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Antiarrhythmic Activity of 3-Amino-3-methyloxindoles

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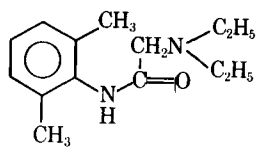
Abstract □ A series of 3-amino-3-methyloxindoles was synthesized from indoles by modification of previously described procedures. All compounds showed activity against chloroform-induced arrhythmias in mice. One member of the series, 3-methyl-3-piperidinooxindole, displayed activity equal to that of lidocaine while showing only one-third the acute toxicity.

Keyphrases □ 3-Amino-3-methyloxindoles—synthesis, antiarrhythmic activity, compared to lidocaine □ Antiarrhythmic activity—3-amino-3-methyloxindoles synthesized and screened

The authors' interest in the synthesis and pharmacological evaluation of lidocaine (I) analogs goes back several years, and initially attention was focused on compounds where the basic group was a hydrazine derivative (1). Recent work in these laboratories on the chemistry of aminooxindoles (2) has led to an expansion of this interest and to the present report concerning the synthesis and antiarrhythmic activity of a series of 3-amino-3-methyloxindoles (II).

Examination of II showed that these compounds incorporate the principal structural moieties of the local anesthetic and antiarrhythmic drug lidocaine—*viz.*, an aromatic nucleus, an amide linkage, and a basic amino group, in a nearly rigid framework. Closer examination revealed that the title compounds possess unique structural features that set them apart from lidocaine and its many congeners. Significant differences can be discerned in the stereochemistry of II and lidocaine. Thus, II has the *cis*-amide configuration, while the protonated form of lidocaine, which is supposed to be the active form of the drug, has the *trans*-configuration (3). Another difference is found in the coplanarity of the amide carbonyl group with respect to the aromatic moiety; steric hindrance precludes such a conformation in the lidocaine molecule (3, 4). Finally, it is worth noting that in II, in contrast to lidocaine, the "activity-controlling distance" (5) between the basic nitrogen and the amide carbonyl and aromatic nucleus is completely fixed.



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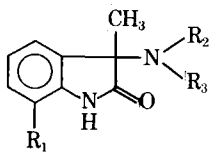


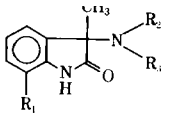
II

Because of its flexible structure, lidocaine can acquire a large variety of conformations and may, therefore, possibly fit a number of different receptor sites (6, 7). On the other hand, if a rigid congener of lidocaine can be found of the proper stereochemical configuration and charge distribution to give a high degree of complementariness to the receptor responsible for antiarrhythmic action, this hypothetical compound should show a more highly specific interaction (8). Since a full coincidence of stereochemical properties for the different receptors involved in the action of a multipotent drug is rather unlikely, this specificity should help to minimize side effects that originate from interaction with other receptor types as well as those that result from degradation of the drug into biologically active metabolites (9). In addition, such a hypothetical compound may be presumed to be highly active (7) and hence efficacious in smaller doses; this, in turn, should lower any associated "physical toxicity" (8) due to nonspecific drug action (10).

RESULTS AND DISCUSSION

The title compounds were obtained from indoles by modification of the procedures described by Hinman and Bauman (11, 12) (Scheme I). They prepared 3-bromo-3-methyloxindole by the *N*-bromosuccinimide bromination–oxidation of skatole (11); but in repeating this work, considerable difficulty was experienced in

Table I—3-Amino-3-methyloxindoles



Compound	R_1	R_2	R_3	Melting Point	Mole Ratio of *N*-Bromosuccinimide to Indole	Yield[a], %	Crystallization Solvent[b]	Formula	Analysis, %	
									Calc.	Found
IIa	H	—$CH_2CH_2CH_2CH_2$—		205–206°	2:1[c]	35[d]	A	$C_{13}H_{16}N_2O\cdot(COOH)_2$	C 58.82 H 5.92 N 9.15	58.70 5.98 9.31
IIb	H	—$CH_2CH_2CH_2CH_2CH_2$—		163–164°[e]	2:1[c]	16	B	$C_{14}H_{18}N_2O$	—	—
IIc	H	—$CH_2CH_2OCH_2CH_2$—		179.5–180°	3:1	29	B	$C_{13}H_{16}N_2O_2$	C 67.22 H 6.94 N 12.06	67.36 6.99 11.88
IId	H	H	C_2H_5	192–193°	2:1[c]	24	C	$C_{11}H_{14}N_2O$	C 69.45 H 7.42 N 14.72	69.67 7.50 14.87
IIe	H	H	*n*-C_3H_7	160°	3:1	83[f]	B	$C_{12}H_{16}N_2O$	C 70.56 H 7.90 N 13.71	70.76 7.91 13.82
IIf	H	H	*n*-C_4H_9	147°	2:1[c]	28	B	$C_{13}H_{18}N_2O$	C 71.53 H 8.31 N 12.83	71.29 8.12 12.56
IIg	CH_3	H	C_2H_5	140–141°	3:1	89	D	$C_{12}H_{16}N_2O$	C 70.56 H 7.90 N 13.71	70.72 8.14 13.61
IIh	CH_3	H	*n*-C_3H_7	117–118°	3:1	76	D	$C_{13}H_{18}N_2O$	C 71.53 H 8.31 N 12.83	71.43 8.15 12.70

