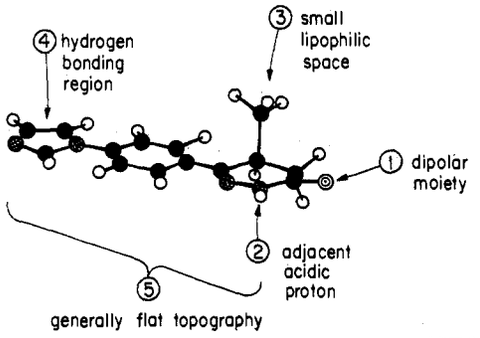


Figure 3. Five-point model illustrated with CAMSEQ-II generated structure of the 4,5-dihydro-3(2*H*)-pyridazinone **2**.

sulmazole, and fenoximone. By applying a linear regression analysis to log (AD-ED_{50}) vs. log (PDE), an equation describing the positive inotropic potency and enzyme inhibition was determined:

$$\log \text{AD-}ED_{50} = 0.56 \log (\text{PDE}) - 1.60$$

$$r = 0.89 \qquad s = 0.32 \qquad n = 6$$

The graphical relationship of the correlation is depicted in Figure 2. This correlation strongly suggests that the principal component of the positive inotropic action of these agents is inhibition of fraction III cardiac phosphodiesterase. This finding is consistent with recently reported studies with amrinone[22,23] and milrinone[9] on crude cardiac phosphodiesterase and with fenoximone on purified cardiac phosphodiesterase.[24] Initial studies with amrinone[25] suggested that inhibition of cAMP–PDE was not a component of its mechanism. It is of considerable significance that the present report is the first to correlate, in a quantitative manner, the in vivo positive inotropic potencies of several different chemical classes with inhibition of a specific molecular fraction of cardiac phosphodiesterase. Sulmazole has been reported to have a direct action on intracellular calcium transport that may also contribute to its positive inotropic action.[26]

Molecular Modeling. Analysis of several new cardiotonic agents by molecular modeling techniques suggests spatial and electronic similarities among cardiotonics of diverse structural classes such as **1**, **2**, amrinone, milrinone, and fenoximone. This observation has resulted in a hypothetical five-point model for positive inotropic activity. Figure 3 denotes the conformational structure of **2** as determined by CAMSEQ-II.[27] The salient features of the five-point model are as follows: (1) the presence of a strong dipole (carbonyl) at one end of the molecule, (2) an adjacent acidic proton, (3) a methyl-sized lipophilic space, (4) a relatively flat overall topography, and (5) a basic or hydrogen bond acceptor site opposite the dipole.[28]

In conclusion, we are reporting two members of a new class of potent positive inotropic agents: 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones, **1** and **2**. Compound **2** is the most potent nonsympathomimetic, noncardiac glycoside cardiotonic agent reported to date. The principal component of positive inotropic mechanism is consistent with inhibition of fraction III cardiac phosphodiesterase, and through the use of molecular modeling techniques, a five-point model has been constructed to rationalize structural features that are necessary for positive inotropic activity in this class of selective cAMP–PDE inhibitors.

Acknowledgment. The technical assistance of G. Bobowski, R. Potoczak, R. McNish, and M. Quade is gratefully acknowledged. Helpful discussions regarding the molecular modeling studies and statistical analyses were provided by J. Topliss, J. Vinson, D. Ortwine, W. Ewy, Y. C. Park, and D. Worth.

Registry No. 1, 84243-58-3; 1·HCl, 89198-09-4; 2, 86798-59-6; 2·HCl, 90791-23-4; 3, 84243-57-2; 4, 88427-81-0; fluorobenzene, 462-06-6; succinic anhydride, 108-30-5; 4-fluoro-γ-oxobenzenebutanoic acid, 366-77-8; imidazole, 288-32-4; 4-(1*H*-imidazol-1-yl)benzaldehyde, 10040-98-9; hydrazine, 302-01-2.

