Cefoxitin Sodium: Solution and Solid-State Chemical Stability Studies

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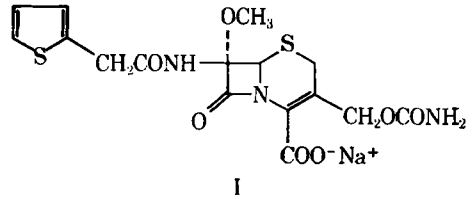
Abstract □ Studies were undertaken to provide the basic physicochemical information necessary for preparing a suitable parenteral formulation of cefoxitin sodium. Emphasis was placed on the physicochemical properties of the compound in solution and in the solid state. Cefoxitin sodium is very soluble in water and exhibits apparent first-order decomposition in this medium at pH 3–9. Maximum stability in water is at pH 5–7. Under these pH conditions, cefoxitin sodium loses about 10% of its activity in 2 days at 25°. Thermal decomposition rates for amorphous and crystalline cefoxitin sodium samples were determined. Amorphous cefoxitin sodium was considerably less stable than its corresponding crystalline form. Solid-state decomposition plots are biphasic, displaying initial rapid losses followed by a slower decay period. The extent of loss in the crystalline solid at the end of the more rapid initial phase can be correlated with the water content of the solid.

Keyphrases □ Cefoxitin sodium—chemical stability, amorphous and crystalline solids, water content □ Antibiotics—cefoxitin sodium, chemical stability, amorphous and crystalline solids, water content □ Stability—cefoxitin sodium, amorphous and crystalline solids, water content

Cefoxitin sodium[1] (I), the sodium salt of 3-(hydroxymethyl)-7α-methoxy-8-oxo-7-[2-(2-thienyl)acetamido] -5- thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate carbamate (ester), is a semisynthetic derivative of a new class of β-lactam antibiotics, the cephamycins. It is chemically unique in that the cephem nucleus is substituted with a 7α-methoxy and 3-carbamoyloxymethyl group. Cefoxitin is active against Gram-positive and Gram-negative bacteria (1) and is therapeutically important because of its resistance to destruction by bacterial β-lactamase (2, 3).

This paper describes preformulation studies undertaken to provide the basic physicochemical information necessary for preparing a suitable parenteral formulation of this antibiotic. Emphasis was placed on the physicochemical properties of the compound in solution and in the solid state. Findings from these preformulation studies are reported herein.



I

EXPERIMENTAL

Materials—Amorphous and crystalline cefoxitin sodium samples were laboratory samples[2] used as received. A selected lot of cefoxitin free acid was used as a reference standard. All other chemicals and solvents were reagent grade and were used without further purification.

Kinetic Procedures—*Solution Studies*—Buffer solutions for kinetic studies were prepared by adding 1.0 *N* NaOH sufficient to attain the desired pH to 50 ml of solutions 0.5 *M* each in acetic, phosphoric, and boric acids and diluting to 250 ml with water (modified Britton–Robinson buffer). In this manner, buffers were prepared at pH 3, 5, 7, or 9, 0.1 *M* in each of the previously named acids.

Aliquots of an aqueous stock solution (5% w/v) of cefoxitin sodium were diluted with sufficient buffer to make 0.5 or 1% solutions. These solutions were stored at 25.0 ± 0.5° in glass-stoppered volumetric flasks, and aliquots were withdrawn periodically for analysis. Aliquots of reaction solutions and reference standard cefoxitin acid were diluted for UV spectrophotometric assay with 1% pH 6.0 phosphate buffer prepared as directed in the USP (4). Each aliquot was diluted to a nominal concentration of 40 μg/ml with 1% pH 6.0 phosphate buffer. For the standard solution, cefoxitin acid was dissolved in 1% pH 6.0 phosphate buffer and diluted to about 40 μg/ml. Absorbances of sample and standard solutions were determined at 262 nm. The true absorbance at 262 nm was obtained by applying a suitable baseline correction.

The reaction solution pH was checked initially and at the end of the 10-day period and did not change significantly during that time.

