REFERENCES

(1) H. Schriftman, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 111(1959).
(2) H. Schriftman and R. C. Shultz, *J. Pharm. Sci.*, **50**, 332 (1961).
(3) C. A. Kelly and M. E. Auerbach, *ibid.*, **50**, 490(1961).
(4) K. O. Montgomery, P. V. Jennings, and M. H. Weinswig, *ibid.*, **56**, 141(1967).
(5) *Ibid.*, **56**, 393(1967).
(6) W. R. Clark and L. R. Rosenberg, *J. Ass. Offic. Agr. Chem.*, **48**, 579(1965).
(7) J. Levine and T. D. Doyle, *J. Pharm Sci.*, **56**, 619(1967).
(8) C. Ponder, *ibid.*, **57**, 467(1968).
(9) P. A. Shore and J. S. Olin, *J. Pharmacol. Exp. Ther.*, **122**, 295(1958).
(10) L. Chafetz, *J. Pharm. Sci.*, **52**, 1193(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 30, 1970, from the *Pharmaceutical Research and Development Laboratories, Warner-Lambert Research Institute, Morris Plains, NJ 07950*

Accepted for publication August 6, 1970.

Antibacterial Activity of Certain Mannich Bases against *Escherichia coli* and *Staphylococcus aureus*

L. G. CHATTEN, G. E. MYERS, K. K. KHULLAR, and G. A. YAGER

Abstract □ A group of 28 Mannich bases comprising four different amino functional groups was tested for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The zones of inhibition were measured, and the results of the tests were tabulated. Postulations are offered to explain the superior activity of one compound when compared with another, and these explanations are combined with suggested modes of action.

Keyphrases □ Mannich bases—antimicrobial activity testing, *Staphylococcus aureus*, *Escherichia coli*, structure–activity relationships, mode of action proposed □ Antimicrobial activity—Mannich bases tested, *Staphylococcus aureus*, *Escherichia coli*

Some of the 5-chloro-2-thienyl-3-dialkylaminoethyl ketones reported by Britton and Nobles (1) possessed *in vitro* activity against certain bacteria. In addition, Taylor and Nobles (2) stated that some of their ketonic Mannich bases demonstrated *in vitro* antitubercular and amoebicidal action. Schlingman *et al.* (3) reported the synthesis of vinylogs of β-aminopropiophenones. These substances were Mannich bases and were shown to possess antibacterial activity *in vitro*. The Mannich bases of rifomycin SV were effective against both Gram-positive and Gram-negative bacteria, possessed low toxicity, and could be maintained at high blood levels (4). Blanton and Nobles (5) prepared Mannich bases using 3-azabicyclo[3.2.2]nonane as an amine moiety, and the resulting compounds had a broad spectrum of antimicrobial activity. In addition, Mannich bases derived from tetracycline retained a high activity (6).

These observations prompted the present survey of the antibacterial activity of a series of Mannich bases, including those reported by Khullar and Chatten (7).

EXPERIMENTAL

Materials—All Mannich bases employed in this study were synthesized by Method A, as reported earlier (7), and are listed in

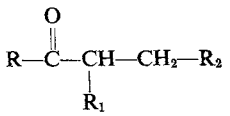


Table I—Mannich Bases Employed in This Study

Compound Number	R	R_1	Melting Ranges, R_2 = 2,6-dimethylmorpholino	R_2 = morpholino	R_2 = piperidino	R_2 = 3-methylpiperidino
1	Me—	H	156–157° (7)[a]	—	175°	—
2	C_6H_5—	H	195–196.5° (7)	186.5° (11)	191–192° (11)	172–174° (11)
3	*p*-Me—C_6H_4—	H	207–208° (7)	224–225° (12)	176.5° (11)	177–178°
4	*p*-MeO—C_6H_4—	H	203–205° (7)	209–210° (11)	209–211° (14)	—
5	*p*-Cl—C_6H_4—	H	203.5–204° (7)	201–202°	187°	186–187.5°
6	*p*-O_2N—C_6H_4—	H	203–204° (7)	206° (13)	193–195° (13)	—
7	*m*-O_2N—C_6H_4—	H	196–197° (7)	186–187° (11)	178–179.5° (15)	—
8	*o*-O_2N—C_6H_4	H	—	192–193°	—	—
9	C_6H_5CH=CH—	H	196–197° (11)	—	—	—
10	C_6H_5—	Me	187–188.5° (7)	—	—	—
11		H	—	200–201.5°	186.5–187.5°	—

[a] Number in parentheses is reference to previously reported melting point.

