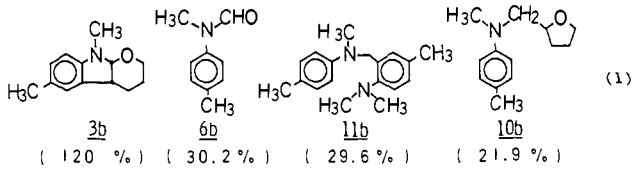
of **6b** and 29.6% of **11b** (eq 1). In the latter reaction, *N*-



(1)

methyl-*N*-(tetrahydrofurfuryl)-*p*-toluidine (**10b**) was isolated in a yield of 21.9%, which was identical with authentic material prepared by an alternative method[12] by IR, NMR, and TLC. The compound **10b** may be noteworthy in relation to the formation of **3b**.

The intermediates of heterotricycle formation were investigated by absorption spectrum measurement and ESR method. Mixing of *N*,*N*-dimethyl-*p*-anisidine (**4c**) and *N*,*N*-dimethylbenzylamine–palladium(II) σ-complex in benzene containing a small amount of acetic acid gave a pale green solution (λ_{max} 604 nm), which was ESR active and showed a fairly resolved signal (*g* value 2.0043). The signal gradually diminished at room temperature. The ESR spectrum consisted of 11 lines, showing high spin density on nitrogen, *N*-methyls, and ortho carbons of **4c**. The result is almost identical with the spin distribution of *N*,*N*-dimethyl-*p*-anisidine radical cation which was generated in acetonitrile by anodic oxidation of **4c**.[13] This may support an initial single electron transfer process in the palladium(II) oxidation of **4**.

In the absence of THF, the major products were cyclodimers; e.g., **4b** gave **5b** in a yield of 52.4%.[6] Therefore, the origin of the 9a carbon of **3** may be the *N*-methyl carbon of **4** and hence the formation of **3** must involve three new bond formations, i.e., *N*-methyl C–THF α-C, *N*-methyl C–O, and aromatic C–THF α-C bonds. The most probable mechanism is shown in Scheme I, which consists of double SET processes, 1,5-radical rearrangement, and double intramolecular radical cyclizations. The first cyclization with oxygen radical might preferentially give tetrahydropyranyl radicals,[14] which could form cis-fused products stereospecifically.[15]

Acknowledgment. We are grateful to Prof. Tsunao Hase for helpful discussion and mass analyses. Partial financial support from the Ministry of Education, Science, and Culture, Japanese Goverment (Grant-in-aid 59550582), is acknowledged.

Registry No. 3a, 98526-33-1; **3b**, 98526-34-2; **3c**, 98526-35-3; **3d**, 98526-36-4; **3e**, 98526-37-5; **4a**, 121-69-7; **4b**, 99-97-8; **4c**, 701-56-4; **4d**, 770-03-6; **4e**, 35113-87-2; **5b**, 7137-79-3; **6a**, 93-61-8; **6b**, 2739-04-0; **6d**, 65772-53-4; **7a**, 101-61-1; **8b**, 73172-84-6; **9c**, 5961-59-1; **9d**, 38036-47-4; **10b**, 98526-38-6; **11b**, 23970-61-8; THF, 109-99-9; $Pd(OAc)_2$, 3375-31-3; [*o*-[(dimethylamino)methyl]phenyl]palladium(II) acetate, 40243-08-1; silver(I) acetate, 563-63-3; iodobenzene, 591-50-4; *N*-methyl-*p*-toluidine, 623-08-5; tetrahydrofurfuryl bromide, 1192-30-9; 1,8-bis(dimethylamino)naphthalene, 20734-58-1.

Supplementary Material Available: Figures for C^{13} NMR and mass fragmentation pattern of the compounds **3** and ESR spectrum of the radical cation intermediate (1 page). Ordering information is given on any current masthead page.

(11) Arylpalladium(II) σ-complexes are formed by the oxidative addition of aryl halides to Pd(0); cf.: Patel, B. A.; Ziegler, C. B.; Cortese, N. A.; Plevyak, J. E.; Zebovitz, T. c.; Terpko, M.; Heck, R. F. *J. Org. Chem.* **1977**, *42*, 3903. Cortese, N. A.; Ziegler, C. B., Jr.; Hornjez, B. J.; Heck, R. F. *Ibid.* **1978**, *43*, 2952.

