Notes

Reductive Amination of 3-Ketoanguidin and Antitumor Activity of the Products

T. Kaneko,* H. Wong, H. G. Howell, W. C. Rose, W. T. Bradner, and T. W. Doyle

Bristol-Myers Company, Pharmaceutical Research and Development Division, Syracuse, New York 13221-4755.
Received November 9, 1984

Amine-containing trichothecanes were prepared by reductive aminations of 3-ketoanguidin. In in vivo tests against P388 leukemia, most of them showed an improved activity compared to anguidin though their potency was decreased. 3-Ketoanguidin also produced some unexpected structures: oxazoline **5**, dioxalane **7**, and α-amino nitrile **13**.

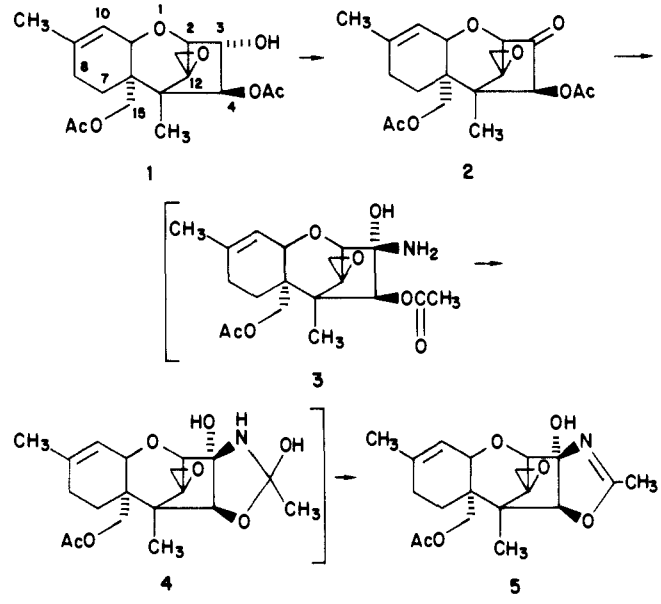
Anguidin (4β,15-diacetoxy-3α-hydroxy-12,13-epoxytrichotec-9-ene, **1**) is a sesquiterpene produced by *Fusarium equiseti* and belongs to the family of trichothecanes.[1] Many members of this family are known to have cytotoxic or antitumor activity. The mechanism of action of trichothecanes is believed to be inhibition of protein synthesis via binding to ribosomes.[2]

We have been interested in structural modifications of anguidin in search of potential antitumor agents and have published antitumor activities of 60 derivatives.[3] Of the derivatives reported by us and others,[4] there is no example of a derivative in which an amino group is directly attached to the trichothecane skeleton.[5] We expected that such compounds might have different pharmacokinetics and biomolecule interactions from anguidin.

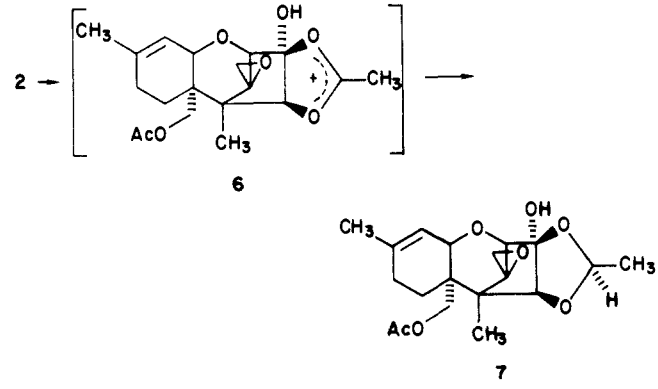
For the synthesis of amine-containing trichothecanes by reductive amination, 3-ketoanguidin (**2**) appeared to be an ideal starting material. This compound could be prepared in high yield by Swern oxidation of anguidin.[3] In this paper we report some unusual chemistry of this ketone and the synthesis and antitumor activity of 3-aminoanguidins.