James A. Bristol,* Ila Sircar, Walter H. Moos
Department of Chemistry

Dale B. Evans, Ronald E. Weishaar
Department of Pharmacology
Warner-Lambert/Parke-Davis Pharmaceutical Research
Ann Arbor, Michigan 48105

Received April 16, 1984

(22) Endoh, M.; Yamashita, S.; Taira, N. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 775.
(23) Honerjager, P.; Schafer-Korting, M.; Reiter, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *318*, 112.
(24) Kariya, T.; Wille, L. J.; Dage, R. C. *J. Cardiovasc. Pharmacol.* **1982**, *4*, 509.
(25) Alousi, A. A.; Farah, A. E.; Lesher, G. Y.; Opalka, C. J. *Circ. Res.* **1979**, *45*, 666.
(26) Solaro, R. J.; Ruegg, J. C. *Circ. Res.* **1982**, *51*, 290.
(27) Potenzone, R., Jr.; Cavicchi, E.; Weintraub, H. J. R.; Hopfinger, A. J. *Comput. Chem.* **1977**, *1*, 187.
(28) Moos, W. H.; Sircar, I.; Bristol, J. A., unpublished results.

Oxidation of Sparteines by Cytochrome P-450: Evidence against the Formation of *N*-Oxides

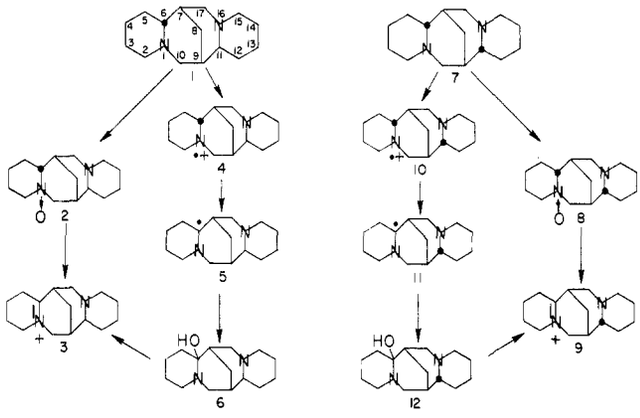
Sir:

(−)-Sparteine (**1**) is an alkaloid with antiarrhythmic properties, which is oxidized by cytochrome P-450 to yield Δ^2- and Δ^5-dehydrosparteine as major products.[1,2] Our interests in the genetic polymorphism of sparteine metabolism[3] led to further investigation of the reported obligate intermediacy of *N*-oxides in the metabolism of sparteine and its derivatives.[1,4] The Δ^5-oxidation of **1** is catalyzed by purified P-450_{UT-H} and anti-P-450_{UT-H} inhibited the formation of >95% of this activity in microsomes,[3] and this reaction was examined as a model for N-oxidation vs. dealkylation with sparteine as well as other substrates. The formation of these and a number of other *N*-oxides, as intermediates in dealkylation reactions or as stable metabolites,[5] is not in accord with either several earlier dealkylation studies[6] or our current view of the mechanism of oxidation in cytochrome P-450 catalyzed reactions.[7]

(1) Eichelbaum, M.; Spannbrucker, N.; Steincke, B.; Dengler, H. J. *Eur. J. Clin. Pharmacol.* **1979**, *16*, 183.
(2) Vinks, A.; Inaba, T.; Otton, S. V.; Kalow, W. *Clin. Pharmacol. Ther.* **1983**, *31*, 23. Otton, S. V.; Inaba, T.; Mahon, W. A.; Kalow, W. *Can. J. Physiol. Pharmacol.* **1982**, *60*, 102.
(3) Distlerath, L. M.; Guengerich, F. P., submitted for publication.
(4) Spiteller, M.; Spiteller, G. In "Biological Oxidation of Nitrogen"; Elsevier-North Holland: New York, 1978; p 109.
(5) Hlavica, P. *CRC Crit. Rev. Biochem.* **1982**, *12*, 39–101.
(6) McMahon, R. E.; Culp, H. W.; Occolowitz, J. C. *J. Am. Chem. Soc.* **1969**, *91*, 3389. McMahon, R. E.; Sullivan, H. R. *Xenobiotica* **1977**, *7*, 377. Misra, A. L.; Pontani, R. B.; Vadlamini, N. L. *Xenobiotica* **1979**, *9*, 189.
(7) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9.

0022-2623/84/1827-1101$01.50/0 © 1984 American Chemical Society

Scheme I



Compounds **3**, **7** (α-isosparteine), **8**, **9**, and sparteine N^{16}-oxide (**13**) were synthesized by variations of literature procedures.[8] Liver microsomes prepared from untreated adult male Sprague–Dawley rats (in 0.1 M potassium phosphate, pH 7.7, with 0.5 mM NADPH and a glucose 6-phosphate/glucose 6-phosphate dehydrogenase generating system at 37 °C)[16] metabolized **1** and **7** to **3** and **9**, respectively (Scheme I).[17] The respective rates were 0.23 and 1.20 nmol of product formed/nmol of cytochrome P-450/30 min. When either **8**, **13**, or a mixture of **2** and **13** (at 0.2 mM) was incubated in the above (aerobic) buffer system in the absence or presence of liver microsomes (with or without NADPH present), neither **3** nor **9** (nor other iminium compounds giving rise to enamines separable by capillary GLC) was formed at rates $\geq 10^{-4}$ h^{-1}, and no other products were found by use of GLC or TLC (vide infra).

