Keyphrases

3,9 - Disubstituted - 2,4,8,10 - tetraoxa - 3,9-diphosphaspiro[5.5]undecane-3,9-dioxides—synthesis

Antitumor activity—screening

IR spectrophotometry—structure

Centrally Acting Isosteric Mannich Bases

By RONALD E. ORTH*, J. W. BENNETT, ON-HOU MA†, and LARRY YOUNG

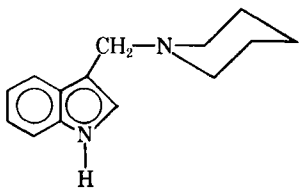
A number of *N*-heteroparaffinomethyl-1*H*-benzazoles is obtained by the Mannich and allied syntheses. Amine salts of all but the indoles are prepared. The degree of motricity and toxic levels are ascertained for the title compounds. Three major molecular modifications investigated cause variations in pharmacodynamic activity: substitution of the isosteric 1*H*-benzimidazole, 1*H*-indazole, and 1*H*-benzotriazole with 1*H*-indole; variation of the *N*-heteroparaffino-side chain moiety; and placement of the *N*-heteroparaffinomethyl substituents in different positions relative to the 1-nitrogen, depending on the particular benzazole. The 3-substituted indoles are most toxic and very active, while the 1-substituted benzimidazoles are best tolerated. Seven benzazoles (at least one from each chemical class) produce unusually high motility. Some general observations are reported. Methylpiperidino derivatives produce the greatest responses studied.

POWELL *et al.* (1) attribute hypertension and *in situ* uterine contraction to 3-(dimethylaminomethyl)indole (gramine). However, Bertaccini and Zamboni (2) report that the indolealkylamines (gramine included) with a single carbon atom lateral side chain have little smooth muscle activity. They find good 5-hydroxytryptamine-like activity exhibited by the indazolealkylamines. Studies by Walshe *et al.* (3) regarding factors influencing cerebral oxidation in relation to hepatic coma, using rat brain cortex slices, indicate gramine and 3-methylindole are active cerebral respiratory inhibitors at concentrations likely to be found in the body. Dubnik *et al.* (4), relating the effect of monoamine oxidase (MAO) inhibitors on brain serotonin of mice in addition to that resulting from inhibition of MAO, find gramine (a MAO-inhibitor) a cause of increased serotonin levels in the mouse brain.

Stimulated by inferences from the literature, this investigation is designed to (*a*) accommodate the synthesis of the title compounds and/or their water soluble salts (Table I) and (*b*) determine the degree of motor involvement and LD_{50}'s they produce in young white mice. Motricity data and subjective observations are noted in Table II.

Three major molecular modifications of gramine are responsible for producing the variations in bioactivity: interchange of the isosteric benzimidazole, 1*H*-indazole, and benzotriazole ring systems for indole; interchange of the *N*-heteroparaffino substituents on the side chain methyleno moiety; and placement of the *N*-heteroparaffinomethyl substituent in different positions relative to the 1-nitrogen, depending on the particular benzazole.

Gramine, synthesized by the Mannich reaction in 1935 (5), is the "open ended" tertiary amino model for the cyclized analogs prepared. Okuda (6), using a Mannich modification, has synthesized 3-(piperidinomethyl)indole (I). The product is found identical to Hellmann's (7) Mannich product, which is formed utilizing the acidic properties of benzenesulfonamide. Henry (8) has prepared the same compound from gramine *N*-oxide.



I

The Mannich reaction in methanol (9) is useful for preparing 1-(*N*-heteroparaffinomethyl)benzimidazole (II). 1-Hydroxymethylbenzimidazole (III) is also formed. The products of the normal Mannich condensation of benzimidazole with HCHO and *N*-heteroparaffins are stable and crystalline. Titration results using $(C_6H_5)_3C^-Na^+$ indicate a side chain attachment to the imidazole nitrogen (Scheme I).