(12) **10b** was prepared by the reaction of *N*-methyl-*p*-toluidine with tetrahydrofurfuryl bromide in the presence of 1,8-bis(dimethylamino)-naphthalene: 40% yield, bp 127 °C (7 mmHg).

(13) Seo, E. T.; Nelson, R. F.; Fritsch, J. M.; Marcoux, L. S.; Leedy, D. W.; Adams, R. F. *J. Am. Chem. Soc.* **1966**, *88*, 3498.

(14) Intramolecular radical cyclization onto ω-olefins have been known preferentially to give five-membered rings.[15] In Pd-mediated cyclization, we have the precedence that the six-membered ring formation is preferred to the corresponding five-membered ring formation; e.g.: Semmelhack, M. F.; Bodurow, C. *Ibid.* **1984**, *106*, 1496. However, further model studies of intramolecular cyclizations of oxygen-centered radicals on an enamine functionality would be necessary to elucidate regioselectivity.

(15) Beckwith, A. L. J. *Tetrahedron* **1981**, *37*, 3073.

Thiolate Additions to Bicyclomycin and Analogues: A Structurally Novel Latent Michael-Acceptor System

Robert M. Williams,* Kohtaro Tomizawa,
Robert W. Armstrong, and Jen-Sen Dung

Department of Chemistry, Colorado State University
Fort Collins, Colorado 80523

Received May 28, 1985

Seven years after the isolation and structure elucidation of bicyclomycin (**1**), Iseki and co-workers[1] reported the regiospecific addition of sodium methane thiolate to the C-5 *exo*-methylene moiety of this structurally unique antibiotic affording the sulfide **3**. Iseki[2] has shown that bicyclomycin irreversibly forms covalent bonds to inner-membrane proteins (BBP's) of *E. coli* that were shown to be distinct from the penicillin-binding proteins; the function of the BBP's are presently unknown. It has been suggested[1] that "...the terminal olefinic group reacts with the sulfhydryl groups of the inner-membrane proteins and covalent bonds are formed. Thus the olefinic double bond seems to be the reactive site or functional site of bicyclomycin..." The chemical mechanism by which bicyclomycin undergoes simple thiolate additions and the intriguing connection between sulfide-forming capacity and BBP-covalent modification (i.e., antimicrobial activity) remains to be established.

We recently proposed[3] two distinct, yet related, possible chemical mechanisms by which **1** could irreversibly undergo alkylation at the C-5 *exo*-methylene. One mechanism[3] (shown) suggests that bicyclomycin may act as a "latent" α,β-unsaturated pyruvamide **2** which should undergo facile Michael-type addition at the C-5 exomethylene (**1** → **2** → **3**, Scheme I).

We have examined the thiolate addition reaction to semisynthetic[4] and totally synthetic[5] bicyclomycin systems in detail and report herein several fundamentally interesting and unexpected observations in this context that are relevant to the mechanism of action of bicyclomycin.

All thiolate reactions were carried out in homogeneous solutions of 0.2 M $NaSCH_3$ in 3:1 THF/H_2O (pH 12.5) at 25 °C. The acetonide derivative **13**[6] was used as a reactivity standard, which underwent clean thiolate addition analagous to **1**. Not surprisingly, the 6-deoxy derivatives **7**–**9**[3,5] were totally unreactive under these conditions. Surprisingly, the C-6 oxygenated derivatives **10**–**12**[3,5] were equally unreactive. Even more curious was the observation that the *N*,*N*′-dialkylated derivatives of the control, **14**[5] and **15**[5] proved to be completely unreactive to thiolate addition. From these simple observations, it can be hypothesized that free N–H amides *and* a C-1′ hydroxyalkyl residue play a subtle yet critical role in facilitating thiolate addition. The hydroxymethyl derivative **16**[4] was prepared and found to cleanly afford the sulfide **17**; thus, compound **16** represents the *minimal* structural requirements for sulfide formation at C-5; this notion is further supported by the following. Conversion of **16** to the *N*,*N*′-dimethyl derivative **18** and the silyl ether **19** afforded, in both cases, an unreactive substrate consistent with the behavior of **10**–**15**. Of the two monomethyl derivatives compound **20** proved unreactive, but compound **21** furnished the adduct **22**. Thus, it is clear that the amide adjacent to the C-6 hydroxyl must be unsubstituted (–N-

(1) Someya, A.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1979**, *32*, 402 and references cited therein concerning isolation and structural elucidation.