Solid-State Studies—Individual samples of about 50 mg each of amorphous and crystalline cefoxitin sodium were stored in tightly closed glass vials in constant-temperature ovens. Separate individual samples were removed periodically for each assay point.

Characterization of Crystalline Form—The primary tool for the differentiation of amorphous and crystalline cefoxitin sodium was a commercial X-ray powder diffractometer[3] supplying CuKα radiation. Solid-state IR spectroscopy, differential scanning calorimetry, and optical

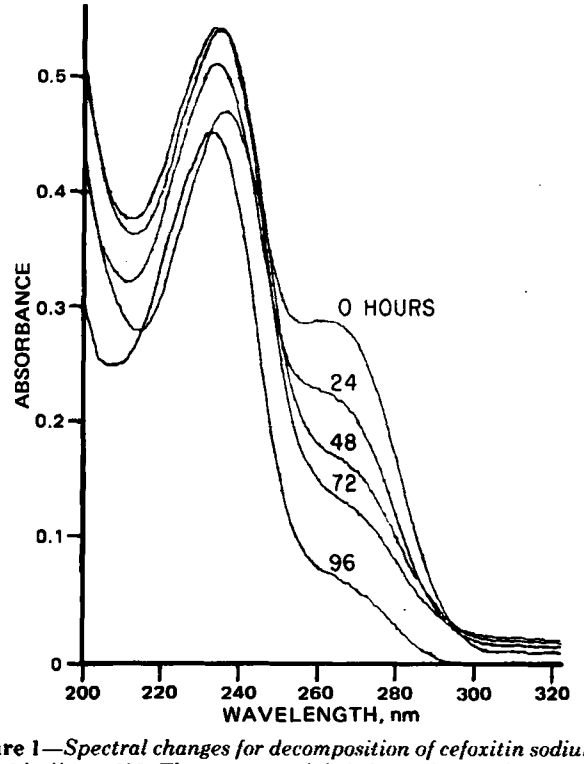


Figure 1—*Spectral changes for decomposition of cefoxitin sodium in pH 9.0 buffer at 25°. The curves are labeled as to hours after the start of the reaction.*

[1] Mefoxin, Merck Sharp and Dohme.
[2] Merck Sharp and Dohme Research Laboratories.
[3] Phillips Electronic Instruments model 12045 full-wave constant potential unit with vertical diffractometer and scintillation counter detector.

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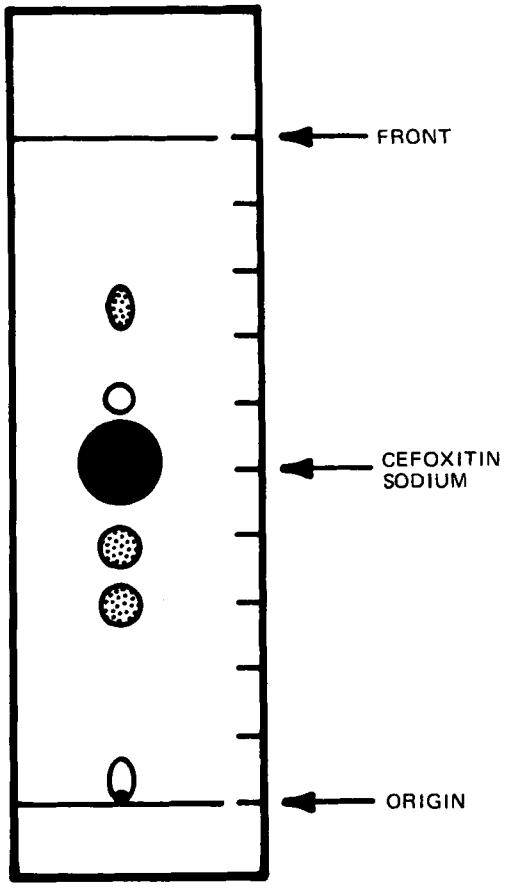

Figure 2—*Thin-layer chromatogram of a 1% solution of cefoxitin sodium following storage at 25° for 72 hr.*

microscopy with polarized light were also employed to determine crystallinity.

