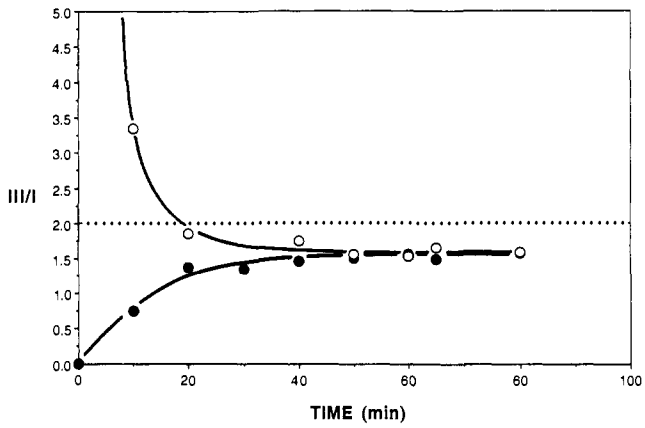

Figure 2. Plot of molar ratio of III/I as a function of equilibration time for vesicles prepared from III plus 40 mol % of cholesterol (○) and a 1/1 mixture of I/II plus 40 mol % of cholesterol (●); the equilibration temperature was maintained at 60 ± 1 °C. In all cases, equal molar ratios of symmetrical dimers were produced (±5%). The percentage of cholesterol is based on phospholipid monomer content.

independent experiments. This level of nearest-neighbor recognition corresponds to a thermodynamic preference for forming homodimers of $\Delta G = 0.17 \pm 0.02$ kcal/mol. Similar equilibration experiments, carried out in the presence of 30 mol % of cholesterol, afforded a molar ratio of I/III/II equaling $1/(1.68 \pm 0.07)/1$ ($\Delta G = 0.12 \pm 0.03$ kcal/mol). In the absence of (or in the presence of 10 mol % of) cholesterol, a completely random distribution of lipid dimers was observed; i.e., the ratio of I/III/II was $1/(2.01 \pm 0.06)/1$ in the absence of cholesterol and $1/(1.98 \pm 0.06)/1$ with 10 mol% of cholesterol. In contrast, equilibrium mixtures that were produced from dimeric analogs of DMPC and DPPC (i.e., I, IV, and V) were not influenced by the presence of 10, 30, or 40 mol % of cholesterol in the fluid phase (53 °C); the ratio of I/IV/V was 1/2/1, within the limits of error in each case.

Why does cholesterol induce nearest-neighbor recognition in fluid bilayers made from DMPC/DSPC analogs but not in those made from DMPC/DPPC analogs? The answer, we believe, lies in the known condensing effect that cholesterol has on the liquid-crystalline phase and in the greater difference in chain length that exists between the monomeric components of I and II, as compared with those of I and IV.[8,9] We have previously shown that I-III favor homodimer formation in the gel-fluid coexistence region, where solid-like domains that are rich in II exist in a fluid "sea" that is rich in I, i.e., two distinct endotherms (23 and 48 °C) are observed by high-sensitivity differential scanning calorimetry.[4] Thus, the thermodynamic preference of the higher melting homodimer II to self-associate in this mixed phase not only drives phase separation but it also leads to nearest-neighbor recognition and domain formation. An analogous situation is likely to exist for cholesterol-rich membranes made from I-III. By increasing the compactness of the bilayer, cholesterol "moves" the liquid-crystalline phase closer toward the gel-fluid coexistence state, where van der Waals forces are greater and where nearest neighbors can be recognized. The inability of cholesterol to alter the dimer distribution of fluid bilayers made from DMPC/DPPC analogs is fully consistent with this interpretation since these lipids favor a random distribution in the gel-fluid coexistence region, i.e., the difference in chain length between the equilibrating monomers is too small to be recognized.[4]

The ability of cholesterol to induce nearest-neighbor recognition in fluid bilayers infers the existence of phospholipid domains. This result further suggests that sterols may influence the suprastructure of biological membranes in ways that have not previously been realized.