The many attempts by Snyder *et al.* (10) at varying the Mannich reaction conditions to induce

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* Present address: College of Pharmacy, University of Houston, Houston, TX 77004. Direct all inquiries.

† Present address: Chemistry Department, Yale University, New Haven, Conn.

TABLE I—*N*-HETEROPARAFFINOMETHYLBENZAZOLES

Name	Ref.[a]	M.p. °C.[b]	% N[c] Calcd.	% N[c] Found	%, Yield	Hydrochloride[d] M.p. °C.[b]
3-(Pyrrolidinomethyl)indole	(6)	126–128	14.0	13.8	21	—
3-(3-Methylpiperidinomethyl)indole	(6)	144–146	12.3	11.4	35	—
3-(4-Methylpiperidinomethyl)indole	(6)	107–109	12.3	12.4	30	—
3-(4-Methylpiperazinomethyl)indole	(6)	158–160	18.3	18.1	41	—
1-(Pyrrolidinomethyl)benzimidazole	(9)	—	20.9	20.2	42	187–188
1-(4-Methylpiperazinomethyl)benzimidazole	(9)	116–117	24.3	23.9	87	151–152
1-(2-Methylpiperidinomethyl)benzimidazole	(9)	—	18.3	18.0	92	180
1-(3-Methylpiperidinomethyl)benzimidazole	(9)	64	18.3	18.2	89	d 213, sub. 160
1-(4-Methylpiperidinomethyl)benzimidazole	(9)		18.3	18.3	92	200–220, sub. 188
1-(Pyrrolidinomethyl)benzotriazole	(9)	83	27.7	27.8	80	148–150
1-(3-Methylpiperidinomethyl)benzotriazole	(9)	62–64	24.3	24.3	95	125–130
1-(4-Methylpiperidinomethyl)benzotriazole	(9)	78	24.3	24.5	92	120–122
1-(4-Methylpiperazinomethyl)benzotriazole	(9)	127	30.3	30.1	95	75
1-(2-Methylpiperidinomethyl)1*H* indazole	(12)	...	18.3	18.7	21	MeI 210–212
1-(3-Methylpiperidinomethyl)1*H* indazole	(12)	71–72	18.3	17.9	34	Hygroscopic
1-(Pyrrolidinomethyl) 1*H* indazole	(12)	130–135	20.9	21.2	16	Hygroscopic

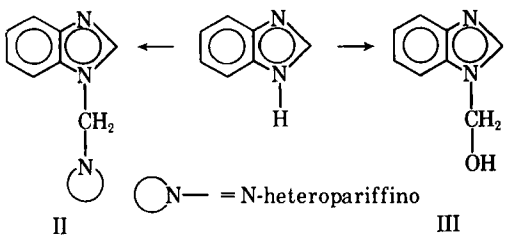
[a] Prepared according to the procedures described in the reference for similar compounds. [b] Uncorrected transition points obtained on Fisher-Johns melting point apparatus. [c] Weiler and Strauss Microanalytical Laboratories, Oxford, England [d] The indoles form red polymers with hydrogen chloride.

indazole to undergo condensation are a classic study of thoroughness. These investigators utilize a circuitous route to prepare 3-piperidinomethyl-1*H*-indazole commencing with methyl-3-indazolecarboxylate. Kochetkov and Dudykina (11) have also prepared this product with as arduous an