The synthetic N-oxides were shown to interact with cytochrome P-450 heme as judged by spectral perturbation experiments with microsomes, in which "type II" difference spectra (λ_{max} 414 nm, λ_{min} 375 nm) with apparent dissociation constants of 0.42–0.60 mM were obtained.

[6-^{3}H]-**1** and [6-^{3}H]-**7** were prepared by stereospecific reduction of **3** and **9**, respectively, with NaB^3H_4[9] at pH 8 (specific radioactivity 56 and 50 mCi/mmol, respectively) and incubated with rat liver microsomes and the NADPH-generating system in the same buffer. TLC analysis (alumina; $CHCl_3/CH_3OH$/28% aqueous NH_4OH, 90/10/1, v/v/v; R_f values 0.59, 0.26, 0.22, 0.06, 0.06, 0.30, and 0.11 for **1**, **2**, **13**, **3**, **9**, **7**, and **8**, respectively) of the incubates indicated that the N-oxides **2**, **8**, and **13** did not accumulate at levels $\geq 5\%$ those of **3** and **9**. Further, ^3H_2O was released in each case (measured after two CH_2Cl_2 extractions of the incubates raised to pH 13). The kinetic tritium isotope effects for removal of the C-6 hydrogens of **1** and **7** were 2.8 and 2.3 (±0.2), respectively.

The results are not compatible with mechanisms involving N-oxides as competent intermediates in the formation of the iminium derivatives, i.e., **1** → **2** → **3** and **7** → **8** → **9**. This view is based upon the observations that (a) N-oxides at the junctures of both chair/boat (N^{16}-oxide of **1**) and chair/chair (N^1-oxide of **1** and N-oxide of **7**) quinolizidine systems[9,11] were stable under the conditions used, (b) the N-oxides did not accumualte during incubations in which the iminium compounds were formed, and (c) the kinetic isotope effects were similar to those calculated for and observed in other cytochrome P-450 mediated N-dealkylation reactions interpreted to proceed via initial aminium ion formation.[18] These kinetic isotope effects are consistent neither with initial carbon hydroxylation nor decomposition of an N-oxide in the overall reaction. In the acid- and ferrous iron catalyzed Polonovski rearrangement of N-oxides to iminium ions,[19] α-hydrogen loss is thought to be slower than subsequent aminium ion oxidation, as rates of proton abstraction vary widely with pH to determine product distribution.[20] Thus, high *intra*molecular kinetic isotope effects (e.g., k_H/k_T = 10–20) would be expected. As an alternative, the mechanisms **1** → **4** → **5** → **6** → **3** and **7** → **10** → **11** → **12** → **9** are preferred, in which oxygenated cytochrome P-450 (formally perferryl) abstracts an electron from the nitrogen, the aminium radical rearranges with loss of an α-hydrogen to form a carbon-centered radical α to the nitrogen, oxygen rebound to the carbon radical occurs, and the resulting carbinolamine collapses by dehydration.[7] This general mechanism for N-dealkylation is supported by other studies involving ring opening of cyclopropylamines[21] and the metabolism of dihydropyridines.[22] The cytochrome P-450 mediated formation of N-oxides can also be initiated by one electron oxidation of the amine, but oxygen rebound to the aminium radical should *only occur when* (a) the radical is rendered unusually stable (e.g., azo com-

