°C, prepared[13] from RCH=C(R')CH_2Br (0.17 mol), aluminum (4.6 g, 0.17 mol), and ether (110 mL) previously decanted off the excess aluminum. The resultant mixture was allowed to reach slowly room temperature (3 h) and was then poured onto a mixture of ice–water. The aqueous layer was extracted with ether (3 × 75 mL) and the combined organic layers were washed with NaOH (5 M) (3 × 50 mL) and water (4 × 70 mL). After drying (K_2CO_3), the dithiane 7 was distilled at once.

Registry No. 1, 39915-66-7; **5**, 57529-04-1; **6a**, 67702-85-6; **6b**, 70688-45-8; **6c**, 12354-30-2; **6d**, 70688-43-6; **6e**, 70688-44-7; **6f**, 95313-92-1; **7a**, 63382-29-6; **7b**, 69178-01-4; **7c**, 95313-87-4; **7d**, 95313-88-5; **7e**, 95313-89-6; **7f**, 95313-90-9; aluminum, 7429-90-5; 1,3-dithiane, 505-23-7; propargyl bromide, 106-96-7; allyl bromide, 106-95-6; methallyl bromide, 1458-98-6; crotyl bromide, 4784-77-4; 2-pentenyl bromide, 20599-27-3; 2-heptenyl bromide, 34686-77-6; 2-nonenyl bromide, 76853-14-0; $(HC{\equiv}C\text{-}CH_2)_3Al_2Br_3$, 61781-91-7; $HC{\equiv}C\text{-}CH_2\overline{CSCCCS}$, 95313-91-0.

Stereochemical Relationship between Mitomycins A, B, and C

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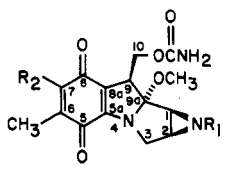
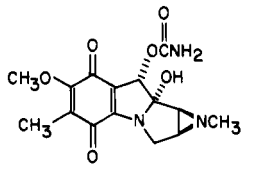
As a consequence of the recent high precision determination of the absolute configuration of mitomycin C (Ic) as C_1 (*S*), C_2 (*S*), C_9 (*S*), C_{9a} (*R*) by X-ray crystallographic analysis,[1] there now exists uncertainty about the stereochemical and perhaps even the biosynthetic relationships among some of the naturally occurring mitomycins.[2] This situation prompts us to report on studies which show that mitomycin A (Ia) and mitomycin B (II) have the same absolute configuration at C_1 and C_2. This result supports the suggestion that all naturally occurring mitomycins are the products of one main biosynthetic pathway differing only in late stages for individual members and are not the end products of two similar but early divergent biosynthetic pathways.



Ia MITOMYCIN A
R_1=H, R_2=OCH_3

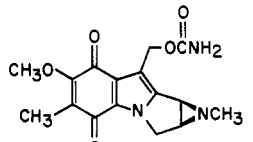


II MITOMYCIN B

Ib N-METHYLMITOMYCIN A
R_1=CH_3, R_2=OCH_3

Ic MITOMYCIN C
R_1=H, R_2=NH_2

Id PORFIROMYCIN
R_1=CH_3, R_2=NH_2



III 7-METHOXY-1,2-(N-METHYLAZIRIDINO) MITOSENE

In several fermentations of *Streptomyces* species[2] including *S. caespitosus*,[3] mitomycin A is often produced in greatest abundance followed by mitomycins B and C, and porfiromycin (Id). Under different fermentation conditions, the clinically useful mitomycin C[4,5] is the most abundant compound in *S. caespitosus*, suggesting a close biosynthetic relationship between mitomycins A and C. Mitomycin A has been converted into mitomycin C by treatment with ammonia.[6,7] The product was identical with mitomycin C by paper chromatography, IR, UV, and X-ray powder diffraction. Mitomycin C can be converted into porfiromycin by methylation, and the latter has been converted into *N*-methylmitomycin A (Ic), which occurs naturally[8] and which can also be readily obtained from mitomycin A by methylation.[2] However, chemical conversions of mitomycin A and C, porfiromycin, and *N*-methylmitomycin A into mitomycin B, or vice versa, have never been reported.