TABLE II—PRELIMINARY PHARMACODYNAMICS OF *N*-HETEROPARAFFINOMETHYLBENZAZOLES IN MICE

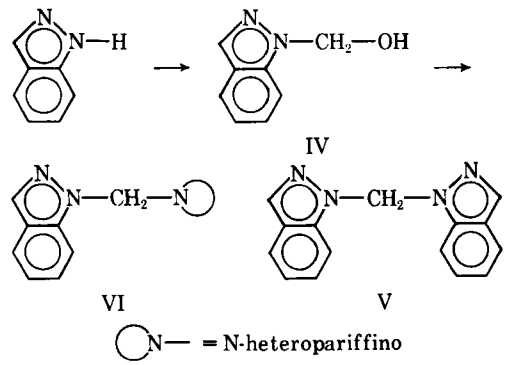
Name[a]	LD_{50}[b]	Dosage and Response[c] Dose	Motricity, 0.5–1 hr.	Dose	Motricity, 0.5–1 hr.	Observations[d]
Placebo	—	0	7–7	0	7–7	Normal behavior
d-Amphetamine sulfate	50/—	4	101–122	—	—	Intense motricity
3-Pyrrolidinomethyl indole	67/35	7	2–4	13	39–1	Cyanosis, convulsions
3-(2-Methylpiperidinomethyl)indole	87/20	8	3–2	17	36–28	Cyanosis, convulsions
3-(3-Methylpiperidinomethyl)indole	—	7	55–8	15	11–3	Normal behavior
1-Pyrrolidinomethyl benzimidazole	870/26	50	18–3	100	11–2	Low response at 250 mg./kg.
1-Piperidinomethyl benzimidazole	818/26	50	13–13	100	17–21	Motricity lost at 250 mg./kg.
1-Morpholinomethyl benzimidazole	960/26	50	11–13	100	4–4	Hypersensitive at 250 mg./kg.
1-(4-Methylpiperazinomethyl)-benzimidazole	368/26	50	6–11	100	5–6	Unsteady at 250 mg./kg.
1-(2-Methylpiperidinomethyl)-benzimidazole	930/26	50	15–3	100	11–3	Low response at 250 mg./kg.
1-(3-Methylpiperidinomethyl)-benzimidazole	806/26	50	9–7	100	6–3	High secretion/all doses
1-(4-Methylpiperidinomethyl)-benzimidazole	1200/26	50	5–53	100	4–4	Hypersensitive/all doses
1-Pyrrolidinomethyl benzotriazole	549–19	50	6–5	100	3–3	Respiratory failure
1-Morpholinomethyl benzotriazole	750/22	50	1–7	100	9–4	Normal at 500 mg.
1-Piperidinomethyl benzotriazole	—	50	3–1	100	2–3	Decreased motricity
1-(3-Methylpiperidinomethyl)-benzotriazole	686/23	50	61–28	100	51–87	Hypersensitive/all doses
1-(4-Methylpiperidinomethyl)-benzotriazole	570/26	50	35–3	100	26–6	Irritable
1-(4-Methylpiperazinomethyl)-benzotriazole	—	50	11–15	100	9–19	Normal
1-(3-Methylpiperidinomethyl)1*H*-indazole	290/20	15	34–14	30	36–112	Hypersensitive at 100 mg.
1-Pyrrolidinomethyl-1*H*-indazole	—	15	21–8	30	2–16	Normal

[a] The indoles are suspended in 0.5% methylcellulose. 1-(4-Methylpiperazinomethyl)benzimidazole is administered as the free base. All of the other benzimidazoles, indazoles, and benzotriazoles are administered as the HCl salt. [b] Administered intraperitoneally. Dose/number of young white mice (20 g.). [c] Three mice are utilized at each dosage level (mg./kg.), requiring an apparatus composed of three squirrel cages mounted on an axis. A spring tension pen, parallel but off-center, projects from the end of the axle to record on a Bird V. H. kymograph (Phipps and Bird, Inc.). The cages are narrow enough to allow unrestricted forward locomotion. Recordings (5 min.) are taken 0.5 hr. and 1 hr. after administration. A Jakar map measurer is used to tabulate the linear, rocking, and rotary tracing distances. [d] The mice are isolated and observed for 1 hr. following LD_{50} measurements. Prodding and tail pinching responses are observed.