Analytical Procedures—Cefoxitin sodium stability studies in solution and in the solid state were monitored by UV spectrophotometry, iodometric titration, microbiological assay, high-performance liquid chromatography (HPLC), and TLC.

UV Spectrophotometry—Intact cefoxitin sodium exhibits a UV absorption band near 262 nm attributed to the O=CNC=C linkage in the molecule. β-Lactam ring opening leads to disappearance of this absorption band. Periodic monitoring of UV spectra of stability samples was carried out to follow the extent of this transformation. This UV spectrophotometric method, with appropriate baseline corrections, yielded assay values essentially identical to those obtained by HPLC and iodometric methods.

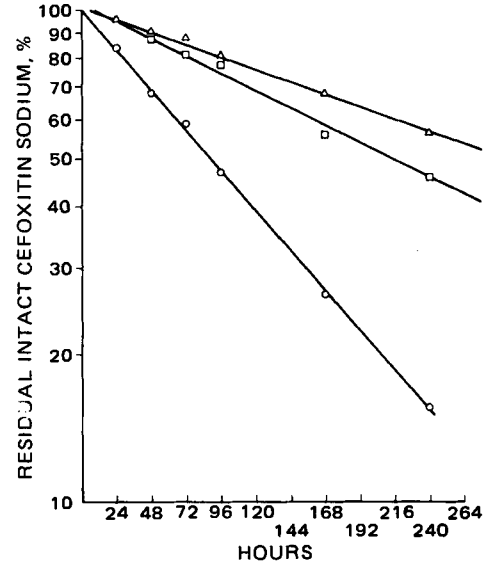

Figure 3—*Apparent first-order plots followed by UV spectrophotometry for decomposition of 0.5 and 1% solutions of cefoxitin sodium at 25° and various pH values. Key: △, pH 5 and 7; □, pH 3; and ○, pH 9.*

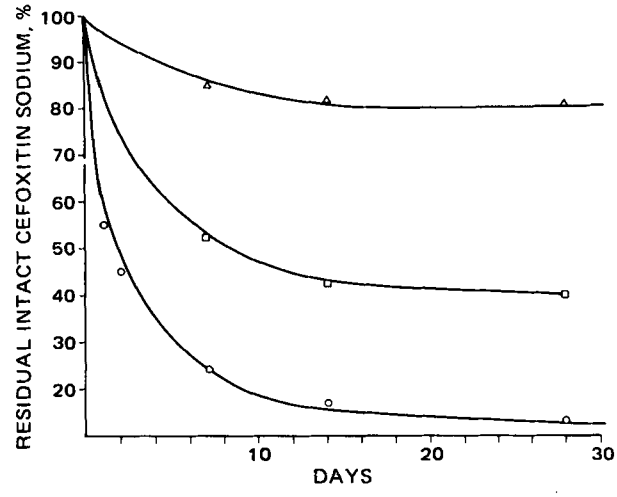

Figure 4—*Typical plots of solid-state decomposition of amorphous cefoxitin sodium at various temperatures. Key: △, 40°; □, 60°; and ○, 80°.*

Spectral changes as a function of time for the decomposition of a cefoxitin sodium solution at pH 9 are illustrated in Fig. 1.

Iodometric Titration—The well-established iodometric titration for β-lactam antibiotics is applicable to the determination of the integrity of the β-lactam moiety in cefoxitin sodium. The procedure used in these studies is a modification of the "Code of Federal Regulations" method (5).

Microbiological Assay—Cefoxitin sodium potency was determined by an agar-diffusion (plate) assay with *Staphylococcus aureus* (NRRL-B11034) as the test organism.

HPLC—HPLC was used to follow cefoxitin sodium decomposition. The liquid chromatograph[4] employed was equipped with a variable-wavelength UV detector[5] set at 254 nm; the separation was carried out on a 30-cm, 10-μm, microporous, octadecylsilane stationary phase[6] with an acetonitrile–water–acetic acid (20:80:1) mobile phase. The column pressure was 800–900 psig, and the column temperature was controlled at 25°. The sample load on the column was 10 μl of a 1-mg/ml solution.

