Anthramycin exhibited *in vitro* cytotoxic activity and *in vivo* antitumor activity against transplantable tumors in mice.[7] It also showed substantial *in vitro* antimicrobial activity, particularly against Gram-positive bacteria and, to a lesser extent, against Gram-negative organisms and fungi. The direct comparison of anthramycin and anthramycin methyl ether showed no significant differences in biological activity. Preliminary clinical trials indicate that the antibiotic possesses appreciable carcinostatic activity in a variety of malignant human tumors.[8,9]

(7) (a) G. Zbinden in "Proceedings of the International Symposium on the Chemotherapy of Cancer," Pl. A. Plattner, Ed., Elsevier Publishing Co., Amsterdam 1964, pp. 303–310; (b) E. Grunberg, H. N. Prince, E. Titsworth, G. Beskid, and M. D. Tendler, to be published.

(8) Initial clinical trials were conducted with various preparations of the crude antibiotic; when crystalline anthramycin methyl ether, also designated as Roche 5-9000/15, became available, it was used exclusively.

(9) S. Korman and M. D. Tendler, to be published.

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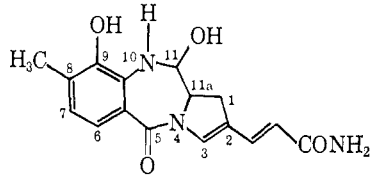
Received October 27, 1965

The Structure of Anthramycin

Sir:

We wish to present evidence which permits the assignment of structure **1** (5,10,11,11a-tetrahydro-9,11-dihydroxy-8-methyl-5-oxo-1H-pyrrolo[2,1-*c*][1,4]benzodiazepine-2-acrylamide) to anthramycin.[1]



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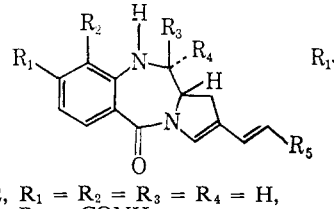
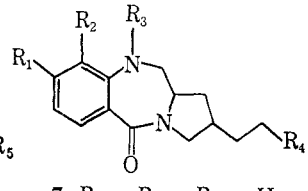
The structure elucidation of this antibiotic was facilitated by the investigation of another fermentation product, designated as "yellow pigment,"[1] which possesses the closely related, but less complex structure **2**.[2]





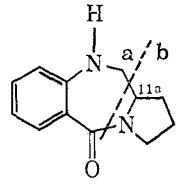
2, $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = CONH_2$
3, $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = CN$
4, $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$, $R_4 = OCH_3$, $R_5 = CONH_2$
5, $R_1 = CH_3$, $R_2 = OH$, $R_3 = OCH_3$, $R_4 = H$, $R_5 = CONH_2$
6, $R_1 = CH_3$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = OCH_3$, $R_5 = CONH_2$
7, $R_1 = R_2 = R_3 = H$, $R_4 = CONH_2$
8, $R_1 = R_2 = R_3 = H$, $R_4 = COOCH_3$
9, $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$, $R_4 = CONH_2$
10, $R_1 = CH_3$, $R_2 = OCOCH_3$, $R_3 = COCH_3$, $R_4 = CONH_2$
11, $R_1 = CH_3$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = CONH_2$

(1) W. Leimgruber, V. Stefanović, F. Schenker, A. Karr, and J. Berger, *J. Am. Chem. Soc.*, **87**, 5731 (1965).

(2) We have not assigned a generic name to the "yellow pigment" since it may be regarded as an anthramycin derivative, namely, desdihydroxydesmethylanthramycin.

