horses three horses weighing between 250 and 300 Kg. were chosen. The animals were kept in a clean stable under veterinary medical observation[3] for 3 days before the administration of the drug and were examined constantly to ascertain their normal state of health. During the 24 hr. prior to administration of amphetamine, urine was collected from the three horses in leather bags tied under their bodies. Each animal was then injected subcutaneously with 100 mg. amphetamine sulfate. The urine of each animal was collected periodically after 3, 12, 24, 48, and 72 hr. by means of a sterile catheter. In addition, a clean leather bag was tied under each horse to collect any urine which might pass off between the specified collection times. The urine samples were pale yellow, turbid, and possessed a pH 5.9–6.3.

The urine samples were made alkaline with ammonium hydroxide T.S., shaken with chloroform, the extract chromatographed by TLC, and the separated amphetamine was colorimetrically assayed as described above.

The urine collected before the administration of amphetamine was used as a blank. The results are shown in Table III, from which it is clear that about 55.6% of the administered amphetamine was excreted in the urine of horses within 48 hr. after the administration of amphetamine. During this period, amphetamine could be detected by thin-layer chromatography; after 48 hr. it was hardly detectable and had almost disappeared after 72 hr.

[3] The authors thank Dr. A. Hanna, Faculty of Veterinary Medicine, Cairo University, for his valuable help.

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Antibacterial Activity of Some Substituted Benzofurans. Preliminary Study of Structure Activity Relationship

By P. K. SHARMA, KALA MEHTA, O. P. GUPTA, M. M. MAHAWAR, and S. K. MUKERJI

Sensitivity of three substituted nitrobenzofurans, 4-methyl-6-phenylbenzofuran, 2(*p*-nitrophenyl)benzofuran, and 2-aceto-5-methyl-7-nitrophenylbenzofuran, against *Staphylococci, Pyocyanea,* and *B. coli* was studied. A possible structure activity relationship has been worked out, which suggested that the sensitivity of benzofurans is due to nitro substitution. Sensitivity is reduced by substitution of aceto and methyl groups.

NITROFURANS have been widely studied for their antibacterial activity (1–3). A series of substituted benzofurans and nitrobenzofurans were synthesized and tested for their antibacterial activity.

The reaction of *p*-nitrobenzyl bromide with an *O*-hydroxy carbonyl compound in presence of anhydrous potassium carbonate in methanol yields a 2-(*p*-nitrophenyl) benzofuran (4, 5). This reaction was utilized to prepare a few benzofurans required for pharmacological studies.

The reaction of benzyl bromide with hydroxymethyl propiophenone and *p*-nitrobenzyl bromide with 2-hydroxypropiophenone and 2-hydroxy-3-aceto-6-methylpropiophenone in methanol, in the presence of anhydrous potassium carbonate from 45 min. to 1 hr., furnishes the respective benzyloxy and *p*-nitrobenzyloxy derivatives. When allowed to react further with anhydrous potassium carbonate in methanol under reflux (time varying from 6–9 hr.), they yield the respective benzofurans—*viz.*, 4-methyl-6-phenylbenzofuran (a pasty substance) (I), 2-(*p*-nitrophenyl) benzofuran (m.p. 193°) (II), and 2-aceto-5-methyl-7-nitrophenylbenzofuran (m.p. 68°) (III). All the three compounds are completely soluble in alcohol, acetone, and ether.

Received August 4, 1966, from R. N. T. Medical College, Udaipur, Rajasthan, India.
Accepted for publication November 7, 1966.

EXPERIMENTAL

Aqueous suspensions of these compounds were prepared in 1% polysorbate 80,[1] the wetting agent being used to compensate for low aqueous solubility. Control experiments were also performed with the same solvent.

The bactericidal property of the test agents (I, II, and III) was studied against *Staphylococci, Pyocyanea,* using nutrient agar media and *B. coli* on MacConkey's agar media, by the use of impregnated filter paper disks to determine the bacterial sensitivity for the compounds under study.

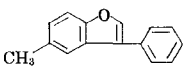
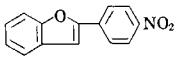
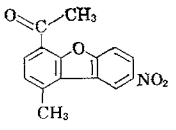
The disks were soaked in the solutions, the activity of which was to be tested, placed on the media, and incubated overnight at 37°. The diameters of the circular areas of inhibited growth were observed and percentage of inhibition was calculated.

RESULTS AND DISCUSSION

The results of these observations are presented in Table I. From the results it is evident that compound II is the most active against all three test

[1] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

TABLE I—RESULTS OF ANTIBACTERIAL ACTIVITY OF COMPOUNDS

No.	Compd.	Chemical Structure	Bacteria	Concn., mcg./ml.	% Inhibition of Growth[a]
I	4-Methyl-6-phenyl-benzofuran		*Staphylococci*	0.25	0
				0.50	0
				1.00	0
			Pyocyanea	0.25	0
				0.50	0
				1.00	10
			B. coli	0.25	0
				0.50	0
				1.00	0
II	2-(*p*-Nitrophenyl) benzofuran		*Staphylococci*	0.25	80
				0.50	100
				1.00	100
			Pyocyanea	0.25	50
				0.50	80
				1.00	100
			B. coli	0.25	100
				0.50	100
				1.00	100
III	2-Aceto-5-methyl-7-nitrophenyl-benzofuran		*Staphylococci*	0.25	50
				0.50	50
				1.00	100
			Pyocyanea	0.25	0
				0.50	0
				1.00	100
			B. coli	0.25	0
				0.50	50
				1.00	100

[a] No inhibition of growth was observed with control.

species of bacteria. Compound III is active against *Staphylococci* but inactive against *Pyocyanea* and *B. coli* in low concentrations (0.25 and 0.50 mcg./ml.) and sensitive in higher concentration (1.0 mcg./ml.). Compound I is practically resistant to all the three types of bacteria. This suggests that the nitro group is essential for antibacterial activity of the benzofurans (compound II) while the sensitivity is reduced by the substitution of methyl and aceto groups (compound III).

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