(8) The dihydrosulfate of **3** was prepared from **1** by mecuric acetate oxidation[9] (mp 237–238 °C);[10] downfield ^{13}C NMR peaks at 190.61, 64.93, 61.70, 56.99, 56.94, and 56.58 ppm vs. DSS (D_2O).[11,12] **13** was formed from **1** by treatment with H_2O_2 in CH_3OH;[13] ^{13}C NMR indicated that a mixture of **2** and **13** was present;[14] crystallization (ethanol) or alumina chromatography (ethyl acetate/CH_3OH) gave **13** as the hydrosulfate (mp 211–213 °C),[10] the dihydrosulfate (mp 244–245 °C),[10] and the hydrate of the free base (mp 108–110 °C);[10] downfield ^{13}C NMR peaks at 78.66, 73.81, 65.55, 61.27, 58.34, and 56.29 ppm (D_2O); IR 2806, 2765 cm^{-1}.[15] Pyrrolysis[15] of the free base of **13** and vacuum distillation yielded the dihydrosulfate of **9** (mp 240 °C);[10] downfield ^{13}C NMR peaks at 190.57, 68.93, 59.18, 56.80, 56.49, and 56.32 ppm (D_2O). $NaBH_4$ reduction[9] of **9** at pH 8 and chromatography on alumina with benzene/ether[13] yielded the dihydrosulfate of **7** (mp 203–206 °C;[10] mp of dipicrate 217–219 °C),[13] which showed a simplified ^{13}C NMR spectrum with downfield peaks at 70.43, 62.68, and 55.69 ppm (D_2O) and only five upfield peaks.[11,12] **8** was prepared from **7** by treatment with H_2O_2 in CH_3OH.[13]

(9) Leonard, N. J.; Thomas, P. D.; Gash, V. W. *J. Am. Chem. Soc.* **1955**, *77*, 1552.

(10) Satisfactory elemental analyses were obtained for C, H, and N (Galbraith Laboratories, Knoxville, TN) and probe mass spectra (electron impact or chemical ionization) were consistent with the assigned structures.

(11) Bohlman, F.; Zeisberg, R. *Chem. Ber.* **1975**, *108*, 1043.

(12) Downfield ^{13}C NMR shifts for **1** (hydrosulfate) were at 69.21, 65.71, 59.94, 59.27, 57.53, and 57.04 ppm (D_2O).

(13) Galinovsky, F.; Fischer, W. *Monatsh. Chem.* **1956**, *87*, 763.

(14) Wiewiorowski, M.; Baranowski, P. *Bull. Acad. Pol. Sci. Chem.* **1962**, *10*, 537.

(15) Wiewiorowski, M.; Baranowski, P. *Bull. Acad. Pol. Sci. Chem.* **1962**, *10*, 543.

(16) Guengerich, F. P.; Martin, M. V. *Arch. Biochem. Biophys.* **1980**, *205*, 365.

(17) Assayed as the extracted Δ^5 enamines by GLC (Durawax capillary column, 160 °C) with flame-ionization detection and 17-ethoxysparteine as an internal standard.[1]

(18) Shea, J. P.; Ford, G. P.; Nelson, S. D. *J. Am. Chem. Soc.* **1983**, *105*, 5451. Miwa, G. T.; Walsh, J. S.; Kedderis, G. L.; Hollenberg, P. F. *J. Biol. Chem.* **1983**, *258*, 14445.

(19) Craig, J. C.; Dwyer, F. P., Glazer, A. N.; Horning, E. C. *J. Am. Chem. Soc.* **1961**, *83*, 1871.

(20) Ferris, J. P.; Gerwe, R. D.; Gapski, G. R. *J. Org. Chem.* **1968**, *33*, 3493.

(21) Macdonald, T. L.; Zirvi, K.; Burka, L. T.; Peyman, P.; Guengerich, F. P. *J. Am. chem. Soc.* **1982**, *104*, 2050. Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048. Guengerich, F. P.; Willard, R. J.; Shea, J. P.; Macdonald, T. L., submitted for publication.

(22) Augusto, O.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 11288.

pounds),[23] (b) no α-hydrogens are available, or (c) the α-hydrogens are inaccessible for abstraction (e.g., Bredt's rule).[7] Thus, not only does cytochrome P-450 not produce *N*-oxides as intermediates in overall dealkylation pathways but the enzyme *should not form these* at all except in the above three special cases. Another mammalian microsomal enzyme, the flavin-containing monooxygenase,[24] can form *N*-oxides from many amines because it operates via a heterolytic mechanism involving a flavin 4a hydroperoxide,[25] which clearly differs from the radicaloid cytochrome P-450 mechanism proposed here.

(23) Prough, R. A.; Brown, M. I.; Dannan, G. A.; Guengerich, F. P. *Cancer Res.* **1984**, *44*, 543.
(24) Ziegler, D. M. *Enzym. Basis Detoxication* **1980**, *1*, 201.

Acknowledgment. This work was supported by NIH Research Grants ES 01590 and ES 00267. Thanks are extended to J. E. Hill, T. M. Harris, and T. L. Macdonald for providing NMR spectra and helpful discussions.

(25) 1 proved not to be a substrate for this enzyme (Ziegler, D. M.; Guengerich, F. P., unpublished results).