The distinctive features of the n.m.r. spectrum[3] of the "yellow pigment" could be interpreted in terms of structure **2** as follows: two doublets at δ 5.81 and 7.37 (J = 15 c.p.s.) for the *trans*-olefinic protons, a singlet at δ 7.47 for C-3–H, a doublet of doublets at δ 7.80 for C-6–H, and a broad multiplet at $\delta \sim 4.2$ for C-11a–H. The presence of a conjugated primary amide was established by the conversion of **2** to the conjugated nitrile **3**[4] [m.p. 233–234°; ν_{max}^{KBr} 2210 cm.$^{-1}$ (intense); δ 5.36 and 7.52 (two 1 H doublets, J = 16 c.p.s.)], effected by the action of phosphorus pentoxide in boiling quinoline. Hydrolysis of **3** with concentrated hydrochloric acid reconverted it to **2**. Catalytic reduction (Pd–C) of **2** resulted in the formation of a mixture of two epimeric (at C-2) tetrahydro derivatives of structure **7**. The presence of a nonconjugated, primary amide was substantiated by the conversion of **7** (ν_{max}^{KBr} 1670 cm.$^{-1}$) to the corresponding saturated ester **8**[5] ($\nu_{max}^{CHCl_3}$ 1735 cm.$^{-1}$), obtained by hydrolysis (6 *N* HCl) and subsequent esterification with diazomethane. The presence of an anthranilamide moiety in compounds **7** and **8** was deduced from their n.m.r. (aromatic region) and ultraviolet spectra (*cf.* Table I) which were very similar to those of anthranilamide.

The evidence presented above accounts for all the hetero atoms and functional groups of the "yellow pigment" and demands a tricyclic expression for its structure. The n.m.r. and ultraviolet data particularly favored two formulas: One is represented by expression **2** which contains the pyrrolobenzodiazepine nucleus **12**; the corresponding alternative possesses the pyridoquinazoline skeleton **13**. The spectroscopic properties of the parent tricyclic compounds **12** and **13**[6] revealed the striking similarity between **12** and **8** (Table I). The presence of a pyrrolobenzodiazepine skeleton in the "yellow pigment" and its transformation products was further supported by their mass spectra, all of which exhibited two intense peaks (**3**, *m/e* 133, 119; **8**, *m/e* 133, 156; **12**, *m/e* 133, 70) which could be attributed to fragments of the type a and b (always observed in



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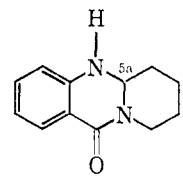

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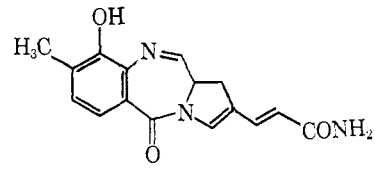

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(3) The n.m.r. spectra were recorded in DMSO-d_6 solution (unless otherwise stated), and the chemical shifts are reported in p.p.m. (δ) downfield from an internal tetramethylsilane reference.

(4) Satisfactory elemental analyses were obtained for all new compounds reported here.

(5) Mixture of epimers.

(6) Compound **12**, m.p. 183–184°, was prepared by lithium aluminum hydride reduction of the dilactam, m.p. 231–232°, obtained from the fusion of proline and isatoic anhydride. Compound **13**, m.p. 135–136°, was prepared from the quinazolinone [E. Späth and N. Platzer, *Ber.*, **68**, 2225 (1935)] by reduction with sodium borohydride in the presence of aluminum chloride.