With respect to their biosynthesis,[2] it is known that these antibiotics are formed from an early intermediate of the shikimic acid pathway which provides the benzoquinone moiety and from D-glucosamine which appears to contribute label to C_1, C_2, C_3, C_{9a}, C_9, and C_{10} as well as the nitrogen atom of the aziridine ring. L-Glucosamine is not a mitomycin precursor and D-galactosamine and D-mannosamine which are two epimers of D-glucosamine are less efficiently incorporated than D-glucosamine. The incorporation of D-[1-^{13}C,^{15}N]glucosamine into mitomycin B occurs essentially without separation of the two labeled atoms. All precursors tested appear to label mitomycins A and B in a constant ratio commensurate with the concentration ratio of these antibiotics in the fermentation broth.

The absolute configurations of mitomycins A and B have been reported on the basis of low precision X-ray crystallographic results as C_1 (*R*), C_2 (*R*), C_9 (*R*), C_{9a} (*S*),[9,10] and as C_1 (*R*), C_2 (*R*), C_9 (*S*), C_{9a} (*S*),[11,12] respectively. If these assignments are correct, the results of the biosynthetic studies make it necessary to postulate the occurrence of an inversion of the chirality of C_2 of D-glucosamine without cleavage of the C_2–N bond during mitomycin biosynthesis.[2] This situation prompted Shirahata and Hirayama to determine the absolute configuration of mitomycin C by X-ray crystallographic analysis. These authors have obtained the best precision of any of the published X-ray determinations of mitomycins and have established the absolute configuration of mitomycin C with high probability. Their results given above make it very likely that mitomycin A also has the same chirality at its four asym-

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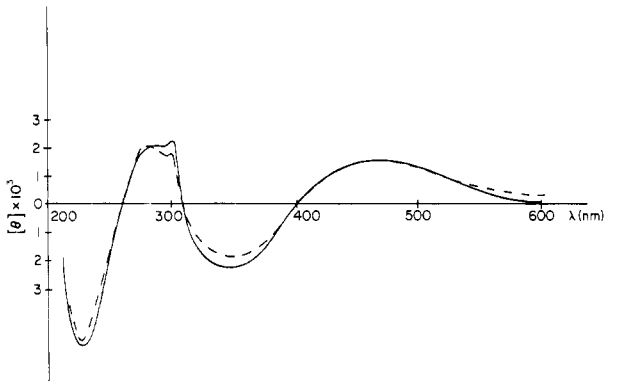

Figure 1.

metric centers since it can be converted into mitomycin C. Furthermore, their results obviate the need to postulate the occurrance of an inversion of the configuration of C_2 of D-glucosamine during the biosynthesis of mitomycins A and C. However, the Shirahata–Hirayama results generate uncertainty concerning the stereochemical relationships during the biosynthesis of mitomycin B since mitomycin B differs in its relative configuration from mitomycins A and C and since it was not certain from the X-ray studies whether this difference is manifest at one or at three chiral centers.