[a] Yield is based on starting indole. [b] A = absolute ethanol–acetone, B = ether, C = benzene, and D = hexane. [c] A 2:1 ratio was used before it was found that 3:1 gave improved yields. [d] The oxalate salt was prepared since the free base is an oil. [e] Lit. (12) mp 160–161°. [f] The yield was 17% when the mole ratio of *N*-bromosuccinimide to indole was 2:1.

separating completely the desired bromo compound from succinimide, the by-product of the reaction. Therefore, the crude 3-bromo compound was not isolated but was allowed to react directly with an amine in ether.

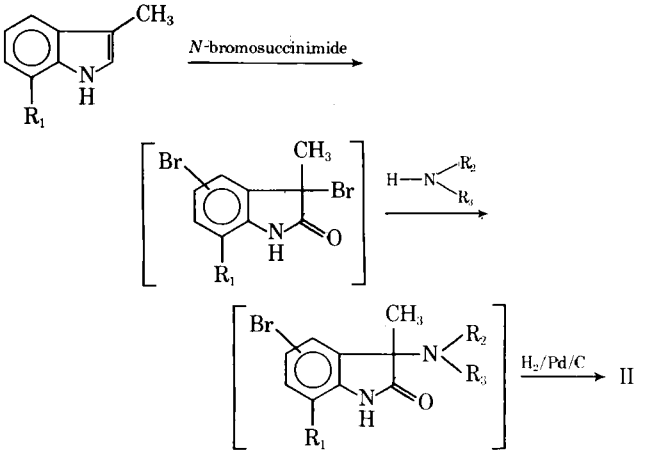
Initial experiments on the preparation of IIe [procedure of Hinman and Bauman, *N*-bromosuccinimide–indole (2:1 mole ratio)] gave a halogen-containing product presumed to be the 5-bromo derivative of IIe. Hydrogenation of this product with palladium-on-carbon catalyst in aqueous acetic acid resulted in the formation of IIe in 17% yield. Since the bromination of the aromatic ring was apparently in competition with bromination of the 3-position (11), the use of a 3:1 mole ratio of *N*-bromosuccinimide to indole followed by hydrogenolysis was tried. With such a proportion of reactants, the conversion of skatole into 3,5-dibromooxindole was expected. Under these conditions, IIe was obtained in 83% yield. These results (Table I) indicate that the optimum procedure for the preparation of II from indoles entails: *(a)* deletion of the isolation of the 3-bromo intermediate, *(b)* use of an *N*-bromosuccinimide to indole mole ratio of 3:1, and *(c)* a final hydrogenolysis step to remove aromatic bromine.

Table I records relevant data for the series of 3-amino-3-methyloxindoles. The IR spectra (KBr) exhibit a strong lactam carbonyl absorption band in the 1700–1705-cm^{-1} range. Table II contains the proton magnetic resonance (PMR) data for these compounds. All compounds shown in Table I, plus a lidocaine standard, were compared for their ability to prevent chloroform-induced arrhythmias by a modification of the procedure described by Lawson (13).

Preliminary experiments established that the chloroform-induced respiratory arrest led to cardiac arrhythmias in healthy female mice. Heart rates[1] averaged 592 ± 29 beats/min (n = 50). This value is in contrast to rates of 134 ± 19 and 137 ± 14 beats/min following ether-induced respiratory arrest at 5 and 10 min after saline injection[2], respectively. These findings are essentially in agreement with the report of Lawson (13) demonstrating the ability of chloroform to induce cardiac arrhythmias.

Subsequent studies indicated that the time of peak effect for protection from chloroform-induced arrhythmias was 10 min for lidocaine and 5 min for IIb and IIc. Therefore, lidocaine was examined 10 min following injection while the test compounds were all examined 5 min following administration.