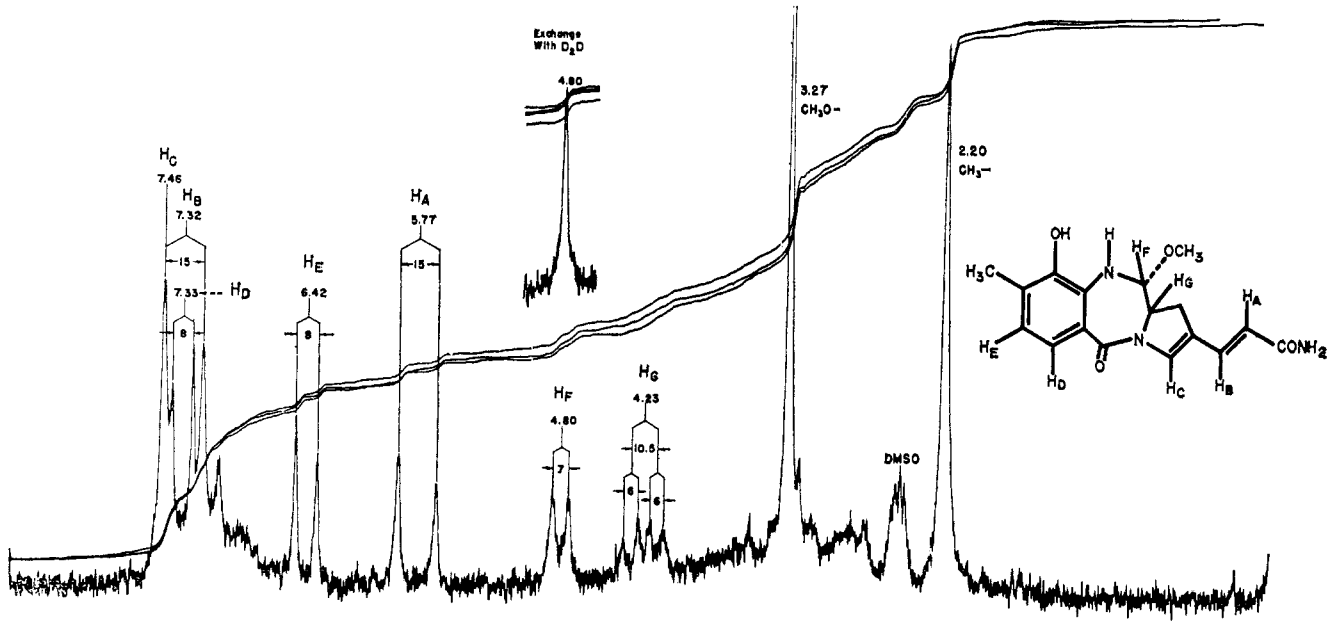

Figure 1. N.m.r. spectrum of anthramycin methyl ether.

protonated form) as illustrated in **12**. More conclusively, the high-resolution mass spectrum[1] of **2** recorded fragment a at *m/e* 133.0514 (Calcd. for C_8H_7NO: 133.0528) and fragment b at *m/e* 137.0702 (Calcd. for $C_7H_9N_2O$: 137.0715). This final evidence secures structure **2** for the "yellow pigment."

diazomethane, afforded methyl 3-methoxy-4-methylanthranilate (m.p. 89–90.5°), identical by comparison with an authentic sample.[8] The relative configuration of the two asymmetric centers in **4** was assigned from its n.m.r. spectrum (Figure 1) which showed a singlet at δ 3.27 for the methoxyl group and, after exchange, a

Table I. Spectroscopic Properties of Compounds **8**, **12**, and **13**

Compd.	$\lambda_{max}^{2\text{-propanol}}$ mμ (ϵ)	$\nu_{max}^{CHCl_3}$ (cm.$^{-1}$)	Chemical shift ($CDCl_3$, δ values)	pK_a (spectroph.)
8	222 (26,800); 257 s (8500) 335 (4800)	3450, 1735, 1620 1600	C-11a–H, 4.2	2.2
12	222 (24,600); 257 s (7800) 335 (4200)	3450, 1620, 1600	C-11a–H, 3.9	2.8
13	229 (32,000); 257 s (4000) 350 (2900)	3420, 1645, 1615	C-5a–H, 4.9	0.1

The comparison of the spectroscopic properties of anthramycin methyl ether[1] and the "yellow pigment" revealed numerous common features. A detailed evaluation of the n.m.r. spectrum (Figure 1) in conjunction with some biogenetic considerations[7] permitted the postulation of structure **4** for this compound, which was fully confirmed by the following evidence: Catalytic reduction (Pd–C) of anthramycin methyl ether (**4**) resulted in the uptake of 3 equiv. of hydrogen and afforded a mixture of two epimeric (at C-2) products of structure **9** [ν_{max}^{KBr} 1670 and 1620 cm.$^{-1}$; $\lambda_{max}^{2\text{-propanol}}$ mμ (ϵ): 229 (21,000), 255 s (6000), and 333 (2600)]. The hydrogenation product **9** gave the diacetate **10**[5] ($\nu_{max}^{CHCl_3}$ 1765 and 1635–1680 cm.$^{-1}$) on acetylation and the ether **11**[5] [δ 3.61 (3 H singlet); *m/e* 177, 141] on treatment with diazomethane. Treatment of **4** with diazomethane gave the phenol ether **6** [m.p. 229°; δ 3.28 and 3.69 (two 3 H singlets)] which, after hydrolysis (6 *N* HCl) and subsequent esterification with