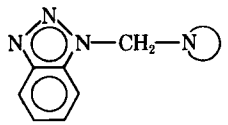
Scheme I—Bachman-Mannich reaction and products.

approach. A facile method of synthesis for 1-(*N*-heteroparaffinomethyl)1*H*-indazole is reported by Pozharskii *et al.* (12). 1-Hydroxymethyl-1*H*-indazole (IV) is first prepared by treating indazole with formalin in HCl. Water-soluble IV and water-insoluble V are obtained and separated. Refluxing IV with the appropriate *N*-heteroparaffin resulted in the formation of the 1-(*N*-heteroparaffinomethyl)-1*H*-indazole (VI) (Scheme II). Methiodide and HCl salts are prepared.



Scheme II—The synthesis of 1-N-heteroparaffinomethyl-1H-indazole and 1,1'-di-indazolylmethane.

The Bachman-Mannich reaction is best used for the synthesis of 1-(*N*-heteroparaffinomethyl)benzotriazoles (VII) (9). Hydrochloride salts are prepared to confer aqueous solubility on these compounds. The benzotriazoles are relatively unstable products and are hydrolyzed in hot water and with $(C_6H_5)_3C^-Na^+$ to give more than 1 mole of active hydrogen/mole of product.



VII

DISCUSSION OF RESULTS

Chemistry—New benzazoles are prepared using adaptations of previously reported procedures. The ease of preparation and percentage yields vary greatly from one class of benzazoles to another. Variation even occurs within a class. For instance, pyrrolidine reacts less favorably with each of the benzazoles than any of the other *N*-heteroparaffins used in this study. The best reactivity and yields, under Mannich conditions, are given in descending order: benzotriazole; benzimidazole; indole. Indazole is not included in this grouping as it (*a*) does not form the 3-substituted Mannich product expected (13), but (*b*) reacts readily with formalin in acid to give two 1-substituted products. Ionization data and electron-density diagrams would lead one to suspect indazole to be more acidic than indole, but less than benzotriazole or benzimidazole (14). Often acylation and alkylation occur at the more electronegative 2-nitrogen, followed by migration of the substituent to the 1-nitrogen position in indazole, to give an isomer which possesses a Kekule-type aromatic ring. Indazole activity may reflect its amphoteric nature, but then benzimidazole is also amphoteric. Electron-density diagrams suggest that the indole nitrogen atom is positive enough that the 1-hydrogen should dissociate to give a magnitude of acidity necessary for a successful Mannich reaction at this position. The 3-position is electronegative and is readily attacked when conditions favor electrophilic substitution. Benzotriazole, with its highly delocalized 1,2,3-triazole electrons, and benzimidazole, with a high degree of delocalization give every indication that their respective acidities are closely related to the excellent yields obtained.

Pharmacodynamics—Placebo i.p. injections of 0.4 ml. of water give the same motricity results as with noninjected animals. A reading of zero indicates no motricity and the extreme value of 122, recorded 1 hr. following the administration of 4 mg./kg. of *d*-amphetamine, create the scale.

The indoles are toxic, and show increased locomotion at 0.2 the LD_{50}. The exceptional indole is the 3-methyl derivative which exhibits questionably high activity at 7 mg./kg. at 0.5 hr. and normal motricity at all other times and doses. The 2-methyl homolog exhibits sustained hypermotricity through both time periods, while the pyrrolidino compound produces hypermotricity at 13 mg./kg. after 0.5 hr. and hypomotricity after 1 hr. The indoles cause death by convulsions and respiratory failure.

The relatively inactive benzimidazoles are the least toxic of the benzazoles studied. The 4-methylpiperidino homolog is the best tolerated of all benzazoles studied, and causes hypermotricity an hour after administration of a 50 mg./kg. dose. One hundred milligrams has no such locomotor stimulant effect. A great variation in class sensitivity is exhibited. Hypersensitivity at all dosage levels is characteristic of this same 4-methylpiperidino product, whereas the 2-methylpiperidino compound increases the pain threshold when 250 mg./kg. is given.