Chemistry. We approached the problem of preparing amino derivatives by employing typical reductive amination conditions. Treatment of 3-ketoanguidin (**2**) with NH_4OAc and $NaBH_3CN$ in methanol, however, did not give the C3-amino compound but a new product with a molecular formula of $C_{19}H_{25}NO_6$ in 40% yield. The molecular formula indicated no reduction took place and the spectroscopic data suggested an oxazoline structure **5**. Indeed, when 4-Å molecular sieves were added to the reaction mixture, the same product was obtained in 90% yield. This result can be rationalized by initial addition of ammonia to the C3-carbonyl group and followed by attack of the amino group upon the C4-acetoxy group and dehydration (Scheme I). The dehydration of **4** occurs specifically as shown in **5** probably because of the strain that would be involved in the alternate structure.

Scheme I



Scheme II



On the other hand, treatment of **2** with $NaBH_3CN$ at pH ~3 (methyl orange as an indicator) gave dioxolane **7** in 37% yield. Its NMR spectrum showed a new doublet (3 H) at δ 1.46 and a quartet (1 H) at δ 5.48 corresponding to the ethylidenedioxy moiety. In an NOE experiment, irradiation of the C4 proton caused 5% signal enhancement of the ethylidene methine proton whereas irradiation of the ethylidene methyl group caused little signal enhancement anywhere. Thus, it was determined that the stereochemistry of the product was as shown in **7**. It was presumed in this case that the C4-acetoxy group attacked

(1) For reviews, see: Doyle, T. W.; Bradner, W. T. In "Anticancer Agents Based on Natural Products"; Cassidy, J. M., Douros, J. D., Eds.; Academic Press: New York, 1980; Chapter 2. Bamburg, J. R.; Strong, F. M. In "Microbial Toxins"; Kadis, S., Ciegler, A., Ajl, C. J., Eds.; Academic Press: New York, 1971; Vol. 7, Chapter 7.

(2) Ueno, Y. *Pure Appl. Chem.* **1979**, *49*, 1737. Carter, C. J.; Cannon, M. *Biochem. J.* **1977**, *166*, 399.

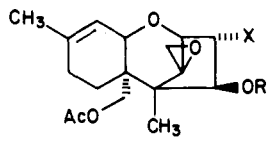
(3) Kaneko, T.; Schmitz, H.; Essery, J. M.; Rose, W. C.; Howell, H. G.; O'Herron, F. A.; Nachfolger, S.; Huftalen, J.; Bradner, W. T.; Partyka, R. A.; Doyle, T. W.; Davis, J.; Cundliffe, E. *J. Med. Chem.* **1982**, *25*, 579.

(4) Sigg, H. P.; Mauli, R.; Flurry, E.; Hauser, D. *Helv. Chim. Acta* **1965**, *48*, 962.

(5) Betainylanguidin in which *N,N,N*-trimethylglycine acid is attached to the C3-hydroxy group has been reported: Hartmann, G. R.; Richter, H.; Weiner, E. M.; Zimmermann, W. *Planta Med.* **1978**, *34*, 231.

0022-2623/85/1828-0958$01.50/0

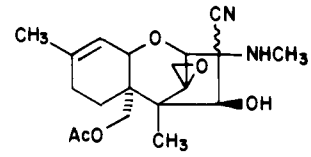
Table I. Synthesis of 3-Aminoanguidin



amine	product no.	X	R	yield, %
dimethylamine	8	$N(CH_3)_2$	H	29
morpholine	9		$COCH_3$	33
methylamine	10	$NHCH_3$	H	27
ethanolamine	11	$NHCH_2CH_2OH$	H	49
propargylamine	12	$NHCH_2C{\equiv}CH$	H	43

the C3-carbonyl group and the resulting acetoxonium ion was reduced (Scheme II). A similar participation of an acetyl group and the subsequent reduction is well-known in carbohydrate chemistry.[6]

With substituted amines the oxazoline formation was not possible, and the expected reductive amination products were obtained. The amines used and the products obtained are listed in Table I. The major byproduct was anguidin, from simple reduction of **2**.[7] In all cases, a single isomer was obtained. The coupling constant between the C2 proton and C3 proton was the same (5 Hz) as that in anguidin. If the C3 proton were on the α face, the molecular model suggests there would be a coupling constant close to 0. Therefore, the amino group was assigned to be on the α face. This was also consistent with the molecular model that showed that the β face was more accessible for the hydride attack. With use of methylamine, compound **13** was obtained in 24% yield in addition to the expected C3-methylamino product (27%). The former presumably results from the addition of cyanide to the intermediate iminium salt. In all cases except morpholine the C4-acetoxy group was hydrolyzed during the reaction.