(7) A possible relationship between anthramycin and the actinomycins was envisaged.

singlet at δ 4.80 for the C-11 proton, thus indicating by the absence of coupling between the protons at C-11 and C-11a a dihedral angle of approximately 90°.[9] In contrast, the epimeric (at C-11) anthramycin methyl ether **5** [m.p. 193–195°; $[\alpha]^{25}D$ +262° (*c* 1.00, DMSO)[10]], which could be obtained by treatment of anhydroanthramycin with methanol at room temperature, showed a singlet at δ 3.44 for the methoxyl group and, after exchange, a doublet at δ 4.62 (J = 9 c.p.s.) for the C-11 proton. Inspection of Dreiding models revealed for structure **4** (but not **5**) that the above mentioned dihedral angle was approximately 90° and that the methoxyl group could be shielded by the π-electron system.

These facts establish structure **4** for anthramycin methyl ether. The structures of anthramycin (**1**[11])

(8) W. H. Hanger, W. C. Horwell, and A. W. Johnson, *J. Chem. Soc.*, 496(1958).

(9) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963); H. Conroy, *Advan. Org. Chem.*, **2**, 308 (1960).

(10) Anthramycin methyl ether, **4**, and the epimer **5** mutarotate in methanol solution.

and anhydroanthramycin (**14**) follow from the interconversions which were discussed previously.[1]

Anthramycin (**1**) possesses some structural features found in the actinomycins.[12] A more pronounced relationship is apparent from the comparison of anthramycin with the 3-hydroxy-4-methylanthraniloyl peptides which are intermediates in the postulated biosynthesis of the actinomycins.[13] Since the oxidative dimerization of these intermediates leads to the formation of actinomycins, conceivably anthramycin could be transformed *in vivo* to "actinomycin analogs" which actually may be responsible for the observed antitumor activity.[14]

Acknowledgment. The authors sincerely wish to thank Professor G. Büchi for many stimulating discussions.

(11) The relative and absolute configurations of anthramycin are being investigated.

(12) H. Brockmann, *Fortschr. Chem. Org. Naturstoffe*, **18**, 1 (1960).

(13) H. Brockmann, *Ann. N. Y. Acad. Sci.*, **89**, 323 (1960); H. Weissbach, B. Redfield, V. Beaven, and E. Katz, *Biochem. Biophys. Res. Commun.*, **19**, 524 (1965).

(14) We have observed that anthramycin is easily oxidized by air under physiological conditions to products containing the actinomycin chromophore.

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On the Role of the Nitroxyl Molecule in the Reaction of Hydrogen Atoms with Nitric Oxide

Sir:

The suggestion that HNO is a reactive intermediate in the hydrogen atom–nitric oxide reaction has often been made on the basis of kinetic studies of stable product formation.[1–5] This suggestion has been confirmed by spectroscopic observation and study[6–10] of a red emission from the reaction, which is attributable to excited nitroxyl molecules formed in a termolecular reaction.[6–10] However, nitroxyl is not a stable product of the reaction[1–5] and, hence, must react further. In this regard, Strausz and Gunning[5] have proposed that nitrous oxide and water are formed by the following mechanism.

$$\mathrm{HNO} + \mathrm{HNO} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathrm{HNO})_2 \qquad (1)$$

$$(\mathrm{HNO})_2 + \mathrm{M} \xrightarrow{k_2} \mathrm{N_2O} + \mathrm{H_2O} + \mathrm{M} \qquad (2)$$

We wish to report direct evidence of the correctness of this proposal[5] and, in addition, an approximate lower limit to k_1.

(1) P. Harteck, *Ber.*, **66**, 423 (1933).
(2) H. A. Taylor and C. Tanford, *J. Chem. Phys.*, **12**, 47 (1944).
(3) A. Serewicz and W. A. Noyes, Jr., *J. Phys. Chem.*, **63**, 843 (1959).
(4) M. Z. Hoffman and R. B. Bernstein, *ibid.*, **64**, 1753 (1960).
(5) O. P. Strausz and H. E. Gunning, *Trans. Faraday Soc.*, **60**, 347 (1964).
(6) J. K. Cashion and J. C. Polanyi, *J. Chem. Phys.*, **30**, 317 (1959).
(7) M. A. A. Clyne and B. A. Thrush, *Trans. Faraday Soc.*, **57**, 1305 (1961).
(8) M. A. A. Clyne and B. A. Thrush, *Discussions Faraday Soc.*, **33**, 139 (1962).
(9) M. J. Y. Clement and D. A. Ramsey, *Can. J. Phys.*, **39**, 205 (1961).
(10) J. L. Bancroft, J. M. Hollis, and D. A. Ramsey, *ibid.*, **40**, 322 (1962).

