It can be seen that, after 30 min., virtually all of the counts administered in 2.5 ml. were found in the plasma; approximately one-half of the counts administered in 5 ml. were found in the plasma.

During the phenol red experiment, large quantities of indicator were found in the bile. Approximately 32% of the initial 500 mcg./ml. was found in the bile after 1 hr. The total amount of indicator recovered by the liver from the blood was 98%, with very little remaining in the hepatic vein plasma. If enterohepatic circulation had not been interrupted, the phenol red concentration in the intestinal lumen would have remained fairly constant after the initial loss due to the amount circulating through the liver. Evidence of phenol red absorption from the rat jejunum was also found by Kunze (6) and indirectly mentioned by Miller and Schedl (2, 7).

No significant effect of water movement was observed in the first part of the drug absorption experiments. It is probable that as the steroid absorption process progressed from the lumen-to-membrane phase to the membrane-to-blood phase, the water effect was magnified by the membrane-to-blood rate constant, which is approximately a factor of 10 less than the initial lumen-to-membrane rate constant. From the water outflow rate, the drug absorption data were corrected for water loss. These corrected results are shown in Fig. 4. After correcting the data for water influence, the drug absorption curve no longer shows a rise in concentration. The resulting graph is very similar to the expected curve for a three-compartment drug absorption model. These expected data were generated from the corrected first-phase data of the absorption curve and the Metzler (8) curve-fit computer program modified for the pharmacokinetic treatment of steroid drug absorption studies presently being done in this laboratory (9). If absorption studies are prolonged, water movement must not be ignored, especially if the compounds investigated show biphasic absorption as described here and by Doluisio *et al.* (10). Misleading concentrations of compounds in the lumen may lead to incorrect conclusions concerning the drug absorption mechanisms and the rate constants.

REFERENCES

(1) H. Heller and F. H. Smirk, *J. Physiol.*, **76**, 1(1932).

(2) D. L. Miller and H. P. Schedl, *Gastroenterology*, **58**, 40 (1970).

(3) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, *J. Pharm. Sci.*, **58**, 1196(1969).

(4) T. Bates and M. Gibaldi, in "Current Concepts in Pharmaceutical Science: Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, p. 88.

(5) G. A. Bray, *Anal. Biol. Chem.*, **1**, 279(1960).

(6) H. Kunze, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **259**, 260(1968).

(7) H. P. Schedl, *Gut*, **7**, 159(1966).

(8) C. M. Metzler, "A Brief Introduction to Nonlinear Least Squares Estimation," Compilation of Symposia Papers, 5th National Meeting of the APHA Academy of Pharmaceutical Sciences, American Pharmaceutical Association, Washington, D. C., 1970.

(9) K. S. Pelzmann and R. N. Havemeyer, to be published.

(10) J. T. Doluisio, W. G. Crouthamel, G. H. Tan, J. V. Swintosky, and L. W. Dittert, *J. Pharm. Sci.*, **59**, 72(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 5, 1971, from the *Institute of Pharmaceutical Sciences, Syntex Research, Stanford Industrial Park, Palo Alto, CA 95304*

Accepted for publication July 26, 1971.

▲ To whom inquiries should be directed.

Substituted 3-Aminomethylbenzoxazoline-2-thiones as Potential Antibacterial Agents

RAJENDRA S. VARMA* and W. LEWIS NOBLES†▲

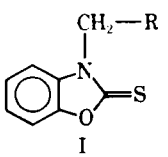
Abstract □ A series of 3-aminomethylbenzoxazoline-2-thiones was tested for possible antibacterial activity. Out of the 17 compounds screened, 11 exhibited some degree of activity.

Keyphrases □ 3-Aminomethylbenzoxazoline-2-thiones, substituted —screened as potential antibacterial agents □ Antibacterial agents, potential—substituted 3-aminomethylbenzoxazoline-2-thiones screened □ Agar diffusion technique—screening of 3-aminomethylbenzoxazoline-2-thiones as antibacterial agents

Several benzoxazoline-2-thiones (I), substituted in position 3, were synthesized (1) for biological evaluation. In this publication, the results of the screening of these compounds by the agar diffusion technique (2) against four different organisms are described.