(2) (a) Tanaka, N.; Iseki, M.; Miyoshi, T.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1976**, *29*, 155. (b) Someya, A.; Iseki, M.; Tanaka, N. *Ibid.* **1978**, *31*, 712.

(3) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Med. Chem.* **1985**, *28*, 733.

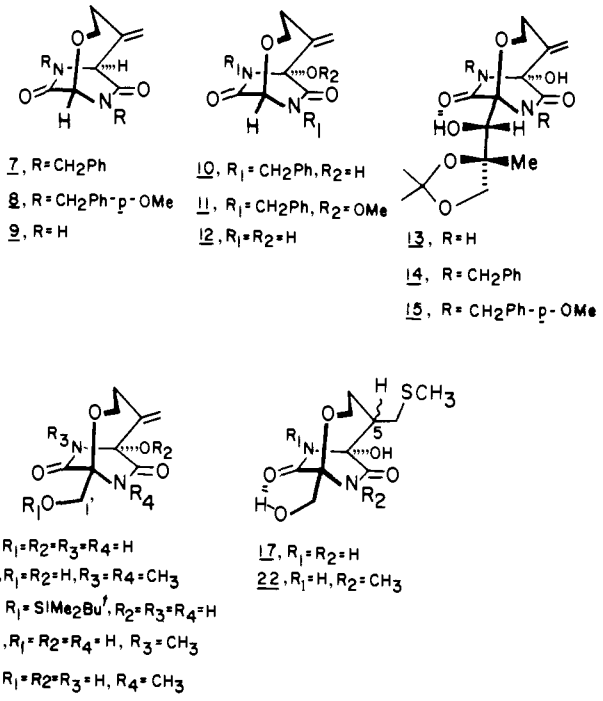
(4) Muller, B. W.; Zak, O.; Kump, W.; Tosch, W.; Wacker, O. *J. Antibiot.* **1979**, *32*, 689.

(5) (a) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1984**, *106*, 5748. (b) *Ibid.* **1985**, *107*, 3253.

(6) Kamiya, T.; Maeno, S.; Kitaura, Y. Belgium Patent 847 475.

(7) All new compounds displayed satisfactory spectroscopic and analytical data in accord with the assigned structures.

0002-7863/85/1507-6419$01.50/0



H–).

In order to rationalize the marked difference in reactivity between the unreactive *N*-alkylated derivatives (**14**, **15**, **18**, **20**) and their reactive counterparts (**1**, **13**, **16**, **21**), an imino alcohol tautomer B would minimize disturbance of the amide π-resonance by situating the forming electron pair on N-10 in an sp^2 hybrid orbital coplanar with the Dunitz ("exit") vector[8] of the forming C-6 carbonyl and C-9/N-10 amide system (see C). The N-alkylated derivatives obviously do not have access to such a tautomer. Due to the orthogonality of the C-6/N-10 bond to the C-9/N-10 π system (Figure 1), N-10 must bear the entire burden of carrying the electron pair resulting from cleavage of the C-6/N-10 bond. Electronically, this can be accommodated best by simultaneous protonation of the imino lone pair from the solvent during cleavage of the C-6/N-10 bond (B). What then, is the role of the C-1′ hydroxyalkyl group? ^{1}H NMR studies of **1**, **13**, and **16** clearly show that both the C-1′-OH and C-6-OH are tightly H-bonded to the C-9 and C-7 carbonyl oxygen atoms, respectively. It is reasonable then, that intramolecular proton transfer from the C-1′-OH to the C-9 carbonyl catalyzes the tautomerization of B → C. Further, inspection of Dreiding molecular models for the *reverse reaction* (i.e., closure of ketone C → B) very clearly shows that this eight-membered ring cannot readily achieve the Dunitz approach vector[8] of ~105°; a "relaxed" vector cone of ca. 60° is defined by the rigidity of the peptide bonds. Therefore, significant distortion of the eight-membered ring must accompany reclosure of C → B (or conversely, ring opening of B → C) via the minimum energy approach vector. These speculations, of course, implicitly assume that sulfide formation proceeds through the agency of the ring-opened species, a potentially experimentally verifiable entity.