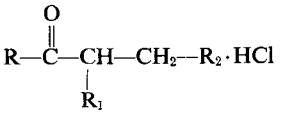


Table II—Antibacterial Activity of Certain Mannich Bases against *E. coli*

Compound Number	R	R_1	Zone of Inhibition, mm. ([a]Dilution, mg./0.1 ml.) R_2 = 2,6-dimethylmorpholino	R_2 = morpholino	R_2 = piperidino	R_2 = 4-methylpiperidino
1	Me—	H	15.8 (1.25)		13.4 (10.0)	
2	C_6H_5—	H	17.5 (1.25)	19.1 (0.625)	14.5 (5.0)	13.5 (5.0)
3	*p*-Me—C_6H_4—	H	25.5 (1.25)	13.9 (0.625)	13.2 (5.0)	11.5 (5.0)
4	*p*-MeO—C_6H_4—	H	—	15.1 (1.25)	Growth (10.0)	—
5	*p*-Cl—C_6H_4—	H	16.0 (0.625)	20.6 (0.625)	13.3 (2.50)	9.6 (0.625)
6	*p*-O_2N—C_6H_4—	H	11.7 (0.625)	—	10.6 (1.25)	—
7	*m*-O_2N—C_6H_4	H	15.6 (5.0)	12.4 (5.0)	8.5 (2.50)	—
8	*o*-O_2N—C_6H_4—	H	—	16.6 (0.625)	11.7 (1.25)	—
9	C_6H_5—CH=CH—	H	11.0 (0.625)	—	—	—
10	C_6H_5—	Me	14.9 (10.0)	—	—	—
11		H	—	15.7 (0.625)	17.9 (10.0)	—

[a] Figures in parentheses represent lowest dilution.

Table I. References to previous literature reports for these substances also are cited wherever available. The following compounds have not been reported previously:

1. Piperidinoethyl methyl ketone HCl—*Anal.*—Calcd. for $C_9H_{18}ClNO$: C, 56.38; H, 9.46; N, 7.31. Found: C, 56.42; H, 9.50; N, 7.31.
2. Morpholinoethyl *o*-nitrophenyl ketone HCl—*Anal.*—Calcd. for $C_{13}H_{17}ClN_2O$: C, 52.00; H, 5.66; N, 9.33. Found: C, 51.30; H, 5.82; N, 9.47.
3. Morpholinoethyl-2-furyl ketone HCl—*Anal.*—Calcd. for $C_{11}H_{16}ClNO_3$: C, 53.76; H, 6.56; N, 5.71. Found: C, 53.60; H, 6.71; N, 5.91.
4. Morpholinoethyl-4-chlorophenyl ketone HCl—*Anal.*—Calcd. for $C_{13}H_{17}Cl_2NO_2$: C, 53.76; H, 5.86; N, 4.82. Found: C, 54.44; H, 6.21; N, 4.83.
5. Piperidinoethyl-4-chlorophenyl ketone HCl—*Anal.*—Calcd. for $C_{14}H_{19}Cl_2NO$: C, 58.33; H, 6.60; N, 4.86. Found: C, 58.39; H, 7.06; N, 4.89.
6. 3-Methylpiperidino-4-chlorophenyl ketone HCl—*Anal.*—Calcd. for $C_{15}H_{21}Cl_2NO$: C, 59.60; H, 6.95; N, 4.64. Found: C, 59.64; H, 7.39; N, 4.50.
7. 3-Methylpiperidino-4-methylphenyl ketone HCl—*Anal.*—Calcd. for $C_{16}H_{24}ClNO$: C, 68.21; H, 8.53; N, 4.97. Found: C, 67.88; H, 8.36; N, 5.19.

The benzalacetone employed for the synthesis of Compound 9 in Tables II and III was obtained from benzaldehyde and acetone in the presence of sodium hydroxide, according to the procedure of Drake and Allen (8).

Microbiological Methods—The test microorganisms used in these experiments were *Staphylococcus aureus* (A.T.C.C. 6538) and *Escherichia coli* (A.T.C.C. 10536). These organisms were chosen because they represent the two principal groups of bacteria commonly causing human infections, namely, the Gram-positive cocci and the Gram-negative bacilli. Furthermore, these particular strains are routinely used in microbiological assays of disinfectants, antiseptics, and antibiotics. The organisms were maintained on nutrient agar[1] slants and transferred once a week between tests.

Qualitative Tests—These were done to determine whether a substance is capable of inhibiting the growth of the test microorganisms.

To ensure that the majority of the cells being tested were of the same physiological age, *i.e.*, in the same growth phase, immediately prior to each experiment the test microorganisms were transferred from the agar slant to 5 ml. of nutrient broth.[1] The inoculated broth was incubated 24 hr. at 37.5°, and a loopful of the culture was transferred to a second tube of broth. The second broth culture was incubated for 24 hr., and 0.1 ml. of this culture was then used to inoculate the surface of a nutrient agar[2] plate in such a manner as to produce confluent growth when the medium was subsequently incubated.