Highest motricity in the well-tolerated benzotriazole series is displayed by the 3-methyl- and 4-methylpiperidino homologs. At low dosages, the 3-methyl derivative exhibits sustained hypermotricity. At 100 mg./kg. the rate of locomotion increases from 51 at 0.5 hr. to 87 after 1 hr. Hypersensitivity is noted at all dosages for this compound. The 4-methyl homolog promotes hypermotricity at 0.5 hr., but not at 1 hr. The mice are irritable at all levels with this product.

1-(3-Methylpiperidinomethyl)-1*H*-indazole is tolerated about four times as well as the indoles and 0.5 as well as the benzotriazoles. It causes the highest motricity of all benzazoles tested. At 0.1 its LD_{50} dose, a response rivaling that of *d*-amphetamine is produced in 1 hr.

The *N*-heteroparaffinomethyl benzazoles of greatest interest are: 3-(pyrrolidinomethyl)indole; 3-(2-

methylpiperidinomethyl)indole; 3-(3-methylpiperidinomethyl)indole; 1-(4-methylpiperidinomethyl)-benzimidazole; 1-(3-methylpiperidinomethyl)benzotriazole; 1-(4-methylpiperidinomethyl)benzotriazole; and 1-(3-methylpiperidinomethyl)-1*H*-indazole. It is interesting to note that in all cases, with one exception, the benzazoles which produce the highest locomotor activity are methylpiperidino derivatives. Death usually occurs coupled with excess secretions, respiratory failure, and cyanosis. Occasionally convulsions are observed.

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Keyphrases

Mannich bases—centrally acting
Benzazole derivatives—Mannich reaction synthesis
Pharmacological screening—benzazoles

Hexahydropyrimidines IX. Synthesis of 2-Substituted-1,3-bis{2-methyl-4-[*N*,*N*-bis(2-chloroethyl)-amino]benzyl}hexahydropyrimidines as Transport Molecules for Tumor Inhibition

By JOHN H. BILLMAN and M. SAMI KHAN

A series of nitrogen mustard hexahydropyrimidines has been prepared by reacting *N*,*N*-bis{2-methyl-4-[*N*,*N*-bis(2-chloroethyl)amino]benzyl}1,3-diaminopropane with various aldehydes and evaluated for antitumor activity against various test systems. Some of the aldehydes used herein for the preparation of hexahydropyrimidines were reported to cause temporary tumor regression in test animals. The majority of compounds were screened against Walker 256 in rats and KB cell culture.

IT IS WELL KNOWN that a majority of tumors contain cells with a lower pH than cells in normal tissues. It has been shown also by Fitch *et al.* (1) that the administration of glucose to tumor-bearing animals can produce an even lower pH value for the tumor cells. Since hexahydropyrimidines are one class of compounds which hydrolyze readily *in vitro* under mild acidic conditions, it is likely that these compounds could be selectively hydrolyzed by the tumor cells to liberate active aldehydes as well as diamines which in themselves might act as antitumor agents. Thus it seems reasonable to expect that properly designed hexahydropyrimidines might act as carrier molecules to direct the nitrogen mustard grouping or other active neoplastic agents into the cellular metabolism.

In an earlier publication (2) the authors have reported the synthesis and the antitumor activity of a number of hexahydropyrimidines containing two aromatic nitrogen mustard groupings in the N-1 and N-3 positions. The primary screening results indicate that all of these compounds were nontoxic at high dose levels and were very active in KB cell culture. (0.1–0.0047 mg./ml.). Against Walker 256 a somewhat moderate activity was observed. In view of these encouraging results, it was of interest to study the effect of the substitution of methyl groups in the 2-positions of the benzene rings containing the nitrogen mustard grouping in order to obtain compounds with better therapeutic indexes and establish more fully their antitumor potentiality. Previous test results have indicated that a methyl group in the 2-position of aldehydes

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