pigeon brain homogenate was found to be 98.2 ± 1.0. Likewise, fluorescence spectra of extracts from brains of mice and pigeons which had been administered apomorphine systemically were identical to spectra obtained for authentic apomorphine in ethyl acetate.

Although several procedures were reported for the quantitative determination of apomorphine, none possesses adequate sensitivity for studies of the compound's CNS distribution. Kaul *et al.* (4) reported that the spectrophotometric assay was unable to detect the presence of apomorphine in tissue homogenates in concentrations less than 4 mcg./g. Preliminary investigations of the compound's CNS distribution in mice using the fluorometric assay reported here indicate that concentrations of apomorphine as low as 0.1 mcg./g. brain tissue can be readily determined. As a result of the increased sensitivity of this fluorometric procedure, studies of apomorphine disposition in brain are now being productively pursued.

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Synthesis and Biological Evaluation of Some 4-Arylazopyrazoles

W. U. MALIK, H. G. GARG*, and VEENA ARORA

Abstract □ A variety of derivatives of N^1-(4-methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles and N^1-hippuryl-4-arylazo-3,5-dimethylpyrazoles were prepared. Preliminary biological data also are described.

Keyphrases □ 4-Arylazopyrazoles—synthesis and biological evaluation as antimicrobial agents □ Antimicrobial agents, potential—synthesis and biological evaluation of 4-arylazopyrazoles □ N^1-(4-Methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazole derivatives—synthesis and biological evaluation as antimicrobial agents □ N^1-Hippuryl-4-arylazo-3,5-dimethylpyrazole derivatives—synthesis and biological evaluation as antimicrobial agents

The interest in the synthesis and biological evaluation of pyrazole derivatives has been renewed by the fact that 1-carbamoyl-3-methyl-4-(2-chloro-4-nitrophenylhydrazono)-2-pyrazolin-4,5-dione possesses anti-*Trichinella spiralis* activity (1).

This report includes the syntheses of N^1-(4-methoxyphenylsulfinyl) - 3,5 - dimethyl - 4 - arylazo-, N^1 - hippuryl-3,5-dimethyl-4-arylazo-, and N^1-hippuryl-3-methyl-4-arylazo-5-phenylpyrazoles. The preparation of others was recently described (2, 3).

The syntheses of N^1-(4-methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles and N^1-hippuryl-4-arylazo-3,5-dimethylpyrazoles were achieved by the condensation of 2,3,4-pentanetrione-3-arylhydrazones (4) with 4-methoxyphenylsulfinylhydrazine (5) and hippurylhydrazine (6), respectively. N^1-Hippuryl-4-arylazo-3-methyl-5-phenylpyrazoles were similarly prepared from 1-phenyl-2-arylhydrazono-1,2,3-butanetrione (7) and hippurylhydrazine (6).

BIOLOGICAL RESULTS

4-Arylhydrazono-1-carbamoyl-3-methyl-2-pyrazolin-5-ones (2) and 3,5-dimethyl-4-arylazo-5-phenyl-N^1-carbamoylpyrazoles (3) were tested for antimicrobial activity (8) against *Staphylococcus aureus* (No. 20390), *Klebsiella pneumoniae* (No. 1200), *Pseudomonas aeruginosa* (No. 1320), *Escherichia coli* (No. 12140), *Trichophyton mentagraphytes* (No. 17410), *Candida albicans* (No. 3470), and *Mycobacterium tuberculosis* (No. H37RV). They were found inactive.

Anti-*Trichinella spiralis* activity of N^1-(4-methoxyphenylsulfinyl)- and N^1-hippuryl-4-arylazopyrazoles was determined by using the method of Garg (1). All these compounds were essentially inactive.

The IR spectra of representatives of all the pyrazoles showed characteristic bands of —C=C—N=N in the range 1480–1540 cm.$^{-1}$, aryl C=C in the range 1580–1670 cm.$^{-1}$, substituted phenyl in the range 690–730 cm.$^{-1}$, and —NH in the range 2700–3400 cm.$^{-1}$ (broad). UV spectra of the representatives showed $\lambda_{max.}^{EtOH}$ between 237–246 and 327–347 nm. These data are summarized in Table I.

EXPERIMENTAL[1]

3-Arylhydrazono-2,3,4-pentanetriones (4), 2-arylhydrazono-1-phenyl-1,2,3-butanetriones (7), hippurylhydrazine (6), and 4-methoxyphenylsulfinylhydrazine (5) were prepared by earlier described procedures.

2-Arylhydrazono-1,3-diphenyl-1,2,3-propanetriones—These were obtained by coupling diazotized anilines with 1,3-diphenyl-1,3-propanedione under conditions used previously (9).

