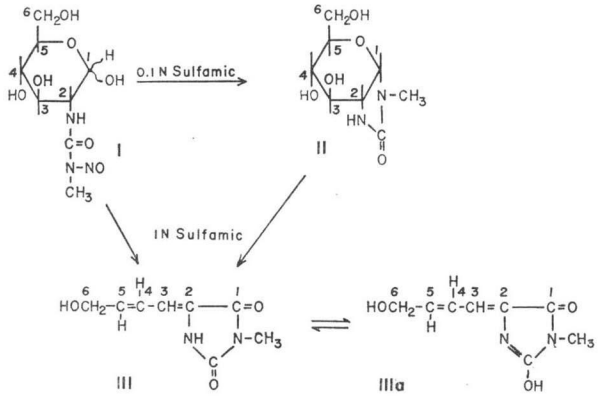
A HYDANTOIN DERIVED FROM THE GLUCOSAMINE-RELATED ANTIBIOTIC, STREPTOZOTOCIN

Sir:

Streptozotocin[1~4] (Fig. 1, **I**), an antibiotic produced by *Streptomyces achromogenes*, is a broad spectrum antibacterial agent and also has antitumor activity in both *in vitro* and *in vivo* systems. The structure of streptozotocin and its synthesis have been described in previous communications[5,6].

We have reported that reaction of streptozotocin with 0.1 N aqueous sulfamic acid yielded **II** (Fig. 1); 1 mole of N_2/mole of **I** is evolved during the reaction[5]. Further studies showed that the products of the reaction of the antibiotic with sulfamic acid depend on the concentration of the acid. Treatment of streptozotocin with 1 N aqueous sulfamic acid resulted in the production of a new compound, assigned structure **III** (Fig. 1). The present paper presents evidence supporting the assignment of this structure.

Fig. 1.



III is produced when a solution of streptozotocin (1 g) in 1 N aqueous sulfamic acid (25 ml) is allowed to stand at room temperature for 24 hours. One mole of N_2 per mole of streptozotocin is evolved during the reaction. **III**, isolated as orange-colored crystals (prisms), has the molecular formula $C_8H_{10}N_2O_3$*, m.p. 215~217°C, $[\alpha]_D^{25}0°$ (*c* 1, methanol). The molecular weight determined by mass spectrometry is 182 (calcd. 182). **III** is also produced when **II** ($C_8H_{14}N_2O_5$) is treated wish 1 N aqueous sulfamic acid, as described above. Carbon dioxide is not evolved during this reaction. This suggests that streptozotocin is transformed to **II** which then yields **III** by loss of 2 molecules of water.

Potentiometric titration of **III** [in methanol-water (60:40, v/v)] suggests the presence of a weakly acidic group, pKa′ 10.6. The UV spectrum (in methanol) showed maxima at 245 (*a* 50) and 310(*a* 178) and a shoulder at 320 nm. The UV maxima shift to 250 and 345 nm in alkaline methanol. The titration and UV data are in agreement with the presence of an enol group as shown in **IIIa** (Fig. 1). This is also supported by NMR data discussed later.

The IR spectrum (Nujol mull) showed characteristic absorptions at 1765, 1700 and 1655 cm^{-1} assigned[7,8] to the hydantoin system $=\overset{2}{C}-\overset{1}{C}=O$ present in **III**.

In addition, the IR spectrum showed absorptions at 3440 (O–H stretch), 3200 (N–H stretch), 1030 (C–O stretch) and 980 cm^{-1} (CH deformation of a *trans* –CH=CH– system).

The NMR spectrum of **III** showed the presence of ten hydrogens, two being exchangeable. A singlet at δ 3.08 (3H) is assigned to the $-N-CH_3$ group. The fragment **IV** (6H) (Figure 2) containing the C-4, C-5 *trans*-system was established by spin decoupling.

Finally, a broad absorption at δ 10.20 (1H) is assigned to the presence of an enolic hydrogen, as shown in **IIIa**, indicating that the "enol" form (**IIIa**) predominates in solution while the "keto" form (**III**) is present in the crystalline material, as shown by IR data.