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DNA Interstrand Cross-Linking by Reductively Activated FR900482 and FR66979

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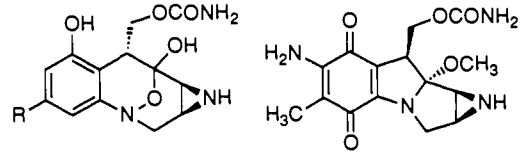
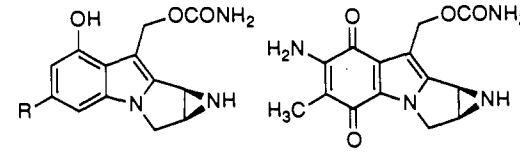
Several hypotheses[1-3] have been offered to account for the in vivo DNA alkylating activity of the antitumor antibiotic substances FR900482[4] (**1**) and FR66979[5] (**2**). We report herein that the DNA interstrand cross-linking reactions of **1** and **2** share in common with mitomycin C (**3**) greatly enhanced efficiency in a reducing medium and selectivity for dG-to-dG cross-linking at 5′-d(CG) with the participation of both dG exocyclic amino groups. We also provide preliminary evidence favoring mitosene-like structures **7** and **8** for these cross-links, analogous to lesion **9** previously derived from mitomycin C.[6] These observations support the hypothesis of reductive activation of **1** and **2** to form mitosene-like, reactive intermediates (e.g., **4**, **5**)[1] which are responsible for interstrand cross-linking.



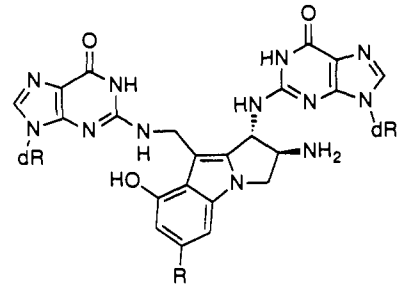
1 R = CHO, FR900482
2 R = CH_2OH, FR66979

3 mitomycin C

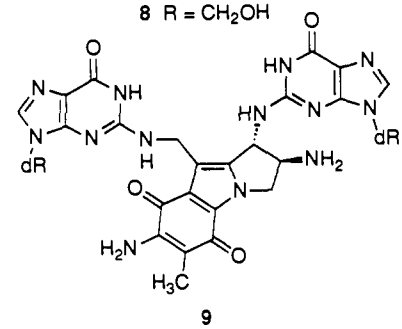


4 R = CHO
5 R = CH_2OH

6



7 R = CHO
8 R = CH_2OH



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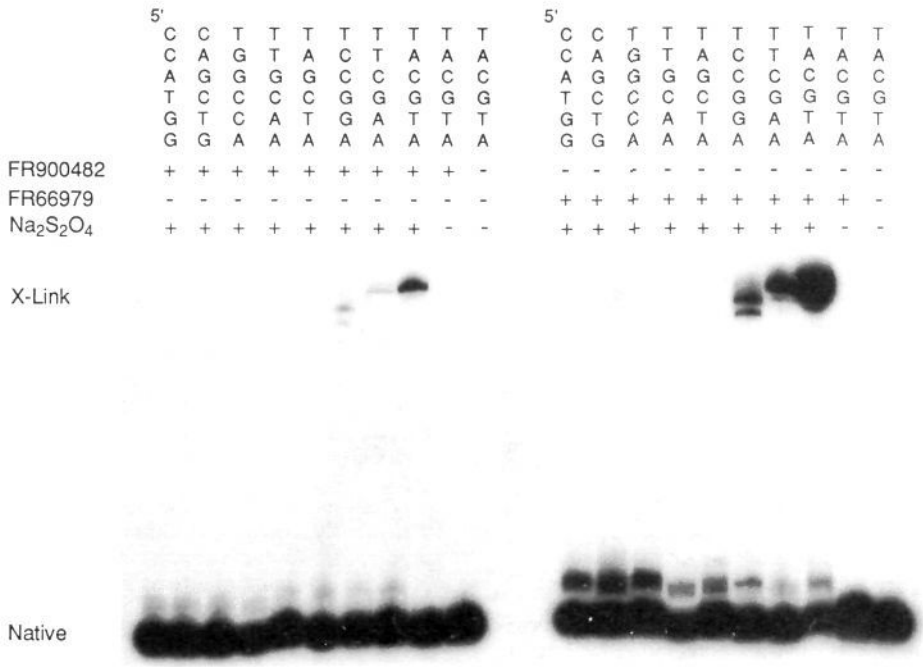

Figure 1. Autoradiogram (DPAGE) of 5′-^{32}P-labeled DNA duplexes [5′-d(TATAAN$_6$TTATA)]$_2$ treated with FR900482 (**1**) or FR66979 (**2**) reveals interstrand cross-linking to have a requirement for $Na_2S_2O_4$ and 5′-d(CG). Bands in drug-treated lanes retarded slightly relative to native DNA are likely monoadducts, but this has not been experimentally established.

