pound I was prepared by the method of Farrar and Levine.[14] The product, which was an azeotrope, was found by gas phase chromatography to consist of 70% of I and 30% of the 2-isomer, in close agreement with the authors. Only chromatographically pure I was used to avoid contamination of the products by the Mannich bases of the 2-isomer. Except for II and III, which were prepared in isoamyl alcohol, each of the β-amino ketones was prepared from formaldehyde and the base in absolute ethanol by the usual procedure.[11]

Method of Testing for Antimicrobial Activity.—The test microorganisms consisted of stock laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae*. The minimal inhibitory concentration (MIC) of each compound, as well as of each of four antibiotics, for the test organisms was determined by the agar dilution method. Appropriate concentrations of each compound were incorporated in 15-ml. portions of liquified nutrient agar, the medium was then poured into Petri plates, and 0.05 ml. of 24-hr. nutrient broth cultures of the microbial species were spread on the solidified agar surfaces. For the yeast species, glucose yeast infusion agar and broth, instead of nutrient agar and broth, were employed.

The lowest concentrations of Mannich bases or of antibiotics that prevented the development of visible growth are listed in Table I. The range of values represents maximum fluctuation in a series of assays. The data were obtained from tests with solutions sterilized by filtration; autoclaving destroyed as much as 90% of the activity of the various bases. Additional tests carried out with the most active of the Mannich bases (VII) demonstrated that the substance is not germicidal at low concentrations nor is its antimicrobial potency inactivated by lecithin. Inasmuch as the compound suppresses the growth of gram-positive and gram-negative bacteria, and yeast cells at similar concentrations, the mechanism of action is probably not associated with suppression of cell wall synthesis. Further speculation concerning the mode of action must await additional observations of microorganisms exposed to the compound.

Experimental

3-Acetylbenzo[*b*]thiophene (I).—To a rapidly stirred mixture of 134.2 g. (1.0 mole) of benzo[*b*]thiophene and 112 g. (1.1 moles) of acetic anhydride was added 10 g. of ferric chloride in one portion. The temperature immediately rose to 96°, and the reaction mixture became very dark. After stirring for 1.5 hr., 400 ml. of cold water was added, and the dark mixture was extracted with three 200-ml. portions of ether. The combined ether phase was washed with 10% sodium carbonate solution and dried with $MgSO_4$. After removal of the solvent and fractionation, 105 g. (70%) of a clear colorless liquid boiling at 129–131° (1 mm.) was obtained. Upon cooling and seeding, a white crystalline mass formed which was recrystallized three times from ethanol to yield a product melting at 64–65°, which showed only one peak in the gas chromatograph. The reported melting point is 64–65°,[13] λ_{max}^{KBr} 6.05 (C=O) and 7.35 μ (CH_3—C=O). The oxime was prepared as white platelets which melted at 123–124°.

Anal. Calcd. for $C_{10}H_9NOS$: C, 62.85; H, 4.75; N, 7.32. Found: C, 62.91; H, 4.69; N, 7.24.

Mannich Bases From I (II–IX).—In a 50-ml. flask containing 25 ml. of absolute ethanol (dry isoamyl alcohol for II and III) was added 0.05 mole of the respective amine, and the pH was adjusted to 3–4 with concentrated HCl. To this was added 8.8 g. (0.05 mole) of the ketone and 2.3 g. of paraformaldehyde. The reaction mixture was allowed to reflux for 4 hr. and was then poured into 100 ml. of dry acetone. After cooling in the refrigerator overnight, the white precipitate was collected and recrystallized from absolute ethanol.

(14) M. W. Farrar and R. Levine, *J. Am. Chem. So.*, **72**, 4433 (1950).

4-(Alkoxystyryl)quinolines[1]

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The activity of 4-(4-aminostyryl)quinolines against Walker 256 tumors in rats and against KB tumor cells in cell culture[2] led us to prepare the series of 4-(methoxystyryl)quinolines listed in Table I for similar testing. In each case a mixture of lepidine hydrochloride and an equimolar quantity of the appropriate aldehyde was heated until reaction had taken place, the mixture was dissolved in hot methanol and neutralized by adding an excess of concentrated ammonium hydroxide, and the oil which separated upon addition of water was recrystallized from isohexane, isooctane, or ethanol until acceptably pure. Samples were submitted for antitumor tests *in vivo* and *in vitro*. None of the compounds tested showed clear-cut activity against the Walker tumor at a single 200 mg./kg. dose level, but several of them produced 50% reduction in growth rate of KB cells in tissue culture at concentrations below 4 γ/ml., which placed them in the same range with 4-(4-dimethylaminostyryl)quinoline.

It has been suggested that there might be a correlation between the ultraviolet absorption maxima of such compounds and their cytotoxicity. Absorption spectra were examined with a Beckman DU spectrophotometer. Most of the peaks were not sharp but there were observable differences between the compounds. One interesting observation was that the wave length and intensity of absorption both diminished when a methanol solution of the compound was allowed to stand 1 day, but the acetic acid solutions did not undergo this change. On the other hand, the acetic acid solutions had an additional peak at a longer wave length than the methanol solutions. The shift in the methanol solutions was least for the dimethoxy and trimethoxy compounds containing a methoxy group at the 2-position, but 4-(2-methoxystyryl)quinoline itself exhibited a decided shift. The greatest difference between log ϵ for the acetic acid solution peak and the peak in methanol was exhibited by the three monomethoxy compounds and the 2,4-dimethoxy compound. The presence of a 2-methoxy group seemed to increase cytotoxicity except in the 2,3-dimethoxy compound.

