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STRUCTURES AND STEREOCHEMISTRY OF MITOMYCIN HYDROLYSIS PRODUCTS

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We have recently described[1] the synthesis of a 1-substituted mitosene analog of the mitomycin antibiotics. An extension of this work lead to an investigation of 1,2-disubstituted mitosenes some of which possess antibacterial and antitumor activity[2,3]. It has been established that these products are formed from the mitomycins under mild aqueous acidic conditions but their structures have not been fully characterized[4,5,6]. Since these mitosenes are important as intermediates in mitomycin synthesis and for understanding the mechanism of aziridine ring opening in the mitomycins, it was necessary to elucidate their structures in detail.

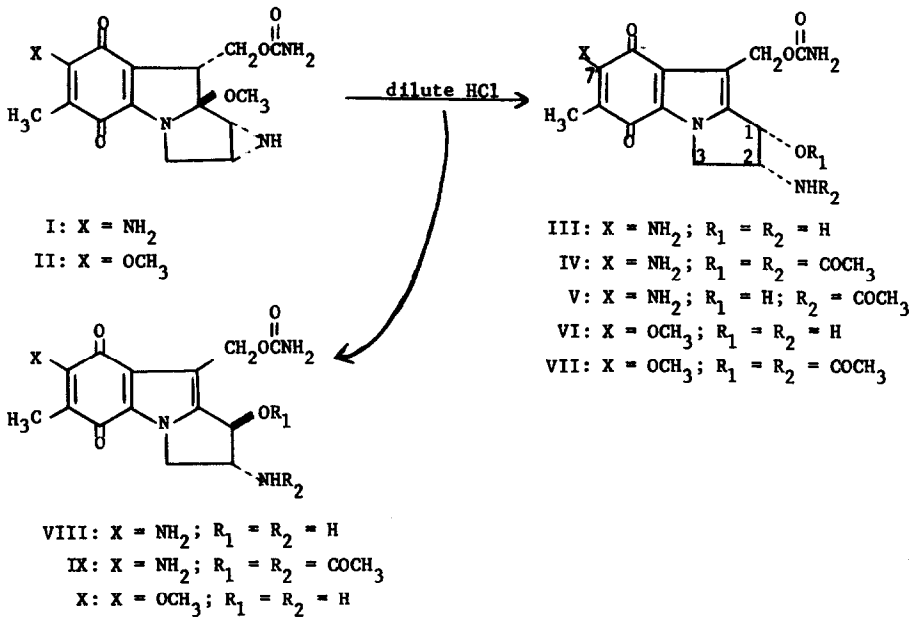
In 1964, Stevens and coworkers[5] reported that hydrolysis of mitomycin C (I) with 0.05 N HCl gave 2,7-diamino-1-hydroxymitosene. On the basis of chemical evidence shown below this compound is assigned the cis configuration (III). We also obtained in low yield the corresponding trans-isomer VIII, which had not been isolated previously. Hydrolysis of mitomycin A (II) in 0.05 N HCl also furnished two products, which are shown below to be the cis-isomer VI and the trans-isomer X of 2-amino-1-hydroxy-7-methoxymitosene. Both of these isomers were isolated previously[4,6] and the major isomer (termed apo-mitomycin A) was given the structure 2-amino-1-hydroxy-7-methoxymitosene without assignment of stereochemistry. However, the minor isomer (iso-apo-mitomycin A) had been assigned the incorrect structure 1-amino-2-hydroxy-7-methoxymitosene[6,7].

The acid hydrolysate from mitomycin C was separated into two pure mitosenes by column chromatography on silica gel. With MeOH as the eluting solvent, the trans-isomer VIII was the first off and was obtained in 4% yield (from MeCN). Elemental analysis suggested that this compound was isomeric with III[5] (III was isolated in 21% yield after rechromatography) which thus excluded the possibility[5,8] that further hydrolysis had occurred to generate the 7-hydroxymitosene or decarbamoylmitosene. The major mitosene VI[4] from mitomycin A was obtained (33%, from MeCN) by extracting the neutralized aqueous hydrolysate with hot EtOAc whereas the minor isomer X[6] was isolated in 5% yield after silica gel chromatography on the MeCN mother liquors. Mitosenes VI and X were converted to the corresponding mitosenes III and VIII by replacing their methoxy groups with NH_2.