A small amount of each compound in powder form was placed on the inoculated surface of the medium. No attempt was made to measure the amount of the compound used in these tests. Five compounds were tested per plate of medium. Upon incubation for 24 hr. at 37.5°, the presence or absence of a zone of inhibition surrounding the test substances was noted as was any alteration in the character or appearance of the medium. Compounds exhibiting antibacterial activity were set aside for quantitative testing.

Quantitative Tests—These were done to establish the antibacterial potency of compounds that showed any activity in qualitative tests. In this instance the test microorganisms were subcultured twice for 24 hr. at 37.5° in 5 ml. of antibiotic assay broth[3] immediately prior to the test. Preliminary experiments, done in the manner described by Kavanagh (9), established that clearcut zones of inhibition were consistently obtained when 0.1 ml. of the second successive broth subculture was added to 4.0 ml. of seed agar[3] solidified as an even layer on the surface of 21 ml. of hardened base agar (Antibiotic Medium 2).[3]

In the actual tests, five stainless steel cylinders (outside diameter 8 mm., inside diameter 6 mm., length 10 mm.) were placed on the surface of the hardened medium approximately equidistant on the perimeter of a circle of 2.8–3.0-cm. radius (9). Plates with cylinders applied were allowed to stand one-half hour at room temperature to ensure proper seating of the cylinders in the agar surface.

One hundred milligrams of the compound to be tested was dissolved in 1.0 ml. of sterile distilled water (ethanol was used for Compound 9, Table II). The "stock" solution so prepared was diluted 1/10, 1/20, 1/40, 1/80, and 1/160 with the same solvent. Subsequently, 0.1 ml. of each dilution was placed in one cylinder. A control cylinder was prepared using 0.1 ml. of the solvent only. Quantitative test plates prepared as described were incubated 24 hr. at 37.5°. The diameter of the zone of inhibition surrounding each cylinder was then measured using a Fisher–Lilly antibiotic zone reader. Initially, only the dilutions previously mentioned were tested. However, when a compound proved to be very active, additional dilutions were tested in an attempt to determine the greatest dilution that would produce a measurable zone of inhibition.

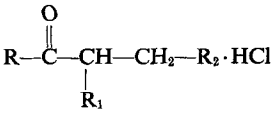
Duplicate samples of each dilution of each compound were tested in each experiment, and at least two complete experiments were done with separately prepared "stock" solutions of each compound.

[1] Difco Laboratories, Detroit, Mich.

[2] Bio-Cert, Fischer Scientific Company (Chemical Manufacturing Division), Fairlawn, N. J.

[3] Baltimore Biological Laboratory (Division of B.D. Laboratories Inc.), Baltimore, Md.

Table III—Antibacterial Activity of Certain Mannich Bases against *S. aureus*



Compound Number	R	R_1	Zone of Inhibition, mm. ([a]Dilution, mg./0.1 ml.) R_2 = 2,6-dimethylmorpholino	R_2 = morpholino	R_2 = piperidino	R_2 = 3-methylpiperidino
1	Me—	H	28.0 (1.25)	—	13.3 (10.0)	—
2	C_6H_5—	H	20.7 (0.625)	25.5 (0.625)	14.8 (5.0)	15.6 (5.0)
3	*p*-Me—C_6H_4—	H	31.0 (0.625)	16.4 (0.625)	13.1 (5.0)	12.8 (2.50)
4	*p*-MeO—C_6H_4—	H	—	10.6 (0.625)	Growth (10.0)	—
5	*p*-Cl—C_6H_4—	H	12.6 (0.078)	14.7 (0.078)	10.3 (0.625)	13.7 (0.625)
6	*p*-O_2N—C_6H_4—	H	11.0 (0.625)	—	10.6 (0.625)	—
7	*m*-O_2N—C_6H_4—	H	14.4 (0.625)	15.2 (1.25)	11.0 (2.50)	—
8	*o*-O_2N—C_6H_4—	H	—	21.7 (0.625)	10.2 (1.25)	—
9	C_6H_5—CH=CH—	H	17.1 (0.625)	—	—	—
10	C_6H_5—	Me	14.1 (10.0)	—	—	—
11		H	—	17.7 (0.625)	11.5 (1.25)	—

[a] Figures in parentheses represent lowest dilution.

RESULTS AND DISCUSSION

Duplicate tests done with a given dilution of a compound gave duplicate inhibition results, and the results obtained in experiments done with separately prepared "stock" solutions were in close agreement.

In no instance did the solvent control produce measurable inhibition of the growth of the test microorganisms.

The test compounds were subdivided according to functional group to permit a study of the effects of each substituent on antibacterial activity. The summarized results of the tests, presented in Tables II and III, are the averages of duplicate determinations.