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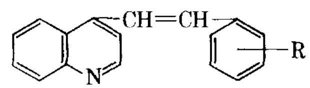
(2) C. T. Bahner, L. M. Rives, and C. Breder, *J. Med. Chem.*, **7**, 818 (1964); C. T. Bahner, D. Brotherton, W. H. Chapman, Jr., W. Longmire, H. B. Orr, L. M. Rives, E. B. Senter, and W. Yee, *ibid.*, submitted for publication.

TABLE I

4-(ALKOXYSTYRYL)QUINOLINE



No.	Alkoxy group	M.p.,[a] °C.	Reaction time, hr.	Reaction temp., °C.	Yield, %	Formula	Calcd., % C	Calcd., % H	Found,[b] % C	Found,[b] % H	λmax mμ (log ε) In methanol: Fresh solution	In methanol: after standing	In methanol: Change on standing	In acetic acid	Difference[c]	ED50,[d] γ/ml.	T/C,[e] 200 mg./kg.
1	2-OCH_3	121[f,g]	2	160	28	$C_{18}H_{15}NO$	82.75	5.79	82.50	5.49	345 (4.3)	315 (4.0)	−30 (−0.3)	390 (3.85) 320 (4.0)	+45 (−0.4)	0.7	1
2	3-OCH_3	95[h,g]	14	145	0.2	$C_{18}H_{15}NO$	82.75	5.79	82.31	5.76	330 (4.3)	310 (4.0)	−20 (0.3)	365 (3.85) 320 (4.0)	+35 (−0.4)	7	
3	4-OCH_3	91[h,i]	4	160	29	$C_{18}H_{15}NO$	82.75	5.79	82.58	5.53	350 (4.3)	315 (4.0)	−35 (−0.3)	405 (3.8) 315 (3.8)	+55 (−0.5)	6	1
4	2,3-$(OCH_3)_2$	116[f,g]	10	150	6	$C_{19}H_{17}NO_2$	78.33	5.88	78.14	5.83	330 (4.4)	320 (4.3)	10 (−0.1)	380 (4.3)	+50 (−0.1)	10	1
5	2,4-$(OCH_3)_2$	130[f,i]	2	145	34	$C_{19}H_{17}NO_2$	78.33	5.88	77.95	5.80	360 (4.1)	350 (3.8)	−10 (−0.3)	430 (3.7) 315 (3.8)	+70 (−0.4)	5	1.2
6	2,5-$(OCH_3)_2$	85[f,i]	2	145	26	$C_{19}H_{17}NO_2$	78.33	5.88	78.68	6.18	360 (4.2) 325 (4.2)	360 (4.1) 320 (4.1)	0 (−0.1) −5 (−0.1)	420 (4.1)[k] 370 (4.1) 330 (4.0)	+60 (−0.1)	1	1.1
7	3,4-$(OCH_3)_2$	157[f,g]	10	150	24	$C_{19}H_{17}NO_2$	78.33	5.88	78.16	5.88	355 (4.2)	310 (3.8)	−45 (−0.4)	420 (4.0) 310 (3.8)	+65 (−0.2)	5	0.5[l]
8	3,4-O_2CH_2	129[f,g]	1.5	155	19	$C_{18}H_{13}NO_2$	78.57	4.86	78.61	4.92	355 (4.2)	305 (3.9)	−50 (−0.3)	418 (4.0) 315 (3.9)	+65 (−0.2)	8	1[l]
9	3,4-$(OC_2H_5)_2$	105[f,g]	4	150	16	$C_{21}H_{21}NO_2$	78.63	4.36	78.63	4.77	360 (4.3)	330 (3.8)	−30 (−0.5)	425 (4.1) 315 (3.9)	+65 (−0.2)	15	1[l]
10	2,4,5-$(OCH_3)_3$	133[f,i]	1.5	145	44	$C_{20}H_{19}NO_3$	74.71	5.95	74.60	5.94	375 (4.2)	370 (3.9)	−5 (−0.3)	460 (4.3)	+85 (+0.1)	1	
11	3,4,5-$(OCH_3)_3$	167–168	3	155	12	$C_{20}H_{19}NO_3$	74.71	5.95	74.88	5.84	345 (4.5)	350 (3.6)	+5 (−0.9)	410 (4.3)	+65 (−0.2)		
12	3,4,5-$(OCH_3)_3$	134[f,i]	3	155	14	$C_{20}H_{19}NO_3$	74.71	5.95	74.96	5.80	347 (4.3)	315 (4.0)	−30 (−0.3)	410 (4.2) 315 (4.0)	+65 (−0.1)	2	1.7[m]

[a] Corrected for thermometer stem exposure; determined with Thiele tube. [b] Average of two analyses by Weiler and Strauss, Oxford, England. [c] Difference between peak in acetic acid solution and in fresh methanol solution. [d] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory and Southern Research Institute. [e] We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in Arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor-bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts are reported as the ratio T/C. [f] Recrystallized from ethanol. [g] White. [h] Recrystallized from isohexanes. [i] Yellow. [j] Recrystallized from isooctanes. [k] The peaks at the lower wave lengths are very small elevations on a long plateau. [l] Killed none of 3 test rats at 400 mg./kg. [m] Killed 1 of 3 test rats at 400 mg./kg.