***N*^{1}-(4-Methoxyphenylsulfinyl)-3,5-dimethyl - 4 - arylazopyrazoles: General Procedure**—A solution of 4-methoxysulfinylhydrazine (0.005 mole) in alcohol (15 ml.), containing a few drops of concentrated sulfuric acid, was added to the appropriate 2,3,4-propane-

[1] Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord using KBr phase. UV spectra were measured with a Bausch and Lomb Spectronic 505 spectrophotometer.

Table I—Spectral Data of N^1-(4-Methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles and N^1-Hippuryl-4-arylazo-3,5-dimethylpyrazoles

Number	IR, cm.$^{-1}$ —C=C—N=N—	IR, cm.$^{-1}$ Aryl C=C	IR, cm.$^{-1}$ Substituted Phenyl	IR, cm.$^{-1}$ —NH	IR, cm.$^{-1}$ —NO_2	UV, μ $\lambda_{max.}^{EtOH}$	
1[a]	1540	1600	715, 690	3400	1375	246	347
2[b]*	1505	1630	730, 690	3100	—	244	243
3[c]	1520	1670	720	2850	—	241	346
4[b]†	1500	1610	720, 730	3000	—	240	335
5[b]‡	1500	1580	720	2850	—	239	331

[a] Table IV, No. 2. [b] Table II: *No. 4, † No. 5, and ‡ No. 2. [c] Table III, No. 1.

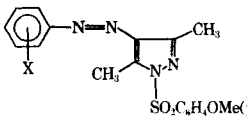

Table II—Characteristics of N^1-(4-Methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles

Number	X	Yield, %	Melting Point	Color[a]	Formula	Analysis, % Calc.	Found
1	H	70	114°	BrYSN	$C_{18}H_{18}N_4O_3S$	C 58.37	58.42
						H 4.86	5.00
						N 15.13	15.02
						S 8.64	8.53
2	2-MeO	72	193°	GYSP	$C_{19}H_{20}N_4O_4S$	N 14.00	13.82
						S 8.00	8.21
3	4-MeO	70	162°	YFlN	$C_{19}H_{20}N_4O_4S$	N 14.00	13.92
						S 8.00	8.13
4	2,4-Cl_2	65	197°	GSp	$C_{18}H_{16}Cl_2N_4O_3S$	Cl 16.17	16.09
						S 7.28	7.22
5	3,4-Me_2	60	180°	YSN	$C_{20}H_{22}N_4O_3S$	N 14.07	14.03
						S 8.04	7.91
6	4-Cl-2,5-$(MeO)_2$	65	240° dec.	MSSp	$C_{20}H_{21}ClN_4O_5S$	Cl 8.50	8.44
						S 6.88	6.82
7	5-Cl-2,4-$(MeO)_2$	55	250° dec.	DYN	$C_{20}H_{21}ClN_4O_5S$	Cl 8.50	8.43
						S 6.98	6.83

[a] B, brown; Br, bright; D, dark; F, fibers; Fl, fluffy; G, golden; M, mustard; N, needles; O, orange; P, plates; S, shiny; Sp, specks; and Y, yellow.

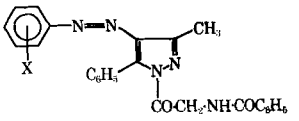

Table III—Characteristics of N^1-Hippuryl-3,5-dimethyl-4-arylazopyrazoles

Number	X	Yield, %	Melting Point	Color[a]	Formula	Analysis, % Calc.	Found
1	2-Me	70	200°	GSSp	$C_{21}H_{21}N_5O_2$	C 67.20	66.52
						H 5.60	5.43
						N 18.66	18.54
2	2-MeO	65	223° dec.	SGYN	$C_{21}H_{21}N_5O_3$	N 17.45	17.82
3	4-MeO	72	160–162°	BYFlN	$C_{21}H_{21}N_5O_3$	N 17.45	17.81
4	4-Cl-2,5-$(MeO)_2$	55	230°	MYSSp	$C_{22}H_{22}ClN_5O_4$	Cl 7.54	7.62
5	5-Cl-2,4-$(MeO)_2$	65	202°	DYSSp	$C_{22}H_{22}ClN_5O_4$	Cl 7.54	7.73

[a] See *Footnote* [a] of Table II.