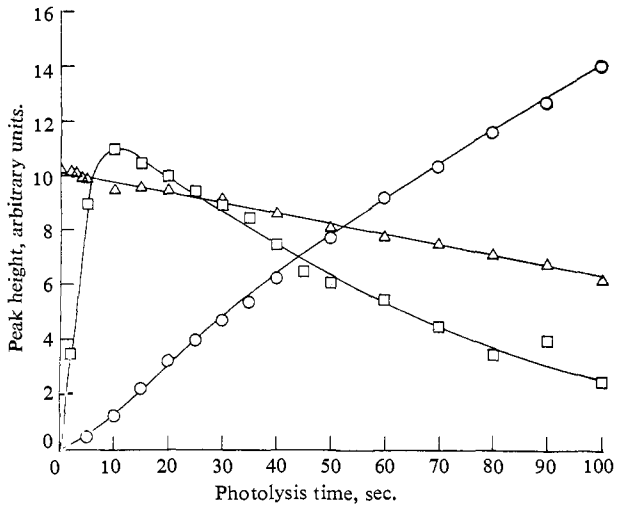

Figure 1. Formation of DNO and N_2O and depletion of NO during photolysis: △, *m/e* 30 (NO^+); □, *m/e* 32 (DNO^+); ○, *m/e* 44 (N_2O^+).

We have studied the D + NO and H + NO reactions by mercury photosensitization (at 2537 Å.) of D_2–NO and H_2–NO mixtures in a cell containing a pinhole leak into a Bendix Model 14-101 time-of-flight mass spectrometer.[11,12] We have observed nitrous oxide and water as stable products of the reaction, while nitroxyl is found as a transient intermediate. In addition, small amounts of hydroxylamine appear to be formed. We do not observe nitrogen, but this is as expected[5] under our conditions of $P_{D_2}/P_{NO} \geq 138$. Masses higher than *m/e* 36 attributable to the dimer $(HNO)_2$ or to nitric acid were not observed.

The results of a typical reaction at $P_{D_2}/P_{NO} = 138$ and $P_{total} = 38$ torr are shown in Figures 1 and 2. Figure 1 shows the variation of *m/e* 30, 32, and 44 with photolysis time. These peaks are measures of the concentration of NO, DNO, and N_2O, respectively. However, since the peak base lines have been greatly suppressed to allow the measurement of small changes, the peak heights, as shown, are not proportional to concentrations. The rapid rise of *m/e* 32 to a maximum while the *m/e* 44 growth is accelerating, followed by the inflection point of *m/e* 44 close to the time of the *m/e* 32 maximum, indicates that N_2O arises from HNO. Actually, the rise of [DNO] is steeper and the fall less steep than shown by the time behavior of *m/e* 32. This is because [NO] decreases continually during photolysis and a significant part of *m/e* 32 is comprised of $N^{14}O^{18+}$. Identical behavior was observed for *m/e* 31, for the case of H_2–NO mixtures, including mass interference; but in this case the interference was from $N^{15}O^{16+}$. The growth curves of H_2O are similar to those of N_2O indicating their formation in a common reaction.

Figure 2 depicts the logarithm of the relative peak heights of *m/e* 30, 32, and 44 as a function of time after the photolysis was stopped. The behavior shows quite conclusively that N_2O is formed by the dimerization of nitroxyl. In this flow apparatus, a stable product, A, which is neither being formed nor reacting, decays by a first-order leakage through the pinhole into the mass

(11) A. Maschke, B. S. Shapiro, and F. W. Lampe, *J. Am. Chem. Soc.*, **86**, 1929 (1964).
(12) J. Heicklen and H. S. Johnston, *ibid.*, **84**, 4394 (1962).