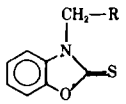
EXPERIMENTAL

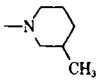
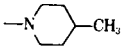
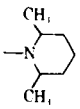
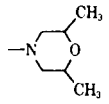
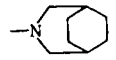
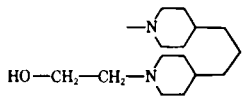
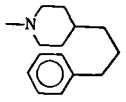
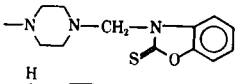
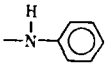
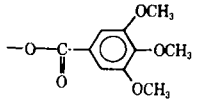
The following test organisms (3) were included in this study: A, *Staphylococcus aureus* K 257; B, *Pseudomonas aeruginosa;* C, *Klebsiella pneumoniae* ATCC 8052; and D, *Mycobacterium smegmatis.* The agar medium was heavily inoculated with the test organism; then filter paper disks (6.35 mm.) saturated with two drops of the solution of the test compound (20 mg./ml. in ethanol) were placed on the agar. The zones of inhibition



I

Table I—Antibacterial Activity of Substituted Benzoxazoline-2-thiones



Compound	R	Microbial Spectrum[a] A	B	C	D
1		+	+	−	−
2		+	+	+	−
3		−	−	−	−
4		+	+	+	−
5		+	+	++	−
6		−	−	−	−
7		−	+	+	−
8		+	+	++	−
9		−	−	−	−
10		−	−	−	−
11		+	−	+	−
12		−	++	+++	+++
13		+	++	++	+++
14		−	−	−	−
15		−	+	+	−
16		−	−	−	−
17	−OH	−	+	+	++

[a] Microbial spectrum: A, *Staphylococcus aureus* K 257 (culture obtained from Dr. James B. Grogan, Department of Surgery, University of Mississippi Medical Center, on October 12, 1962); B, *Pseudomonas aeruginosa* L Ba 160 (culture obtained from Carolina Biological Supply Co.); C, *Klebsiella pneumoniae* ATCC 8052; and D, *Mycobacterium smegmatis* (original culture obtained from Carolina Biological Supply Co.). Inhibition: +, zone size 7–10 mm.; ++, zone size 11–15 mm.; and +++, zone size > 15 mm.

around the disks were measured after an incubation period of 48 hr. Samples on which the zone sizes were smaller than 6.35 mm. were considered devoid of activity.

DISCUSSION

Seventeen compounds, obtained by varying the substituents at position 3 of the benzoxazoline-2-thiones, were subjected to preliminary antibacterial screening. The activity against four organisms is described in Table I. The substituents consisted of dimethylaminomethyl, heterocyclic aminomethyl (with or without substituents in the heterocyclic ring), anilinomethyl, 3,4,5-trimethoxybenzoxymethyl, and hydroxymethyl groups. Compounds having 2-substituted heterocyclic groups as substituents were inactive (Compounds 3 and 6). Compounds with six-membered heterocyclic substituents (with or without substitution in the 3- or 4-position in the heterocyclic ring) were active (an exception being Compound 14); whereas Compounds 9 and 10, with five- and seven-membered heterocyclic groups, were devoid of activity. Therefore, on the basis of these preliminary results, it would appear that the ring size of the substituent plays some part in antibacterial activity of compounds of this type. Lack of activity of 14 and demonstration of activity by 11 may possibly be attributed to electronic and steric factors. The highest activity was recorded when the substituents at position 3 of the benzoxazoline-2-thione were either 1-(*N*-2-hydroxyethyl-4-piperidyl)-3-(4-piperidinomethyl) propane (Compound 12) or 4-phenylpropylpiperidinomethyl (Compound 13). Of all the aminomethylbenzoxazoline-2-thiones, only two compounds (Compounds 12 and 13) inhibited the growth of *M. smegmatis*. 3-Hydroxymethylbenzoxazoline-2-thione showed inhibition against three organisms (B, C, and D). Thus, it was to some extent identical with Compounds 12 and 13 but quite different from the other compounds with respect to antibacterial activity. Moderate inhibition was observed when the heterocyclic amino function was replaced by an anilino group. Replacement of the aminomethyl group by an ester group demonstrated no activity. The most active substance in this study was 3-(4-phenylpropylpiperidinomethyl)benzoxazoline-2-thione.

SUMMARY

Seventeen benzoxazoline-2-thiones were evaluated against four organisms by use of the agar diffusion method. Eleven compounds of the study demonstrated antibacterial activity.

REFERENCES

(1) R. S. Varma and W. L. Nobles, *Can. J. Chem.*, **45**, 3012(1957).
(2) R. S. Varma and W. L. Nobles, *J. Med. Chem.*, **10**, 972(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 29, 1971, from the *Department of Pharmaceutical Chemistry, School of Pharmacy, University of Mississippi, University, MS 38677*

Accepted for publication August 26, 1971.

Supported in part by Grant AI-04701 from the National Institutes of Health, Bethesda, MD 20014

The authors express their appreciation to Dr. Lyman A. Magee and Mr. William Bing of the Biology Department, University of Mississippi, for facilities and assistance provided during the screening of these compounds.

* Present address: Department of Chemistry, University of Lucknow, India.

† Present address: Office of the President, Mississippi College, Clinton, MS 39056

▲ To whom inquiries should be directed.