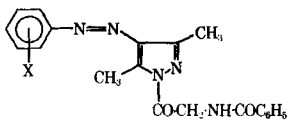

Table IV—Characteristics of N^1-Hippuryl-3-methyl-4-arylazo-5-phenylpyrazoles

Number	X	Yield, %	Melting Point	Color[a]	Formula	Analysis, % Calc.	Found
1	4-NO_2	55	177°	OSN	$C_{25}H_{20}N_6O_4$	C 64.10	64.01
						H 4.27	4.22
						N 17.90	17.83
2	3-NO_2	60	175°	GYSp	$C_{25}H_{20}N_6O_4$	N 17.90	17.72
3	2-MeO	70	118°	YOSp	$C_{26}H_{23}N_5O_3$	N 17.62	17.33
4	4-MeO	60	158°	DYSN	$C_{26}H_{23}N_5O_3$	N 17.62	17.24

[a] See *Footnote* [a] of Table II.

trione-3-arylhydrazone (0.005 mole) dissolved in an alcohol–acetic acid mixture. The resultant solution was boiled under reflux for several hours and then cooled. The reaction mixture was diluted with water, and the crystals which separated were collected and purified by recrystallization from ethanol. Characteristics of N^1-(4-methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles are listed in Table II.

N^1-Hippuryl-3,5-dimethyl-4-arylazopyrazoles—These compounds were obtained from hippurylhydrazine (0.005 mole) and 3-arylhydrazono-2,3,4-pentanetrione (0.005 mole) by the same procedure as was adapted for N^1-(4-methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles. The yields and physical constants of these pyrazoles are listed in Table III.

N^1-Hippuryl-3-methyl-4-arylazo-5-phenylpyrazoles—Treatment of 2-arylhydrazono-1-phenyl-1,2,3-butanetriones with hippurylhydrazine under conditions similar to those used in other cases gave the pyrazole derivatives listed in Table IV.

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* To whom correspondence should be addressed. Present address: Harvard University Medical School, Laboratory for Carbohydrate Research, Massachusetts General Hospital, Boston, MA 02114

Classification of Nicotine Block at Neuromuscular Junction

S. D. COHEN*, B. A. NICHOLAS†, and H. J. JENKINS

Abstract □ The effect of nicotine on the electrical threshold of the neuromuscular junction in a rat sciatic-gastrocnemius preparation was studied and compared with the effects of a true curarizing agent and those of a pseudocurarizing agent on the threshold of the like structure of the same preparation. Low doses of both nicotine and succinylcholine chloride caused a decrease in the predrug electrical threshold level of the neuromuscular junction, while high doses of these drugs caused an elevation of the predrug threshold level. Both low and high doses of dimethyl tubocurarine chloride, on the other hand, caused an elevation in this threshold level. Eserine salicylate enhanced the early blockades caused by nicotine and succinylcholine chloride but opposed the late blockades caused by these same drugs. Eserine salicylate opposed both the early and the late stages of a neuromuscular blockade brought about by dimethyl tubocurarine chloride. Nicotine and succinylcholine chloride induced a spastic paralysis in chicks, whereas dimethyl tubocurarine chloride induced a flaccid paralysis in other chicks of the same age and weight. On the basis of these studies, nicotine is classified as a neuromuscular blocking agent of the pseudocurare type which does not exert its effect through acetylcholine release.

Keyphrases □ Nicotine—effect on electrical threshold, neuromuscular junction, rat sciatic-gastrocnemius preparation, classified as neuromuscular blocking agent of pseudocurare type □ Neuromuscular blockage—nicotine effects studied, compared to other agents, classified, using rat sciatic-gastrocnemius preparation

Nicotine is thought to bring about a stimulation of ganglionic and related receptors, causing a depolarization of the postsynaptic membrane and a transient response. If the dose is adequate, it is thought to block somehow these same receptors as a result of, first, a prolonged depolarization and, later, a stabilized polarization phase.

The block of nicotine at the ganglion was considered by Pelikan (1) to result from an ability to prevent the release of acetylcholine at this site and not from its ability to compete with acetylcholine for the site receptors. On the other hand, Lundberg and Thesleff (2) recognized the ability on the part of nicotine *per se* to stimulate at the ganglionic receptor sites, but they stated that there is no complete correlation between the ganglion blocking action of nicotine and its prolonged ganglion cell depolarization because the blocking action outlasts the depolarization. The work of Paton and Perry (3) supported the view that the ganglionic block of nicotine has a second phase which occurs after the membrane becomes repolarized.

Langley (4), in 1909, discovered an antagonism by curare of nicotine's effect at the neural region of the amphibian skeletal muscle fiber. Thesleff (5), in 1955, found that the neuromuscular blockades of nicotine, succinylcholine, decamethonium, and acetylcholine develop during the depolarization of the muscle sole plate and continue after the transmembrane potential of this structure is spontaneously restored, without removal of the depolarizing agent. He concluded that the neuromuscular blocking action of these drugs is the result of a decrease in the sensitivity of the endplate to the neuronal transmitter.

Beani and Bianchi (6, 7), on the basis of their work in the guinea pig phrenic nerve-diaphragm preparation, classified nicotine as a stimulatory neuromuscular blocking agent. However, they agreed with Thesleff that its competitive block of cholinergic receptors at the