Location of the hydroxy and amino groups at C-1 and at C-2 respectively was established by a spin decoupling experiment on the diacetates IV[5] and VII[6]. Irradiation of the signal containing the C-2 proton collapsed the doublets for the C-1 and amide protons to singlets.

This NMR experiment proved that the nitrogen atom was at C-2 in both diacetates. The previously reported[9] semipinacolic deamination of apo-mitomycin A also supported the 2-amino-1-hydroxy arrangement of substituents (with cis stereochemistry) since VI gave 7-methoxy-1-oxomitosene. Treatment of the O,N-diacetate IV from mitomycin C with methanolic NH_4OH gave the mono-N-acetate V[5]. Oxidation of V with MnO_2 gave a product which was identical by TLC to the compound obtained from N-acetylation (Ac_2O in MeOH) and MnO_2 oxidation of the minor isomer VIII from mitomycin C. This identity established the 2-amino-1-hydroxy structural feature for both VIII and X, since they were interrelated. Further evidence for this assignment was obtained by decoupling the C-2 proton signal in a mixture of O,N-diacetates IV and IX derived from mitomycin C. It was observed that the amide proton and the C-1 proton signals sharpened to singlets.

The hydrolysis products from mitomycin C and mitomycin A were therefore diastereoisomeric. The fact that apo-mitomycin A (VI) gave mainly the 1-one with aqueous nitrous acid and also formed a cyclic carbamate with phosgene[9] established VI, as well as III, to possess the cis configuration. Semipinacolic deamination of iso-apo-mitomycin A (X) gave mostly a 1,2-diol



I: X = NH_2
II: X = OCH_3

III: X = NH_2; R_1 = R_2 = H
IV: X = NH_2; R_1 = R_2 = $COCH_3$
V: X = NH_2; R_1 = H; R_2 = $COCH_3$
VI: X = OCH_3; R_1 = R_2 = H
VII: X = OCH_3; R_1 = R_2 = $COCH_3$

VIII: X = NH_2; R_1 = R_2 = H
IX: X = NH_2; R_1 = R_2 = $COCH_3$
X: X = OCH_3; R_1 = R_2 = H

and no 1-one[6] which shows X (and VIII) to have the trans configuration. The absolute stereochemistry assigned to these mitosenes follows from that of the mitomycins[10] since it is unlikely that the 2-amino substituent would undergo epimerization upon aziridine ring opening. Structures and stereochemistry of numerous products resulting from more drastic hydrolysis of mitomycins A and C, such as the indicator quinones and decarbamoyl indicator quinones, follow from their previously established[4,5,8] interrelationships with the compounds described above.

All of the compounds described above had IR and NMR spectra consistent with their indicated structures and the new mitosenes gave correct microanalyses for C, H, N.

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References

1. D. L. Fost, N. N. Ekwuribe, and W. A. Remers, Tetrahedron Letters, 131 (1973).
2. S. Kinoshita, K. Uzu, K. Nakano, M. Shimizu, T. Takshashi, and M. Matsui, J. Med. Chem., 14, 103 (1971).
3. S. Oboshi, M. Matsui, S. Ishii, N. Masago, S. Wakaki, and K. Uzu, Gann, 58, 351 (1967).
4. J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyers, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, J. Amer. Chem. Soc., 84, 3185 (1962).
5. C. L. Stevens, K. G. Taylor, M. E. Munk, W. S. Marshall, K. Noll, G. D. Shah, L. G. Shah, and K. Uzu, J. Med. Chem., 8, 1 (1964).
6. J. H. Mowat, J. B. Patrick, R. P. Williams, D. B. Cosulich, and J. S. Webb, U. S. Patent 3, 179, 671 (1965).
7. Dr. J. S. Webb informed us prior to the completion of this study of unpublished results on the hydrolysis of mitomycin B followed by nitrosation in which a pair of cis- and trans-1-hydroxy-7-methoxy-2-methylamino-N-nitrosomitosenes were isolated. He suggested that iso-apo-mitomycin A might also be a trans isomer. These results were obtained subsequent to the issue of U. S. Patent 3, 179, 671 and they were communicated to us in February, 1974.
8. K. Uzu, Y. Harada, S. Wakaki, and Y. Yamada, Agr. Biol. Chem. (Japan), 28, 388 (1964).
9. J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyers, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, J. Amer. Chem. Soc., 84, 3187 (1962).
10. A. Tulinsky and J. H. van den Hende, J. Amer. Chem. Soc., 89, 2905 (1967).