TLC—Solution and solid degraded samples (100 μg) were evaluated qualitatively by TLC. Cefoxitin sodium was chromatographed using butanol–acetic acid–water (4:1:1) with precoated silica gel plates containing a fluorescent indicator[7]. Visualization was accomplished by fluorescence quenching under shortwave UV light or by iodine staining.

RESULTS AND DISCUSSION

The results of experimental studies with cefoxitin indicated that crystalline cefoxitin sodium has properties that make it a suitable chemical and physical form of the antibiotic for use as a sterile powder for reconstitution prior to parenteral administration.

Physical Properties—Crystalline and amorphous cefoxitin sodium forms are colorless to off-white solids with a slight characteristic odor. The compound is optically active and, as a 1% solution in methanol, exhibits a specific rotation of $[\alpha]^{25°}_{589\,nm}$ +210°, calculated on a water and solvent-free basis. The UV spectrum in pH 7.0 phosphate buffer is characterized by maxima at 235 and 262 nm with approximate log ε values of 4.17 and 3.97, respectively.

Solution Properties—*Aqueous Solubility*—Cefoxitin sodium is soluble in water in excess of 1000 mg/ml. This value more than satisfies

[4] Dupont model 841 with model 833 flow controller.
[5] Perkin-Elmer model LC-55.
[6] Waters Associates μBondapak C_{18}.
[7] Quantum Industries QIF.

Table I—Apparent First-Order Constants, $T_{10\%}$, and $T_{50\%}$ for 0.5 and 1.0% Solutions of Cefoxitin Sodium at 25° and Various pH Values

pH	$K_{obs} \times 10^3$, hr^{-1}	$T_{10\%}$, hr	$T_{50\%}$, hr
3.0	3.42	40	212
5.0	2.44	50	291
7.0	2.46	49	288
9.0	7.83	14	89

the required solubility for use in injection products based on the intended dose and desired volumes for administration.

Solution Stability—The β-lactam ring of cefoxitin sodium undergoes cleavage in water. Subsequent fragmentation of the β-lactam hydrolysis product yields a complex product mixture, as evidenced by the TLC hydrolysis product pattern (Fig. 2).

As with other β-lactam antibiotics, long-term solution stability at 25° is not sufficient at pH 3–9 to permit formulation of cefoxitin sodium as a preconstituted solution (Table I). However, these studies established the viability of a formulation consisting of a readily soluble sterile solid for the extemporaneous preparation of a solution for injection. As the data indicate, such solutions, under proper pH control, exhibit about a 10% loss ($T_{10\%}$) in potency when maintained at 25° for 48 hr.

The studies summarized in Table I and Fig. 3 establish the pH–rate profile for cefoxitin sodium at 25°. The semilogarithmic data plots obtained at various pH values were reasonably linear (Fig. 3), indicating that cefoxitin sodium degradation follows first-order kinetics. The apparent first-order rate constants, K_{obs} (Table I), were calculated from the slopes of log percent remaining cefoxitin sodium concentration–time data by means of a computer-programmed least-squares regression analysis. The β-lactam hydrolysis rate was nearly identical at pH 5.0 and 7.0. Under these pH conditions, the time for 10% loss ($T_{10\%}$) was approximately 50 hr at 25° and the half-life ($T_{50\%}$) was about 290 hr. Reactivity of the β-lactam was significantly greater under basic conditions. At pH 9.0, the half-life was approximately 89 hr. Sensitivity to acid was less pronounced than to base, as evidenced by the degradation rate at pH 3.0 which was not dramatically greater than the rate in the pH 5.0–7.0 range.

No adverse physical changes, other than some yellowing, were observed in the solutions employed for solution stability studies. All solutions remained free of precipitates during the study.