Unfortunately, UV spectra of these bicyclic compounds at various pH conditions were uninformative. However, incubation of **16** in $^{18}OH_2$ at pH 12.5, removal of aliquots, and analysis by mass spectroscopy showed between 40% and 50% ^{18}O incorporation at the C-6 position after 30 min at 25 °C. In marked contrast, the unreactive compounds **18** and **19** incorporated less than 10% ^{18}O after as long as 47 h. When the thiolate reaction (**16** → **17**) was carried out in $^{18}OH_2$, the product **17** showed no ^{18}O incorporation. This is due to the much faster rate of sulfide

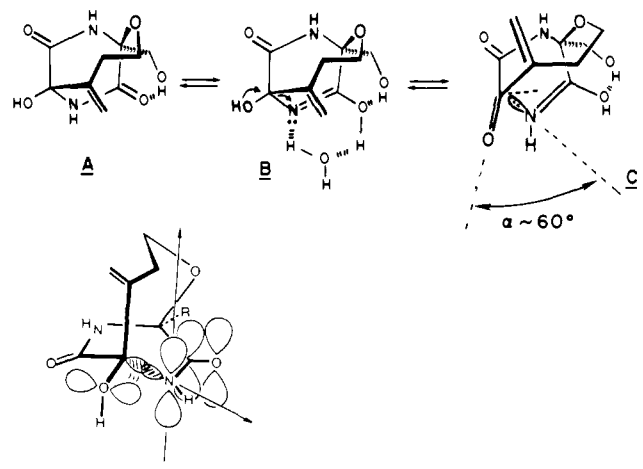


Figure 1.

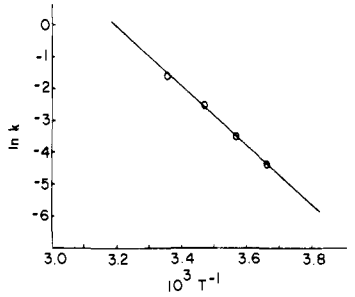
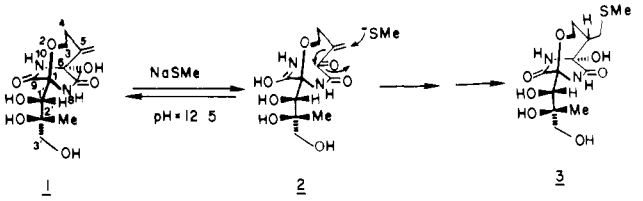
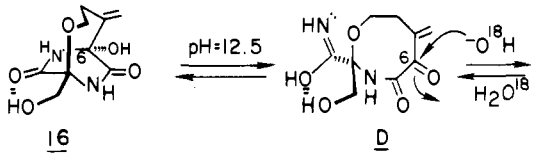


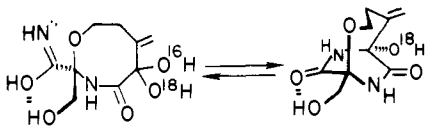
Figure 2. Plot of ln k vs. reciprocal temperature for the conversion of **16** → **17** at 0, 7, and 25 °C with [**16**] = 0.0039 M and [NaSMe] = 0.0098 M.

Scheme I



formation relative to that of hydration and exchange[9] of the putative eight-membered ring ketone (D → E). This result is



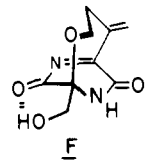


significant, however, in that it *excludes* the base-promoted expulsion of the C-6-OH in forming a C-6/N-10 amidine (F) as a possible reactive electrophilic intermediate, since such an intermediate would necessarily incorporate a significant amount of ^{18}O from the solvent at C-6 in forming **17**.

Subjecting the sulfide **17** to incubation in D_2O at pH 12.5 showed no trace of reversal to **16**, nor was there any H/D exchange

(8) Burgi, H. B.; Dunitz, J. D.; Lehn, J. M.; Wipff, G. *Tetrahedron* **1974**, *30*, 1563. Burgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065.