An examination of Tables II and III reveals that the nature of the R group is not vital for antibacterial activity; those compounds where R is methyl, furyl, phenyl, or substituted phenyl all exhibit measurable activity.

Burckhalter and Johnson (10) synthesized various vinylogs of β-aminopropiophenones with the idea of producing compounds with enhanced antibacterial effect. They noticed that the structural variations failed to improve antibacterial effectiveness. This observation prompted the present investigation of the use of substituted morpholines in the Mannich reaction to determine whether the resulting vinylog of β-2,6-dimethylmorpholinopropiophenone possessed improved antibacterial activity. However, no dramatic change in activity was noted. Again, this finding indicates that the nature of the R group and its distance from the amine moiety are not crucial for antibacterial action.

However, while analysis of the data indicates that the substituents on the phenyl ring do exert some influence, by far the greatest effect results from differences in the amine moiety. In general, a *p*-chloro group exhibits a marked beneficial effect on the antibacterial activity of the compound while a *p*-nitro group generally appears to enhance the activity of that substance when compared to many others in the same series. Those functional groups that have the general effect of reducing the activity of the Mannich bases when tested against both *E. coli* and *S. aureus* are the *p*-methoxy and *m*-nitro. The adverse influence of these two moieties may be entirely due to steric effects. They may simply prevent the respective compounds from making a good contact on the receptor side of either *E. coli* or *S. aureus*. Moreover, differences in potencies observed on introducing the ring substituents at various positions in the benzene ring may be assigned to their influence on transport phenomena (absorption, distribution, excretion, *etc.*).

A comparison of the effects of the amine moiety on activity against *E. coli* (Table II) reveals that there is not much difference between the potency of dimethylmorpholino and morpholino compounds. In addition, the piperidino and 3-methylpiperidino were quite comparable. However, those substances with either a morpholino or dimethylmorpholino group were generally considerably more potent than those in either of the piperidino series. The reason for this situation is not readily apparent but may be due to improved contact with the receptor site because of hydrogen bonding by the oxygen in the morpholino ring. The methyl groups in the dimethylmorpholino may also provide a means of increased contact with the bacterium receptor site through Van der Waals' forces.

Examination of Table III reveals that the same general situation obtains with regard to the potency of the compounds against *S. aureus* as was described for *E. coli*. It is worthy of mention that the *p*-chloro compounds of both the morpholino and dimethylmorpholino series exhibit remarkable activity against *S. aureus* when compared with all other substances included in this study.

Introduction of a methyl group in the α-position to the carbonyl results in a decrease in activity, as noted from Compound 10 in both Tables II and III.

REFERENCES

(1) S. B. Britton and W. L. Nobles, *J. Amer. Pharm. Ass., Sci. Ed.*, **44**, 717(1955).

(2) E. D. Taylor and W. L. Nobles, *ibid.*, **49**, 317(1960).

(3) A. S. Schlingman, unpublished results; through *J. Amer. Chem. Soc.*, **73**, 483(1957).

(4) S. P. A. Lepetit, Belgian pat. 661,981; through *Chem. Abstr.*, **65**, 5475b(1966).

(5) C. D. Blanton and W. L. Nobles, *J. Pharm. Sci.*, **53**, 1130(1964).

(6) W. J. Gottstein, W. F. Minor, and L. C. Cheney, *J. Amer. Chem. Soc.*, **81**, 1198(1959).

(7) K. K. Khullar and L. G. Chatten, *J. Pharm. Sci.*, **56**, 328 (1967).

(8) N. L. Drake and P. Allen, "Organic Synthesis," coll. vol. 1, Wiley, New York, N. Y., 1941, p. 77.

(9) F. Kavanagh, "Analytical Microbiology," Academic, New York, N. Y., 1963, pp. 274–276.

(10) J. H. Burckhalter and S. H. Johnson, *J. Amer. Chem. Soc.*, **73**, 4835(1951).

(11) K. K. Khullar, M.Sc. thesis, University of Alberta, Edmonton, Alberta, Canada, 1966.

(12) J. J. Denton, R. J. Turner, W. B. Neier, V. A. Lawson, and H. P. Schedl, *J. Amer. Chem. Soc.*, **71**, 2048(1949).

(13) W. B. Wheatley, W. E. Fitzgibbon, and L. C. Cheney, *ibid.*, **76**, 4490(1954).

(14) C. Mannich and D. Lammering, *Chem. Ber.*, **55**, 3510(1922).

(15) C. Mannich and M. Dannehl, *Arch. Pharm.*, **276**, 206(1938).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 30, 1969, from the *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton 7, Alberta, Canada.*

Accepted for publication August 18, 1970.