Solid-State Properties—*Physical Forms*—An amorphous and a crystalline form of cefoxitin sodium were evaluated during preformulation studies. The amorphous material was the first form investigated during preclinical and initial clinical studies. As solid-state stability deficiencies in the amorphous material became apparent, attention was directed toward preparation of a more highly ordered structure, a crystalline solid. When this goal was accomplished, the properties of the crystalline solid were investigated and compared to the amorphous materials.

The amorphous form is a white to off-white solid and does not exhibit any well-defined thermal transitions by differential scanning calorimetry below 350°. Gradual solid decomposition is observed by differential scanning calorimetry as temperatures increase over 150°. The amorphous form shows no distinct X-ray powder diffraction pattern and is nonbirefringent when examined microscopically under polarized light.

Crystalline cefoxitin sodium is a white to off-white solid and exhibits a characteristic X-ray powder diffraction pattern and a decomposition exotherm by differential scanning calorimetry at approximately 190–200°. The solid is birefringent when examined microscopically under polarized light.

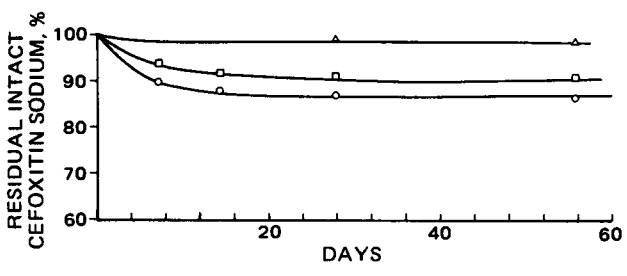

Figure 5—*Typical plots of solid-state decomposition of crystalline cefoxitin sodium at various temperatures. Key: △, 40°; □, 60°; and ○, 80°.*

Solid-State Stability—Accelerated chemical and physical stability testing of amorphous and crystalline cefoxitin sodium solids was carried out as part of a screening program to guide selection of the proper physical form for use in a formulation. Both solid forms, when exposed to elevated temperature and subsequently examined qualitatively by TLC, revealed degradation to a complex mixture of decomposition products. The complexity of the degradation pattern also was reflected in quantitative studies. Typical data plots from these studies used to determine reaction order indicate that degradation did not follow a simple kinetic relationship. A rough reaction order screening was carried out by data plotting. If the amount of intact cefoxitin is plotted as a function of time, a straight line does not result for zero- or first-order plots.

The amount of intact cefoxitin sodium in the two different solid forms as a function of time and temperature is depicted graphically in Figs. 4 and 5. Both solids exhibited a biphasic decomposition pattern typified by an initial accelerated decomposition period followed by a slower decay period. This trend suggests that cefoxitin sodium exhibits two or more parallel decomposition pathways, one or more of which is rapid and the others slower. The initial more rapid degradation rate of crystalline material at elevated temperature and of amorphous material at low temperatures to a level of ~85–90% of the initial concentration is consistent with partial solid hydrolysis; the hydrolysis apparently is limited only by the concentration of water, an exhaustible solid constituent. Thermal degradation reactions occurring at a slower rate most likely account for the additional losses in the subsequent slow decay period.

In cefoxitin sodium decomposition by hydrolysis, water is consumed when the β-lactam, R-CO-N-R′R″, is opened to RCOOH + HNR′R″. Since the cefoxitin sodium samples studied contained about 0.5% water, which corresponded to approximately 12 mole % water, about 12% of the drug could be expected to decompose by hydrolysis. In fact, as the data in Fig. 5 indicate, crystalline cefoxitin sodium subjected to 60 and 80° thermal stress suffered drug loss to that extent in the initial more rapid phase of its decomposition. Drug loss to that extent also occurred in crystalline material at 40° when the study was extended to periods beyond those shown in Fig. 5 and to samples of amorphous solid maintained at 40° as indicated in Fig. 4. The fact that the loss in the initial phase of accelerated studies on the amorphous solid at 60 and 80° is more extensive suggests that the decomposition pathway for this material is not the same as for the crystalline solid at higher temperatures and that the observed rates must reflect a substantial contribution from nonhydrolytic reactions. The activation energy for these thermal, nonhydrolytic processes is most likely considerably less for amorphous materials than for their corresponding crystalline forms.