We sought to clarify the stereochemical relationship between mitomycin A and B by converting both compounds into the common optically active relay compound 7-methoxy-1,2-(*N*-methylaziridino)mitosene (III) following known procedures[13] and by comparing the optical properties of the two specimens by circular dichroism studies. A mixture of mitomycin A (20 mg, 57.4 μmol), dry acetone (2 mL), methyl iodide (1 mL), and anhydrous K_2CO_3 (83 mg, 600 μmol) was refluxed for 4 h to afford *N*-methylmitomycin A (Ib) in excellent yield. The reaction product was purified by column chromatography on silica gel (Merck, washed with NaH_2PO_4–Na_2HPO_4, 0.2 M, pH 7.0 buffer) using the system hexane/ethyl acetate/isopropyl alcohol (2:2:3) for elution. The identity of the product was proven by its facile conversion into porfiromycin (Id) by treatment with 3% ammonia. Subsequently, Ib (10 mg, 27.5 μmol), PtO_2 (1.25 mg), and ethyl acetate (3 mL) were treated with bubbling hydrogen gas for 15 min which yielded a yellow solution. N_2 was then bubbled through this solution for 5 min followed by O_2 which caused the appearance of the orange color of III. The reaction mixture was subjected to column chromatography in the system mentioned above, and the main compound isolated was recrystallized from acetone/hexane. This afforded III (3.8 mg) in 42% yield. The IR spectrum of this material showed the same absorptions as those reported for III by Patrick et al.[13] The CD spectrum of this specimen (1 mg, 3 μmol) dissolved in methanol (3 mL) and water (1 mL) was recorded on a Cary 60 spectropolarimeter equipped with a CD attachment. The resulting spectrum is presented in Figure 1 as a solid line. Mitomycin B (10 mg, 2.87 μmol) was converted directly into III by catalytic reduction and was isolated by column chromatography as described above. III (2.9 mg) was obtained in 30% yield. The IR spectrum of this material also showed exactly the same absorptions as reported for III by Patrick et al.[13] This specimen (1 mg, 3 μmol) was dissolved in methanol (3 mL) and water (1 mL), and its CD spectrum was recorded. The spectrum is shown in Figure 1 as a dotted line.

It is apparent from Figure 1 that both specimens of III have essentially superimposable CD spectra. The only chiral centers present in III are at C_1 and C_2 which must therefore have the same chiralty in both mitomycins A and B. It can be concluded that the fate of the chiral center at C_2 of D-glucosamine is the same for all mitomycins during their biosynthesis. Furthermore, it follows that mitomycin B differs stereochemically from mitomycins A and C only at C_9. Similar results, which have not been published, were obtained by W. A. Remers and J. S. Webb and their co-workers.

Acknowledgment. We gratefully acknowledge receipt of samples of mitomycins A and B from Dr. S. Wakaki, Kyowa Hakko Kogyo, Tokyo, Japan, of mitomycin C from Dr. W. Bradner, Bristol Laboratories, Syracuse, NY, of profiromycin from Dr. G. B. Whitfield, Upjohn Company, Kalamzoo, MI, of information by Dr. Remers on his results, and of financial support by Grant CA14378 from the National Cancer Institute.

Registry No. Ia, 4055-39-4; Ib, 18209-14-8; Id, 801-52-5; II, 4055-40-7; III, 15973-07-6.

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New S-Protection from Known N-Protection: Thio Esters of *N*-Urethanyl-*N*-methyl-γ-aminobutyric Acid as a Class of Protective Groups for Thiols in Peptide Synthesis

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For the last few years we have been engaged in the development of a novel methodology for peptide synthesis which relies heavily on the chemistry of thiols and unsymmetrical disulfides.[1] A major concern in this work has been the problem of temporary protection of reactive arene and cysteine thiol intermediates generated by the chain elongation and acyl-transfer processes, respectively. Since most of the existing S-blocking functions require for removal either harsh condtions (e.g., HF, Hg(II), Na/NH_3) or reagents (e.g., phosphines) which are incompatible with our synthetic scheme,[2] we sought to advance new methodology. In 1973 Geiger reported a stragety in which S-deprotection is triggered by the liberation of a neighboring N-blocked amine.[3] Although promising, this tactic does not meet all of our requirements and has been cited only in reviews[2,4] without experimental details. Consequently, we describe in full a form of thiol protection, similar to Geiger's, that offers versatility and requires exceptionally mild deblocking conditions.

Saponification of *N*-methylpyrrolidone-generated zwitterion 1 (58%)[5] which was functionalized at its N-terminus

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