13

Biological Results

The derivatives were tested ip in mice implanted ip with 10^6 P388 leukemia cells. The results are summarized in Table II. Anguidin was used concomitantly as a standard in each test and its activity is also shown.

From the table, oxazoline **5** is approximately 30% more active than anguidin although it is 4 times less potent. Dioxolane **7** is less active but slightly more potent. Both of these compounds can act as a latent form of 3-ketoanguidin, which is quite active against P388 cell growth.[3] The amine-containing derivatives show activities slightly higher or equivalent to anguidin; however, their potency is reduced several-fold. Variation in amine structure hardly makes any difference in the antitumor activity. In the C3-hydroxy series we found that substituents on the C3-oxygen atom (e.g., THP ether) eliminated activity. In the C3-amino series, however, a fairly bulky substituent is tolerated on the nitrogen atom without loss of activity. Otherwise, it appears that introducing a basic site at C3 has little effect on altering the antileukemic activity.

Table II. P388 Activity of Anguidin Derivatives

no.	T/C[c]	dose,[a] mg/kg	anguidin[a] T/C (OD)
5	228	6.4[b]	172 (1.6)[b]
7	150	0.8[b]	178 (1.6)[b]
8	181	16	163 (0.5)
9	156	4	144 (1.0)
10	156	8	144 (1.0)
11	144	16	144 (1.0)
12	139	16	144 (1.0)

[a] QD 1 → 5. [b] QD 1 → 9. [c] Effect based on median survival time (MST), T/C = (MST treated/MST control) × 100, and OD = optimum dose of anguidin (in milligrams/kilogram per injection) in the same experiment.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. NMR spectra were obtained on a Varian HA-100 or XL-100 spectrometer using tetramethylsilane as internal standard. IR spectra were obtained on a Beckman 4240 spectrophotometer. Elemental analyses were performed by the Analytical Department of these laboratories. Column chromatography was run with either Mallinckrodt SilicAR CC-7 (100–200 mesh) or Merck silica gel 60 (230–400 mesh).

(5′α)-2′-Methyl-15-acetoxy-3α-hydroxy-12,13-epoxytrichotheceno[3,4-*d*]oxazole (5). Sodium cyanoborohydride (1.56 g, 24.8 mmol) was added to a solution of 4β,15-diacetoxy-12,13-epoxytrichothec-9-en-3-one (9.03 g, 24.8 mmol) and ammonium acetate (19.25 g, 250 mmol) in 400 mL of methanol. After 22 h of stirring at room temperature, approximately 20 g of 4-Å molecular sieves was added. The reaction mixture was further stirred at room temperature for 20 h and then filtered and concentrated to 100 mL. The resulting solution was diluted with 500 mL of CH_2Cl_2 was washed with water and brine. The combined aqueous layers were reextracted with CH_2Cl_2. The CH_2Cl_2 layers were combined and dried over Na_2SO_4. The foam obtained after evaporation of the solvent was crystallized from diethyl ether to give 9.06 g (90%) of **5**: mp 136–140 °C; NMR ($CDCl_3$) δ 0.97 (s, 3 H), 1.75 (s, 3 H), 2.01 (m, 4 H), 2.08 (s, 3 H), 2.10 (s, 3 H), 2.63 (d, 1 H, J = 4 Hz), 2.95 (d, 1 H, J = 4 Hz), 3.63 (s, 1 H), 3.95 (d, 1 H, J = 13 Hz), 4.24 (d, 1 H, J = 13 Hz), 4.32 (br d, 1 H, J = 6 Hz), 4.89 (s, 1 H), 5.54 (br d, 1 H, J = 5 Hz); IR (KBr) 3350, 1742, 1665, 1432, 1371, 1335, 1238, 1175, 1073 cm^{-1}. Anal. ($C_{19}H_{25}NO_6$) C, H, N.