A comparison of the thermal decomposition data for amorphous and crystalline cefoxitin sodium demonstrates that crystalline material is more stable. While an increasing water content generally increased the net decomposition rate of both forms, it could be shown by maintaining equivalent water levels that stability differences between the two forms did not reflect differences in water content. The data in Figs. 4 and 5 were obtained for samples of approximately equal water content (0.5%).

It is concluded that the stability differences noted between the solids relate to differences in the degree of crystallinity. Stability differences between crystalline and amorphous solids have been the subject of prior publications. Among β-lactam antibiotics, amorphous forms of penicillin potassium (6), epicillin (7), and a number of cephalosporins (8, 9) are less stable chemically than their crystalline counterparts.

In addition to chemical stability differences between amorphous and crystalline forms of cefoxitin sodium solids, physical stability differences have been noted. Upon aging, amorphous cefoxitin sodium develops considerably more color than its crystalline counterparts, as reflected in both solid-state and solution color measurements.

Crystalline cefoxitin sodium is thus both chemically and physically more stable than the amorphous form.

REFERENCES

(1) H. R. Onishi, D. R. Daoust, S. B. Zimmerman, D. Hendlin, and E. O. Stapley, "Abstracts, XII Interscience Conference on Antimicrobial Agents and Chemotherapy," Atlantic City, N.J., 1972, p. 77 and preceding two abstracts.

(2) H. R. Onishi, D. R. Daoust, S. B. Zimmerman, D. Hendlin, and E. O. Stapley, *Antimicrob. Agents Chemother.*, **5**, 38 (1974).

(3) H. C. Neu, *ibid.*, **6**, 170 (1974).

(4) "The United States Pharmacopeia," 19th rev., Mack Publishing

Co., Easton, Pa., 1975, p. 596.
(5) "Code of Federal Regulations," U.S. Government Printing Office, Washington, D.C., Title 21, 436.204, Apr. 1, 1977 Revision, p. 241.
(6) A. G. Mathews, C. J. Schram, and D. Minty, *Nature*, **211**, 959 (1966).
(7) J. P. Hou and A. Restivo, *J. Pharm. Sci.*, **64**, 710 (1975).
(8) R. Pfeiffer, G. L. Engel, and D. Coleman, *Antimicrob. Agents Chemother.*, **9**, 848 (1976).
(9) M. J. Pikal, A. L. Lukes, and J. E. Lang, *J. Pharm. Sci.*, **66**, 1312 (1977).

Synthesis and Evaluation of Benzylfluorenyl and 1-Arylethyl Quaternary Ammonium Salts for Antimicrobial and Antineoplastic Activities

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Received September 11, 1978, from the **College of Pharmacy and the* ‡*Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, Canada.* Accepted for publication January 18, 1979.

