95; H, 4.82; N, 19.71. Found: C, 44.37; H, 4.48; N, 20.20.

**Reaction of Adenosine (19) with Glycidaldehyde (1) at pH 5.** Adenosine (**19**) (0.280 g, 1.0 mmol) was allowed to react with glycidaldehyde (1) (0.122 g, 1.7 mmol) for 36 h at pH 5. Separation yielded 0.090 g (0.3 mmol, 30%) of 3-$\beta$-D-ribofuranosyl-7-(hydroxymethyl)-3*H*-imidazo[2,1-*i*]purine (**20**) as blunt transparent crystals: mp 214–216 °C; UV ($H_2O$) $\lambda_{max}$ 231 nm ($\epsilon$ 2.9 × $10^4$), 268 (6.7 × $10^3$), 279 (6.4 × $10^3$), 300 (3.0 × $10^3$); mass spectrum, *m*/*z* (relative intensity) 321 ($M^+$, 4.2), 189 ("base" + H, 100.0), 188 ("base", 22.9), 172 ("base" + H − OH, 91.8), 135 (50.7), 133 (14.7); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 3.67 (m 2 H), 4.00 (m, 1 H), 4.22 (t, 1 H, *J* = 4.4 Hz), 4.61 (t, 1 H, *J* = 4.9 Hz), 4.91 (s, 2 H), 5.1–5.5 (brs, 4 H), 6.08 (d, 1 H, *J* = 5.4 Hz), 7.48 (s, 1 H), 8.59 (s, 1 H), 9.15 (s, 1 H).

Anal. Calcd for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.70; H, 4.83; N, 21.69.

**Reaction of Adenosine (19) with 2,3-Epoxybutanal (2) at pH 5.** Adenosine (**19**) (0.282 g, 1.1 mmol) was stirred for 48 h with 0.094 g (1.1 mmol) of 2,3-epoxybutanal (**2**). Separation yielded 0.022 g (0.07 mmol, 6% yield, 7% conversion based on unreacted adenosine) of **21** as fluffy white crystals: mp 219–221 °C; UV ($H_2O$) $\lambda_{max}$ 231 nm ($\epsilon$ 3.00 × $10^4$), 268 (5.9 × $10^3$), 279 (5.9 × $10^3$), 300 (sh, 3.1 × $10^3$); mass spectrum, *m*/*z* (relative intensity) 203 ("base" + H, 14.2), 188 ("base" + H − $CH_3$, 25.0), 159 ("base" − $C_2H_4O$, 100.0); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.64 (d, 3 H, *J* = 6.4 Hz), 3.68 (m, 2 H), 4.00 (d, 1 H, *J* = 3.4 Hz), 4.19 (m, 1 H), 4.60 (m, 1 H), 5.24–5.05 (m, 3 H), 5.51 (m, 2 H), 6.07 (d, 1 H, *J* = 5.9 Hz), 7.44 (s, 1 H), 8.57 (s, 1 H), 9.19 (s, 1 H).

Anal. Calcd for $C_{14}H_{17}N_5O_5{\cdot}H_2O$: C, 47.59; H, 5.42; N, 19.82. Found: C, 48.02; H, 5.43; N, 19.25.

**Preparation of 9-Ethyl-1,$N^6$-ethenoadenine-10-carboxaldehyde (23).** This compound was prepared as described previously[22] and was obtained in 38% yield as white crystals: mp 223–225 °C; UV (95% ethanol) $\lambda_{max}$ 230 nm ($\epsilon$ 2.06 × $10^4$), 328 (1.51 × $10^4$), 339 (1.50 × $10^4$); mass spectrum, *m*/*z* (relative intensity) 216 ($M^+$ + 1, 12.2), 215 ($M^+$, 100.0), 187 ($M^+$ − CO, 34.4), 186 ($M^+$ − $C_2H_5$, 34.4); $^1$H NMR ($CDCl_3$) $\delta$ 1.62 (t, 3 H), 4.44 (q, 2 H), 8.13 (s, 1 H), 8.37 (s, 1 H), 10.02 (s, 1 H), 10.08 (s, 1 H).

**Reduction of 9-Ethyl-1,$N^6$-ethenoadenine-10-carboxaldehyde (23).** To a solution of 0.042 g (1.1 mmol) of $NaBH_4$ in 20 mL of cold ethanol was added 0.052 g (0.24 mmol) of 9-ethyl-1,$N^6$-ethenoadenine-10-carboxaldehyde (**23**). The reaction was stirred for $^1/_2$ h at room temperature and then the solvent removed in vacuo. Separation on silica gel preparative layer plates with 13% MeOH/$CHCl_3$ yielded 0.023 g (0.11 mmol, 46%) of 3-ethyl-7-(hydroxymethyl)-3*H*-imidazo[2,1-*i*]purine (**22**) as off-white crystals: mp 195 °C dec; UV ($H_2O$) $\lambda_{max}$ 233 nm ($\epsilon$ 2.7 × $10^4$), 269 (6.5 × $10^3$), 279 (9.2 × $10^3$), 300 (3.9 × $10^3$); mass spectrum *m*/*z* (relative intensity) 218 ($M^+$ + 1, 6.7), 217 ($M^+$, 50.4), 200 ($M^+$ − OH, 100.0), 172 (39.4); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.48 (t, 3 H, *J* = 7.3 Hz), 4.35 (q, 2 H, *J* = 7.3 Hz), 4.91 (m, 2 H), 5.34 (m, 1 H), 7.44 (s, 1 H), 8.33 (s, 1 H), 9.11 (s, 1 H).

Anal. Calcd for $C_{10}H_{11}N_5O{\cdot}H_2O$: C, 51.05; H, 5.57; N, 29.77. Found: C, 51.22; H, 5.70; N, 29.13.

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# Exserohilone: A Novel Phytotoxin Produced by *Exserohilum holmii*

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A novel phytotoxin, exserohilone (**1**), was isolated from the culture broth of *Exserohilum holmii*, a pathogenic fungus of the weedy plant *Dactyloctenium aegyptium*. The structure of exserohilone (**1**) was elucidated by X-ray diffraction analysis of the bis(*p*-bromobenzoate) derivative **3**. 9,10-Dihydroexserohilone (**2**) was also isolated, and its structure was determined by spectral methods.

Bacterial and fungal pathogens of plants often produce disease symptoms by elaborating phytotoxins in the host.[3] There are relatively few studies on phytotoxins affecting weedy plants, but such compounds could be useful herbicides,[4] or serve as models for new herbicides.[3] *Exserohilum holmii* is a fungal pathogen on *Dactyloctenium aegyptium* (crowfoot grass) which is a serious grasseous

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