15-Acetoxy-3α-hydroxy-3β,4β-*O*,*O*-ethylidene-12,13-epoxytrichothec-9-ene (7). Sodium cyanoborohydride (126 mg, 2 mmol) was added to a solution of 4β,15-diacetoxy-12,13-epoxytrichothec-9-en-3-one (364 mg, 1 mmol) and 15 mL of isopropyl alcohol containing a small amount of methyl orange. Isopropyl alcohol saturated with HCl was added dropwise until the pH of the reaction media remained ca. 3. After 3 h of stirring at room temperature, the resulting mixture was diluted with 30 mL of CH_2Cl_2 and washed with water. Drying over Na_2SO_4 and removal of the solvent gave 338 mg of oil. This oil was purified by chromatography on silica gel, eluting with pentane–ethyl acetate (1:1), followed by recrystallization from diethyl ether to give 136 mg (37%) of colorless crystals: mp 172–173 °C; NMR ($CDCl_3$) δ 0.86 (s, 3 H), 1.46 (d, 3 H, J = 5 Hz), 1.73 (s, 3 H), 1.84–2.16 (m, 4 H), 2.07 (s, 3 H), 2.64 (d, 1 H, J = 4 Hz), 2.94 (d, 1 H, J = 4 Hz), 3.54 (s, 1 H), 3.95 (d, 1 H, J = 13 Hz), 4.03 (s, 1 H), 4.17 (d, 1 H, J = 4 Hz), 4.20 (d, 1 H, J = 13 Hz), 4.52 (s, 1 H), 5.48 (m, 2 H); IR (KBr) 3410, 1745, 1442, 1405, 1306, 1233, 1124 cm^{-1}. Anal. ($C_{19}H_{26}O_7$) C, H.

Synthesis of 15-Acetoxy-3α-(dimethylamino)-4β-hydroxytrichothec-9-ene (8) as a General Procedure. To a solution of **2** (364 mg, 1 mmol) in 5 mL of 2-propanol were added dimethylamine (270 mg, 6 mmol) and 0.69 mL of 10% HCl solution. After addition of $NaBH_3CN$ (44 mg, 0.7 mmol) the reaction

(6) Paulsen, H. In "Methods in Carbohydrate Chemistry"; Whistler, R. L., BeMiller, J. M., Eds.; Academic Press: New York, 1972; Vol. 6, Chapter 20.
(7) Wallace, E. M.; Pathre, S. V.; Mirocha, C. J.; Robinson, T. S.; Fenton, S. W. *J. Agric. Food Chem.* **1977**, 25, 836.
(8) Geren, R. I.; Greenberg, N. H.; MacDonald, M. D.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 8.

mixture was stirred for 18 h at room temperature. The solvent was then evaporated and the residue was dissolved in CH_2Cl_2 and washed with brine. The residue obtained after drying ($MgSO_4$) and evaporating CH_2Cl_2 was chromatographed on silica gel (1% CH_3OH–CH_2Cl_2) to give 190 mg of 1 and 100 mg (29%) of the title compound: mp 138–140 °C; NMR ($CDCl_3$) δ 0.88 (s, 3 H), 1.73 (s, 3 H), 2.0 (m, 4 H), 2.08 (s, 3 H), 2.78 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.64 (d, 1 H, J = 5 Hz), 3.90 (d, 1 H, J = 13 Hz), 4.19 (d, 1 H, J = 13 Hz), 4.28 (m, 2 H), 5.50 (br d, 1 H, J = 6 Hz); IR (KBr) 3480, 1715, 1390, 1260, 1040 cm^{-1}. Anal. ($C_{19}H_{29}NO_5$) H, N; C: calcd, 64.93; found, 64.47.

4β,15-Diacetoxy-3α-morpholinotrichothec-9-ene (9): amorphous solid, mp 73–75 °C; yield 33%; NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.72 (s, 3 H), 2.00 (m, 4 H), 2.06 (s, 3 H), 2.16 (s, 3 H), 2.43–2.69 (m, 4 H), 2.77 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.61–3.80 (m, 4 H), 3.90–4.21 (m, 3 H), 5.14, (d, 1 H, J = 3 Hz), 5.53 (br d, 1 H, J = 6 Hz); IR (KBr) 1742, 1403, 1245, 1119 cm^{-1}. Anal. ($C_{23}H_{33}NO_7 \cdot 0.5H_2O$) C, H, N.