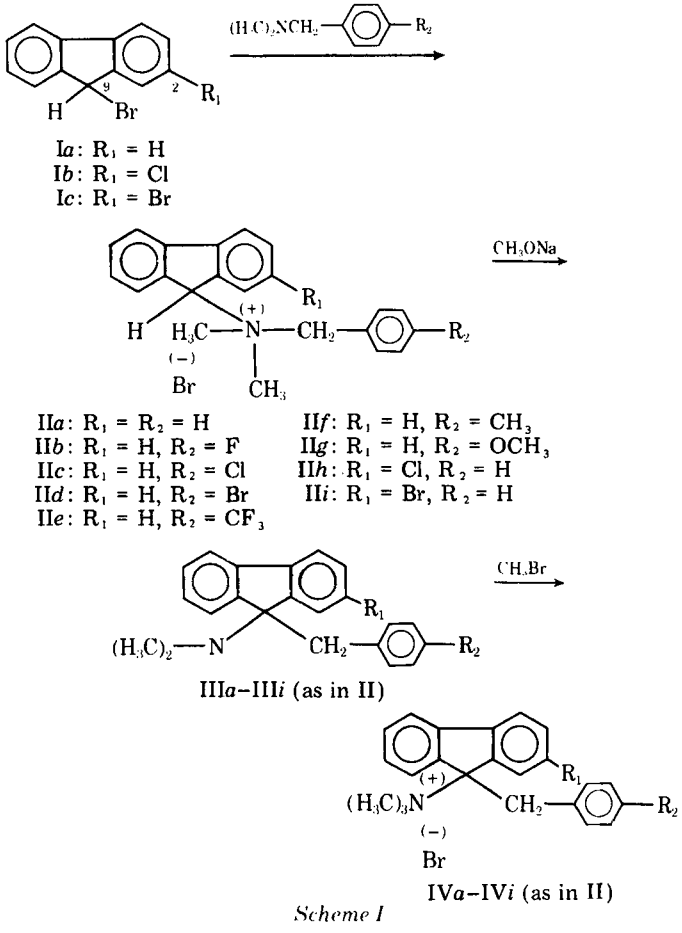
Abstract □ A number of substituted benzyldimethyl-9-fluorenylammonium bromides (II) and 9-benzylfluorenyl-9-trimethylammonium bromides (IV) were synthesized and examined for antimicrobial and anticancer activities. Series IV showed greater antimicrobial activity than Series II while some corresponding acyclic fluorene analogs were bereft of antimicrobial activities. Significant antineoplastic activity was not found in Series II and IV. Representative fluorenes subjected to a preliminary screen for various pharmacological activities revealed marked anti-inflammatory and analgesic properties coupled with some antihistaminic activities. The acyclic quaternary ammonium compounds demonstrated substantial pressor activities.

Keyphrases □ Benzylfluorene—quaternary ammonium derivatives, synthesis, antimicrobial and antineoplastic activity, structure-activity relationships □ Arylethylamines—quaternary ammonium derivatives, synthesis, antimicrobial and antineoplastic activity, structure-activity relationships □ Antimicrobial agents—benzylfluorene, arylethylamines, quaternary ammonium derivatives □ Antineoplastic agents—benzylfluorene, arylethylamines, quaternary ammonium derivatives

As a continuation of studies on new antimicrobial (1–4) and antineoplastic (5–8) agents, it was decided to examine the biological activities of some novel substituted fluorenes, representatives of which have demonstrated antimicrobial (9–11) and anticancer (12–14) activities. Since quaternary ammonium compounds are known to possess antiseptic properties, especially when bulkyl groups are attached to the quadrivalent nitrogen center, which may reduce adsorption onto serum proteins (15), the preparation of fluorenes attached to a quaternary nitrogen atom was contemplated.

The reactivity of benzylic derivatives is documented clearly (16); in the two planned structural isomer series (Scheme I), a benzyl group was attached either to a quaternary nitrogen atom (II) or directly to the fluorene ring (IV). In both cases, facile proton loss from the benzylic methylene group may occur. Furthermore, in Series II, the 9-fluorenyl proton may be considered labile. Thus, both II and IV have the potential for forming carbanions, which would be available for electrophilic attack by biological macromolecules.

In addition, it was proposed to synthesize some fluorene II and IV analogs so that structural requirements for bioactivities might be discerned. In the case of V, which is similar in structure to antifungal 9-fluorenol (9), both the benzyl and quaternary ammonium groups were removed. The quaternary ammonium compound VI was an analog of IV with the benzyl function replaced by a methyl group. Previous investigations compared the antimicrobial activities of flexible acyclic analogs of more rigid cyclic structures with the corresponding cyclic compounds (1–4). For this reason, the synthesis of several quaternary ammonium compounds derived from 1-arylethylamines (VII and VIII) was contemplated.



Scheme I

RESULTS AND DISCUSSION

The synthetic pathway for the prepared fluorenes is illustrated in Scheme I. The appropriate *N,N*-dimethylated benzylamines, prepared by the Eschwieler-Clark procedure, were quaternized with 9-bro-

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