(9) ^{18}O exchange is routinely used for detection of keto tautomers; see, for example: Sue, J. M.; Knowles, J. R. *Biochemistry* **1978**, *17*, 4041 and references cited therein.



F

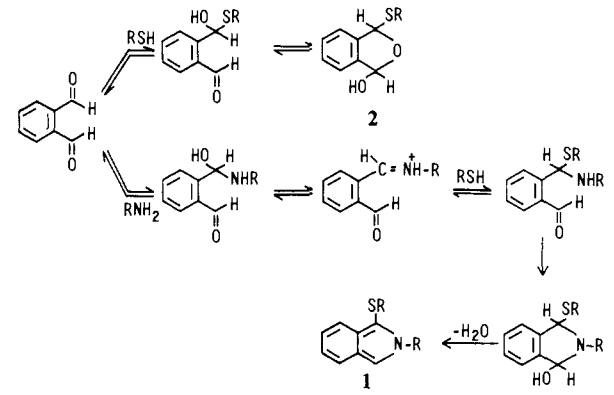
at C-5. For the specific case of **16** → **17**, we have obtained second-order rate constants $k = 1.95 \times 10^{-1}$ M^{-1} s^{-1} (measured over several half-lives, $t_{1/2} \sim 8$ min) at pH 12.5, 25 °C. At pH 7 the reaction is considerably slower; no reaction is observed at pH 3.5. The kinetics at pH 12.5 showed a significant temperature dependence (Figure 2) with an apparent $E_a = 18$ kcal/mol, $\Delta H^{\ddagger} = 17.5$ kcal/mol, ln $A = 28$, $\Delta S^{\ddagger} = -5$ eu, and $\Delta G^{\ddagger} = 19$ kcal/mol. A solvent deuterium isotope effect $K_{H_2O}/K_{D_2O} \sim 2.4$ is indicative of proton transfer in the rate-limiting step, and offers further support that tautomeric ring opening is obligate.

Our findings are consistent with the hypothesis that the bicylomycin ring system is capable of ring opening to an α,β-unsaturated ketone that requires a minimal structure containing (1) a C-5 *exo*-methylene, (2) a C-6-OH, (3) N–H amide at N-10, and (4) a hydroxyalkyl group at C–1. The irreversibility of the reaction may reflect a small energy gain from conjugating the newly formed ketone at C-6 with the C-5 olefin that is not enjoyed by the corresponding sulfide adducts. This is evidenced by the lack of ^{18}O incorporation in the sulfide adducts such as **17** (vide infra).

The compounds reported in this study have been evaluated for antimicrobial activity;[3] only **1** and **10** displayed activity. The lack of correlation between simple thiolate susceptibility and biological activity indicates that this interesting reaction alone cannot be used as the biomechanistic template. An alternative mechanism we have proposed[3] involving suicide inactivation of bacterial proteases by the bicyclomycin system is presently under scrutiny in our laboratories.

Acknowledgment. We thank the National Institutes of Health (Grant RO1AI18957) for financial support of this work and a Research Career Development Award (to R.M.W.). We are indebted to Fujisawa Co. for providing a gift of natural bicyclomycin. Professors Ken DeBruin and Lou Hegedus are acknowledged for helpful discussions.