15-Acetoxy-3α-(methylamino)-4β-hydroxytrichothec-9-ene (10): amorphous solid, mp 92–94 °C; yield 27%; NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.74 (s, 3 H), 2.11 (s, 3 H), 2.62 (s, 3 H), 2.78 (d, 1 H, J = 4 Hz), 3.08 (d, 1 H, J = 4 Hz), 3.70 (d, 1 H, J = 5 Hz), 3.80–4.34 (m, 4 H), 5.48 (d, 1 H, J = 4 Hz). Anal. ($C_{18}H_{27}NO_5 \cdot 0.5H_2O$) C, H, N.

An earlier fraction in the silica gel chromatography produced 260 mg (24%) of 15-acetoxy-3-cyano-3-(methylamino)-4β-hydroxytrichothec-9-ene (13): mp 92–94 °C; NMR ($CDCl_3$) δ 0.88 (s, 3 H), 1.72 (s, 3 H), 2.00 (m, 4 H), 2.07 (s, 3 H), 2.64 (s, 3 H), 2.79 (d, 1 H, J = 4 Hz), 3.12 (d, 1 H, J = 4 Hz), 3.88 (d, 1 H, J = 12 Hz), 3.89 (s, 1 H), 4.11 (d, 1 H, J = 12 Hz), 4.29 (s, 1 H), 4.41 (d, 1 H, J = 4 Hz), 5.43 (d, 1 H, J = 5 Hz); IR (KBr) 3450, 2230, 1740, 1450, 1417, 1242, 1095, 1042 cm^{-1}; MS (CI), m/e 362 (M^+, 9%), 335 (M_+ – HCN, 100%). Anal. ($C_{19}H_{25}N_2O_5 \cdot 0.25H_2O$) C, H, N.

15-Acetoxy-3α-[(2-hydroxyethyl)amino]-4β-hydroxytrichothec-9-ene (11): amorphous solid, mp 54–56 °C; yield 49%; NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.73 (s, 3 H), 1.95 (m, 4 H), 2.08 (s, 3 H), 2.85 (d, 1 H, J = 4 Hz), 2.96 (t, 2 H, J = 5 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.16 (dd, 1 H, J = 5, 4 Hz), 3.54–3.78 (m, 3 H), 3.86 (d, 1 H, J = 13 Hz), 3.98 (d, 1 H, J = 5 Hz), 4.16 (d, 1 H, J = 4 Hz), 4.18 (d, 1 H, J = 13 Hz), 5.45 (br d, 1 H, J = 6 Hz); IR (KBr) 3440, 3300, 1740, 1447, 1366, 1245, 1070, 1045 cm^{-1}. Anal. ($C_{19}H_{29}NO_6 \cdot 0.5H_2O$) C, H, N.

15-Acetoxy-3α-(propargylamino)-4β-hydroxytrichothec-9-ene (12): amorphous solid, mp 59–61 °C; yield 43%; NMR ($CDCl_3$) δ 0.86 (s, 3 H), 1.72 (s, 3 H), 1.80–2.10 (m, 4 H), 2.08 (s, 3 H), 2.25 (t, 1 H, J = 2 Hz), 2.67 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.35 (dd, 1 H, J = 5, 4 Hz), 3.61 (d, 2 H, J = 2 Hz), 3.65 (d, 1 H, J = 5 Hz), 3.86 (d, 1 H, J = 13 Hz), 4.03 (br d, 1 H, J = 5 Hz), 4.16 (d, 1 H, J = 13 Hz), 4.17 (d, 1 H, J = 4 Hz), 5.45 (br d, 1 H, J = 5 Hz); IR (KBr) 3460, 3300, 2430, 1742, 1454, 1370, 1250, 1070 cm^{-1}. Anal. ($C_{20}H_{27}NO_5 \cdot 0.5H_2O$) C, H, N.