F. Peter Guengerich*
Department of Biochemistry and Center in Molecular Toxicology Vanderbilt University Nashville, Tennessee 37232

Received May 7, 1984

Articles

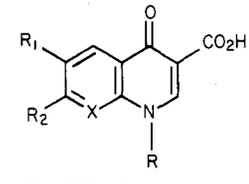
Novel Amino-Substituted 3-Quinolinecarboxylic Acid Antibacterial Agents: Synthesis and Structure–Activity Relationships[1]

Mark P. Wentland,* Denis M. Bailey, James B. Cornett, Richard A. Dobson, Ronald G. Powles, and Roland B. Wagner

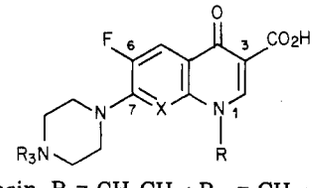
Departments of Medicinal Chemistry and Microbiology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.
Received January 23, 1984

A series of novel 3-quinolinecarboxylic acid derivatives have been prepared and their antibacterial activity evaluated. These derivatives are characterized by fluorine attached to the 6-position and substituted amino groups appended to the 1- and 7-positions. Structure–activity relationship studies indicate that antibacterial potency is greatest when the 1-substituent is methylamino and the 7-substituent is either 4-methyl-1-piperazinyl, **16**, or 1-piperazinyl, **21**. Derivatives **16** and **21**, the 1-methylamino analogues of pefloxacin and norfloxacin, respectively, show comparable in vitro and in vivo antibacterial potency to these two known agents. The activity (vs. *Escherichia coli* Vogel) of **16** (amifloxacin) is the following: in vitro MIC (μg/mL) = 0.25; in vivo (mice) PD_{50} (mg/kg) = 1.0 (po), 0.6 (sc).

Since the introduction in 1963 of nalidixic acid[2] as a systemic Gram-negative antibacterial agent, many related derivatives[3] have been made. The newest derivatives, which include pefloxacin,[4] norfloxacin,[5] AT-2266,[6] DL-8280,[7] and Bay o 9867[8] are considerably more potent and have a broader spectrum of antimicrobial activity than their predecessors. These five agents share several common structural features among which are fluorine and piperazinyl groups attached to the quinoline or naphthyridine ring. Additionally, pefloxacin, norfloxacin, and AT-2266 along with most other therapeutically interesting antibacterials[3] in this class (e.g., nalidixic acid, cinoxacin, oxolinic acid, rosoxacin, and pipemidic acid) have an ethyl group appended to the ring nitrogen (commonly position 1).



nalidixic acid, R = CH_2CH_3; R_1 = H; R_2 = CH_3; X = N
oxolinic acid, R = CH_2CH_3; R_1, R_2 = OCH_2O; X = CH
rosoxacin, R = CH_2CH_3; R_1 = H; R_2 = 4-pyridinyl, X = CH
miloxacin, R = OCH_3; R_1, R_2 = OCH_2O; X = CH



pefloxacin, R = CH_2CH_3; R_3 = CH_3; X = CH
norfloxacin, R = CH_2CH_3; R_3 = H, X = CH
AT-2266, R = CH_2CH_3; R_3 = H; X = N
DL-8280, X, R = $COCH_2CH(CH_3)$, R_3 = CH_3
Bay o 9867, R = c-C_3H_5, R_3 = H; X = CH

Structure–activity relationship studies have indicated that antibacterial potency is closely related to the steric bulk of the 1-substituent.[3] In the case of 1-alkyl naphthyridine/quinoline antibacterial agents, the ethyl ana-

(1) This work was presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Astr 375, Oct 24–26, 1983, Las Vegas, NV.
(2) Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. *J. Med. Chem.* **1962**, *5*, 1063.
(3) Albrecht, R., *Prog. Drug Res.* **1977**, *21*, 9.
(4) Goueffon, Y.; Montay, G.; Roquet, F.; Pesson, M., *C. R. Hebd. Seances Acad. Sci.* **1981**, *292*, 37.
(5) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
(6) Nakamura, S.; Nakata, K.; Katae, H.; Minami, A.; Kashimoto, S.; Yamagishi, J.; Takase, Y.; Shimizu, M. *Antimicrob. Agents Chemother.* **1983**, *23*, 742.
(7) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1982**, *22*, 548.
(8) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.

0022-2623/84/1827-1103$01.50/0 © 1984 American Chemical Society