The IR and NMR data, combined with the observation that **III** results from **II** by loss of $2H_2O$, suggests the presence of fragments **IV** and **V** (Fig. 3) in the molecule of **III**.

Combination of **IV** and **V** in the only possible way establishes the hydantoin **III** as the

* Analytical values for the compounds described in this paper are consistent with the indicated formulas.

Fig. 2.

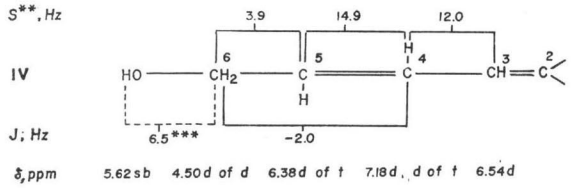


Fig. 3.

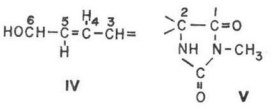
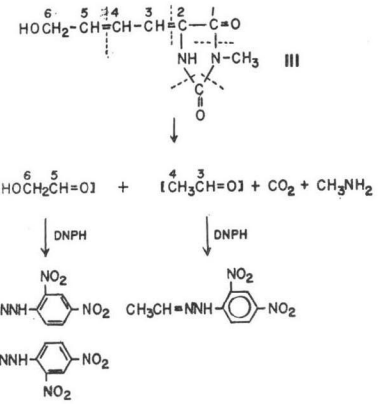


Fig. 4.



structure of the crystalline material obtained by degradation of streptozotocin by 1 N sulfamic acid.

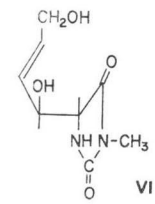
Additional evidence for structure **III** was obtained by ^{13}C NMR spectroscopy****. The proton noise decoupled ^{13}C NMR spectrum of **III** showed eight carbon resonances. The off-resonance decoupled spectrum showed a quartet at δ 24.1 assigned to the $N-CH_3$ carbon, a triplet at δ 61.2 assigned to C-6, three singlets, and three doublets. The singlets at δ 163.7 and 154.3 have been assigned[10] to $-\overset{|}{C}=O$ and $N=\overset{|}{C}-N$ of **IIIa**, respectively. The third singlet at δ 127.3 then must be assigned to C-2. Selective proton irradiation demonstrated that the doublet at δ 141.6 is due to C-4. The remaining doublets at δ 122.5 and 110.0 are assigned[11] to C-5 and C-3 respectively.

Furthermore, reflux of **III** with 1 N aqueous sodium hydroxide yielded one mole of carbon dioxide and one mole of methylamine isolated as the hydrochloride salt. In addition glycolaldehyde and acetaldehyde were formed during the reaction and were isolated as the crystalline glyoxal *bis*-(dinitrophenylhydrazone) and acetaldehyde dinitrophenylhydrazone derivatives (Fig. 4).

Glycolaldehyde originates from C-5 and C-6 and acetaldehyde from C-3 and C-4 of **III**. The formation of these two compounds can be explained by a retro-aldol reaction. Glycine is expected to be formed as one of the products of the reaction originating from C-1 and C-2. However, no attempt was made to isolate this material.

It appears that conversion of **I** to **III** proceeds through **II** which can yield **VI** (Fig. 5) by protonation at the 4-hydroxyl followed by dehydration. Subsequently, **VI** can lose an additional mole of water to yield **III**. The insolubility of **III** in 1 N sulfamic acid could be the driving force for the reactions leading to its formation.

Fig. 5.



The hydantoin **III** is inactive *in vitro* against a variety of Gram-positive or Gram-negative organisms sensitive to streptozotocin.

Acknowledgements

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* The proton NMR spectra were observed in pyridine-d_5 on a Varian XL-100-15 spectrometer operating at 100 MHz, using internal tetramethylsilane as reference.

** (Fig. 2) Line separation[9].

*** (Fig. 2) J_{OH,CH_2}, 6.5 Hz can be observed in DMSO-d_6 as the solvent.

**** ^{13}C NMR spectra were observed in DMSO-d_6 on a Varian XL-100-15 spectrometer operating in the C. W. mode at 25.2 MHz. Tetramethylsilane was used as an internal reference.

helpful discussions.

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