The DNA duplexes [5′-d(TATAAN$_6$TTATA)]$_2$ (Figure 1) admixed with FR900482 or FR66979[7] were treated with sodium dithionite,[8] the reagent of choice for in vitro mitomycin C activation.[6] The largest nucleotide sequence unique to the most efficiently interstrand cross-linked DNAs (N$_6$ = TACGTA, TTCGAA, TCCGGA) was the dinucleotide 5′-d(CG), the preferred site of cross-linking for reductively activated mitomycin C.[9,10] Cross-linking efficiency with FR66979 greatly exceeded that with FR900482. In the absence of dithionite, interstrand cross-linking was negligible (Figure 1). The absence of deoxyguanosine in enzymatic digests[6,8,11] of electrophoretically homogeneous samples of N$_6$ = TACGTA cross-linked with either substance indicated that deoxyguanosine residues on opposite strands at 5′-d(CG) were bridged in the cross-links, as with mitomycin C.[6] Substitution of *one* deoxyinosine residue, which lacks the N^2 amino group of dG, for one of the two dG residues at the duplex sequence 5′-d(CG) in N$_6$ = TACGTA greatly reduced cross-linking with both substances, revealing that *both* N^2 amino groups of dG residues at 5′-d(CG) participate in cross-linking as for mitomycin C[6] and other pyrrole-derived, bifunctional electrophiles.[11] A further similarity of both agents to mitomycin C was the relative efficiency of cross-linking as a function of flanking sequence, 5′-d(ACGT) ≫ 5′-d(TCGA) ≈ 5′-d(CCGG) (Figure 1).[12]

The enzymatic hydrolysates of electrophoretically homogeneous N$_6$ = TACGTA interstrand cross-linked with FR900482 or FR66979 were separately analyzed by RP-HPLC (snake venom phosphodiesterase, calf intestinal alkaline phosphatase, DNase I). Each returned, in addition to dA, dC, and dT, a predominant, single, more strongly retained substance, tentatively proposed herein to be **7** and **8**, respectively. Electrospray ionization MS of these substances afforded molecular ions (proton and sodium ion adducts) as required for **7** and **8**, the analogs of **9** derived from mitomycin C.[6] The longest wavelength UV absorption of **7** was bathochromically shifted by some 40 nm (λ_{max} = 370 nm, n → π^*) relative to **1** as expected for conversion of a hydroxy*benzene*carboxaldehyde to a hydroxy*indole*carboxaldehyde (**1** → **7**). No absorbance of comparable wavelength was present in **8**. $NaBH_4$ treatment of **7** returned **8** (RP-HPLC analysis). Preparation of larger samples of **7** and **8** to permit more rigorous structure assignment is underway.

The DNA interstrand cross-linking activities of reductively activated FR900482, FR66979, and mitomycin C thus share features most simply accounted for by the intermediacy of **4** and **5**, respectively,[1] the analogs of **6**.[13] The existence of significant alternative alkylation pathways involving nucleophile-activated[2] or other[3] intermediates of unspecified structure remains for now a topic of speculation.

Acknowledgment. This work was supported by the NIH (GM45804, GM32681, and AG00417). P.B.H. is a Cope Scholar.

Supplementary Material Available: DPAGE of dI substitution experiment, RP-HPLC of digest, and mass and UV spectra of the putative **7** and **8** (4 pages). Ordering information is given on any current masthead page.

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(8) Partially 5′-^{32}P-radiolabeled synthetic DNA (0.5 OD_{260}, 2 nmol duplex) and 64 μg (200 nmol) of FR900482 or 13 μg (40 nmol) of FR66979 in 25 μL of aqueous pH 7.6 Tris buffer (200 mM) were combined, sparged at 25 °C with argon, and then treated sequentially with five aliquots of 5 μL of 40 mM (FR900482) or 5 μL of 8 mM aqueous (FR66979) $Na_2S_2O_4$ (200 nmol and 40 nmol per aliquot, respectively) at 5-min intervals. The solution stood at 25 °C for 16 h. The nucleic acid products were precipitated with ethanol and analyzed by DPAGE.

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