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Note

High-pressure liquid chromatography of the antibiotics mitomycin A, B and C and of polar mitomycin C conversion products

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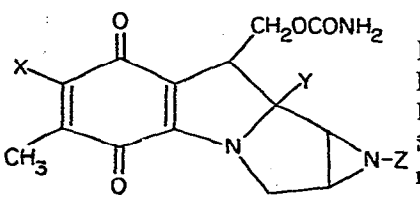
The mitomycins (Ia–c)[1] are a group of antitumor antibiotics produced by a number of *Streptomyces* strains. Mitomycin C is currently of clinical interest as an anticancer agent[1]. These antibiotics are structurally unique due to the simultaneous presence of an aziridine ring, an aminoacetal moiety and a carbamoyl group on a pyrrolizidine ring system fused to a quinone moiety. Several of these functional groups render the mitomycins sensitive to decomposition especially by acid, and these features place limitations on the methods of isolation and purification. Paper chromatography[2], thin-layer chromatography[3] and column chromatography[4] have been used to separate mitomycins but the potential of high-pressure liquid chromatography (HPLC) has apparently not previously been explored. We show that these antibiotics can be separated very efficiently by HPLC and that this method can also be used for the isolation and purification of polar conversion products of mitomycin C. The latter compounds were obtained during our investigation of the nature of chemical transformations that occur when reduced mitomycin C interacts with nucleophiles in aqueous medium. A blue compound, sodium-7-aminomitosane-9a-sulfonate (Id)[5], and red compounds of unknown structure were used in this investigation.



		X	*Y*	*Z*
Mitomycin A	Ia	OCH_3	OCH_3	H
Mitomycin B	Ib	OCH_3	OH	CH_3
Mitomycin C	Ic	NH_2	OCH_3	H
Sodium-7-amino-mitosane-9a-sulfonate	Id	NH_2	SO_3Na	H

EXPERIMENTAL

A Waters Assoc. single-detector Model ALC/GPC 242 liquid chromatograph equipped with a Model 660 solvent flow programmer and Model U6K universal

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injector in conjunction with a Model 202 UV detector was used throughout this work. Stainless-steel analytical columns packed with Corasil II (37–50 μm particle size, 61 cm × 2 mm I.D.), μPorasil (10 μm particle size, 30 cm × 4 mm I.D.), and for preparative work Porasil A (37–75 μm particle size, 122 cm × 7 mm I.D.) were obtained prepacked from Waters Assoc., Milford, Mass., U.S.A. The eluents used were either chloroform–methanol (9:1) or ethyl acetate–methanol (95:5). All solvents were of spectral grade purity and were filtered through a 0.5-μm Millipore filter. The pump was generally run at a flow-rate of 1 ml/min at a pressure of 1000 p.s.i. and the column effluent was monitored at 254 nm. Stock solutions were prepared in analytical grade methanol to contain approximately 10^{-2} mmole/ml of mitomycins A, B or C or compound Id or the red compounds and were stored at 4°. Samples were injected with a 10-μl Hamilton syringe in the range of 0.5–1 μl for analytical samples and in the range of 3–5 μl for preparative-scale work.

The results of this investigation are presented in Figs. 1–3. As shown in Fig. 1, mitomycins A, B and C can be separated cleanly. It appears that the retention time

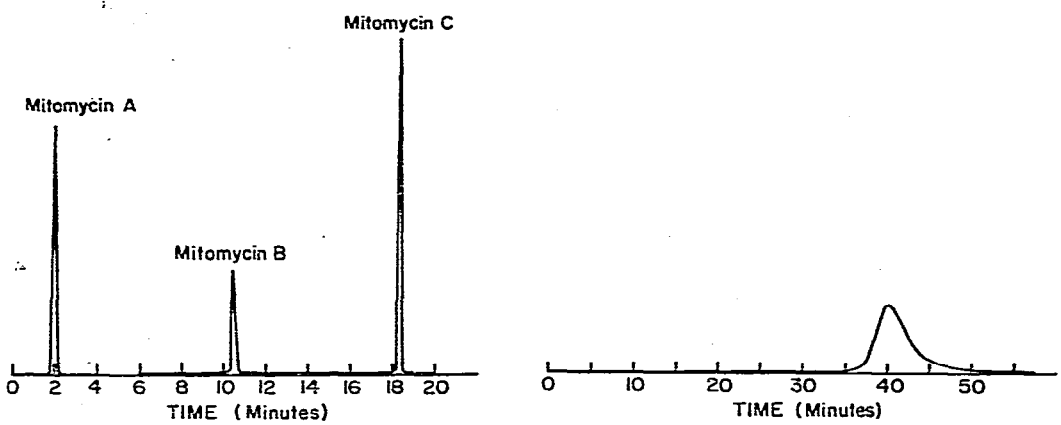

Fig. 1. HPLC separation of mitomycins A, B and C on a Corasil II column. Solvent, chloroform–methanol (9:1); flow-rate, 1 ml/min; chart speed, 10 min/in.

Fig. 2. Check of purity of Id on a Corasil II column. Solvent, chloroform–methanol (9:1); flow-rate, 1 ml/min; chart speed, 5 min/in.

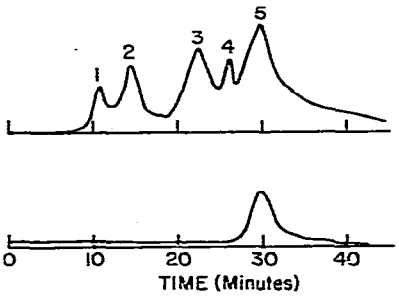

Fig. 3. Separation of red conversion products of mitomycin C on a Porasil A column. Solvent, chloroform–methanol (92:8); flow-rate, 2 ml/min; chart speed, 5 min/in. The lower part of the graph shows a rerun of the isolated peak No. 5 under identical conditions.

correlates very well with the polarity of these compounds. The solvent system chloroform–methanol (9:1) rather than ethyl acetate–methanol (95:5) gave the best separation. In contrast to the narrow peaks obtained with the natural antibiotics the mitomycin C conversion product sodium-7-aminomitosane-9a-sodium sulfonate (Id) gave a broad peak as shown in Fig. 2. This is in accord with the presence of the highly polar sulfonyl group in this compound. Fig. 3 shows the separation of red mitomycin C conversion products which are also highly polar, presumably due to the presence of sulfonyl groups. Both Id and the red compounds were obtained upon reduction of mitomycin C with $Na_2S_2O_4$ in Tris buffer. It is assumed that sodium bisulfite which is generated during the reduction interacted with the mitomycin nucleus and thus participated in the formation of these conversion products[5]. The compound representing the major peak (peak No. 5) of Fig. 3 was concentrated from pooled eluate fractions and the material was run again under identical conditions. As shown in the lower-half of Fig. 3 a homogeneous peak was obtained.

It is concluded from these results that HPLC can be readily used to separate the naturally occurring mitomycins and that this technique is also useful for the isolation and purification of compounds arising by the interaction of reduced mitomycin C with nucleophiles.

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