Registry No. 1, 2270-40-8; 2, 2269-44-5; 5, 79320-86-8; 7, 79320-89-1; 8, 96110-50-8; 9, 96110-51-9; 10, 96110-52-0; 11, 96110-53-1; 12, 96110-54-2; 13, 96110-55-3; $HNMe_2$, 124-40-3; NH_2Me, 74-89-5; $NH_2(CH_2)_2OH$, 141-43-5; $NH_2CH_2C{\equiv}CH$, 2450-71-7; morpholine, 110-91-8.

4-Deoxypyrido[1′,2′:1,2]imidazo[5,4-*c*]rifamycin SV Derivatives. A New Series of Semisynthetic Rifamycins with High Antibacterial Activity and Low Gastroenteric Absorption

Egidio Marchi,*† Laura Montecchi,† Anna Paola Venturini,† Giuseppe Mascellani,‡ Mario Brufani,§ and Luciano Cellai⊥

Alfa Farmaceutici S.p.A., Via Ragazzi del '99 n. 5, 40133 Bologna, Opocrin S.p.A., 41040 Corlo, Modena, Gruppo di Chimica Biologica e Strutturistica Chimica, Università di Roma, 00185 Rome, and Istituto di Strutturistica Chimica "Giordano Giacomello", C.N.R., 00016 Monterotondo Stazione, Rome, Italy. *Received February 13, 1984*

A series of 4-deoxypyrido[1′,2′:1,2]imidazo[5,4-*c*]rifamycin SV derivatives (6–11) were prepared that demonstrated high antibacterial activity suitable for an intestinal disinfectant. These compounds are zwitterionic in nature and are poorly absorbed through the gastroenteric tract but maintain the ability to cross the bacterial cell wall. X-ray crystallographic data are presented to demonstrate the zwitterionic nature of these compounds. The structure–activity relationship of this novel series of antibiotics is discussed and the derivative with the highest ratio between subcutaneous and oral activity (6) was selected for clinical development. At the outset of this work several 3-(quaternary ammonium bromides) (1–5) were prepared and tested for antibacterial activity. These compounds were demonstrated to be too polar to even cross the bacterial cell wall but led to the synthesis of 6–11.

New derivatives of rifamycin SV[1] (Figure 1), with a pharmacokinetic behavior different from that of rifamycin SV itself and from that of rifampicin[2] (Figure 1), were sought with the aim of exploring new specific applications of this type of antibiotics. As references, rifamycin SV undergoes rapid elimination via the biliary route,[3] while rifampicin is not used for urinary or gastroenteric infections because of its low urinary elimination and good oral absorption.[4] Therefore it was planned to synthesize new, highly active, broad-spectrum rifamycin SV derivatives that are able to cross the bacterial cell wall but are not absorbed at the gastroenteric level and are thus suitable for the oral therapy of bacterial intestinal infections.

While structure–activity relationships (SAR) are well defined for both intrinsic and antibacterial activity,[5] no detailed study on the transport of rifamycins across the gastroenteric membranes is yet available. Nonetheless, it

† Alfa Farmaceutici S.p.A.
‡ Opocrin S.p.A.
§ Università di Roma.
⊥ Istituto di Strutturistica Chimica "Giordano Giacomello".

(1) Sensi, P.; Timbal, M. T.; Maffii, G. *Experientia* **1960**, *16*, 412.
(2) Maggi, G.; Pasqualucci, C. R.; Ballotta, R.; Sensi, P. *Chemotherapia* **1966**, *11*, 285.
(3) Bergamini, M.; Fowst, G. *Arzneim.-Forsch.* **1965**, *15*, 951.
(4) Binda, G.; Domenichini, E.; Gottardi, A.; Orlandi, B.; Ortelli, E.; Pacini, B.; Fowst, G. *Arzneim.-Forsch.* **1971**, *21*, 1907.
(5) (a) Brufani, M. In "Topics in Antibiotic Chemistry"; Sammes, P.G., Ed.; Ellis Horwood Ltd: Chichester, 1977; Vol. 1, pp 91–217 and references cited therein. (b) Lancini, G.; Zanichelli, W. In "Structure–Activity Relationships Among the Semisynthetic Antibiotics"; Perlmann, D., Ed.; Academic Press: New York, 1977; pp 531–600 and references cited therein.

0022-2623/85/1828-0960$01.50/0