Scheme I. Probable Reaction Paths for the Reaction of OPA with Amines and Thiols



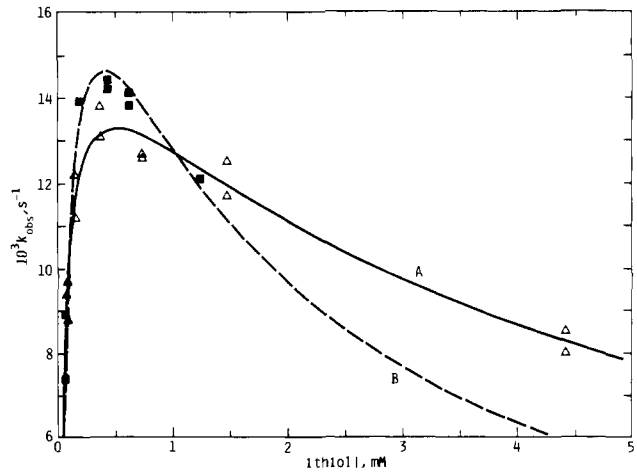


Figure 1. Plot of first-order rate constants for the reaction of OPA with Ala vs. 2ME and 3MPA concentrations in 80 mM borate buffer (pH 9.3) at 25 °C. Reaction progress determined by monitoring fluorescence intensity at 450 nm ($\lambda_{ex} = 340$ nm). Solid lines through the data points were generated by using best-fit parameters obtained from nonlinear least-squares analysis. Line A (thiol = 2ME) [OPA] = 0.144 mM, [Ala] = 3.2 μM. Line B (thiol = 3MPA) [OPA] = 0.179 mM, [Ala] = 2.1 μM.

Reaction of *o*-Phthalaldehyde with Alanine and Thiols: Kinetics and Mechanism

Osborne S. Wong,*‡⊥ Larry A. Sternson,†‡⊥ and Richard L. Schowen§

Department of Pharmaceutical Chemistry, Department of Chemistry, and Center for Bioanalytical Research University of Kansas, Lawrence, Kansas 66045

Received July 1, 1985

The reaction of OPA with a primary amine (RNH_2) in the presence of a thiol (RSH) produces 1-(alkylthio)-2-alkylisoindole **1** which is intensely fluorescent.[1-4] This unique reaction provides the basis for a highly sensitive and specific method for the detection of low levels of primary amines.[5-10] In this report we present results from kinetic studies of the reaction of *o*-phthalaldehyde (OPA) with alanine (Ala) in the presence of 2-mercaptoethanol (2ME) and 3-mercaptopropionic acid (3MPA). Our results show that, under the conditions studied, this OPA reaction follows the kinetic model shown below (eq 1 and 2), and the overall reaction

$$\text{OPA} + \text{Ala} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{I} \xrightarrow{k_2[\text{thiol}]} \text{P} \qquad (1)$$

$$\text{OPA} + \text{thiol} \overset{K}{\rightleftharpoons} \text{L (nonproductive equilibrium)} \qquad (2)$$

can be described by the mechanism depicted in Scheme I. Despite some speculation, to date the mechanism for the formation of the isoindole has not been established.[4,11,12] As such, the findings presented here are important not only for the fundamental understanding of the reaction mechanisms of carbonyl groups but also for the application of OPA chemistry for analysis and design

* Present address: Oread Laboratories, Inc., Lawrence, KS 66046.
† Present address: Smith Kline and French Laboratories, Philadelphia, PA 19101.
‡ Department of Pharmaceutical Chemistry.
§ Department of Chemistry.
⊥ Center for Bionanalytical Research.

(1) Roth, M. *Anal. Chem.* **1971**, *43*, 880–882.
(2) Simons, S. S., Jr.; Johnson, D. F. *J. Am. Chem. Soc.* **1976**, *98*, 7098–7099.
(3) Simons, S. S., Jr.; Johnson, D. F. *J. Chem. Soc., Chem. Commun.* **1978**, 374–375.
(4) Simons, S. S., Jr.; Johnson, D. F. *J. Org. Chem.* **1978**, *43*, 2886–2891.
(5) Lee, K. S.; Drescher, D. G. *Int. J. Biochem.* **1978**, *9*, 457–467.
(6) Joys, T. M.; Kim, H. *Anal. Biochem.* **1979**, *94*, 371–377.
(7) Benson, J. R.; Hare, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 619–622.
(8) Lindroth, P.; Mopper, K. *Anal. Chem.* **1979**, *51*, 1667–1674.
(9) Allison, L. A.; Mayer, G. S.; Shoup, R. E. *Anal. Chem.* **1984**, *56*, 1089–1096.
(10) Hodgin, J. C. *J. Liq. Chromatogr.* **1979**, *2*, 1047–1059.
(11) Trepman, D.; Chen, R. F. *Arch. Biochem. Biophys.* **1980**, *204*, 524–532.
(12) Stobaugh, J. Ph.D. Dissertation, University of Kansas, Lawrence, KS, 1983.

0002-7863/85/1507-6421$01.50/0