The ability of the eight test compounds and lidocaine to counteract chloroform-induced arrhythmias is summarized in Table III. None of the test compounds was as potent as lidocaine in preventing arrhythmias, but several did display considerable activity at the 0.346-mmole/kg dose level tested. In an effort to determine more closely how compounds of this class compared to lidocaine, the median effective dose (ED_{50}) and median lethal dose (LD_{50}) of one of the more active compounds of the series, *i.e.*, 3-methyl-3-piperidinooxindole (IIb), were determined and compared to those of the standard. Graphs of the percent protection and the percent lethality *versus* dose for both compounds are shown in Figs. 1 and 2. Tests for parallelism, as determined by the method of Litchfield and Wilcoxon (14), indicated no significant difference between the curves for the two compounds either in percent protection *versus* dose or in percent lethality *versus* dose. Parallel effect curves for



Scheme I

[1] Mean ± *SE*.
[2] n = 5 in each case.

Table II—PMR Data of 3-Amino-3-methyloxindoles[a]

Compound	1-NH	3-NH	3-Methyl	ArH[b]	Other Substituents
II*a*[c]	—	—	1.93	7.35–8.18	1.82–2.32 (m) ($NCCH_2$); 3.33–3.84 (m) (NCH_2)
II*b*	9.62	—	1.61	7.04–7.81	1.32–1.90 (m) ($CH_2CH_2CH_2$); 2.58 2.98 (m) (NCH_2)
II*c*	9.68	—	1.61	7.07–7.77	2.83 (m) (NCH_2); 3.85 (m) (OCH_2)
II*d*	9.31	1.98	1.54	7.08–7.77	1.06 (t), 2.32 (q) (ethyl)
II*e*	9.40	1.94	1.47	6.86–7.53	0.82 (t), 1.08–2.67 (m) (propyl)
II*f*	9.71	1.98	1.53	7.07–7.77	0.84 (t), 0.96–2.68 (m) (butyl)
II*g*	9.96	1.96	1.50	7.04–7.51	1.03 (t), 2.29 (q) (ethyl); 2.40 (s) (benzylic CH_3)
II*h*	10.12	2.04	1.51	7.13–7.61	0.86 (t), 1.08–2.56 (m) (propyl); 2.43 (s) (benzylic CH_3)

[a] Chemical shift is given in δ units. Multiplicity is reported as: s = singlet, t = triplet, q = quartet, and m = multiplet. Spectra were determined in $CDCl_3$ with tetramethylsilane as the internal reference. [b] Multiplets. [c] Spectrum was recorded in D_2O with Tier's salt as the internal reference.

the two compounds support the probability that the aminooxindole derivative suppresses arrhythmias by a mechanism similar to that of lidocaine. The parallel toxicity curves indicate that the two compounds may also produce lethality by similar mechanisms. Since II*b* and lidocaine are related structurally, it is not surprising that they may also have a number of similarities pharmacologically.

The ED_{50}, LD_{50}, 95% confidence interval, and therapeutic indexes for II*b* and lidocaine are shown in Table IV. Although lidocaine appears to be slightly more potent in its protection from arrhythmias than II*b*, potency ratio = 1.2 (0.5–2.0)[3], this difference is not statistically significant. The marked difference in toxicity between lidocaine and II*b*, potency ratio = 3.8 (3.4–4.2)[3], is significant at the 95% level and it is this difference that accounts for the much more desirable therapeutic index seen with II*b*.

Any explanation for differences in acute toxicity between the two compounds is only speculative. Differences in ability to penetrate into the central nervous system (CNS), as well as to activate CNS receptors, may exist. Conformationally rigid molecules such as II*b* would probably be less likely to interact well with a number of different receptor groups than a compound that can assume several conformations.

Additionally, toxicity differences may be related to the eventual disposition of the compounds. Lidocaine is metabolized by oxidative deethylation of the tertiary amine to 2-ethylamino-2′,6′-acetoxylidine in rats, dogs, and humans (15). Although this metabolite is only one-third as potent as the parent compound at preventing chloroform-induced arrhythmias in mice, it is slightly more potent in producing convulsive lethality (16). If this metabolite is also formed in mice, then the parent drug and the metabolite would contribute to the toxicity demonstrated by lidocaine. Similar conversions to a metabolite that could add to the toxicity of the parent compound may not occur with II*b*.

Although these rigid compounds do not show an increase in activity, the therapeutic index for II*b* is more favorable than the corresponding value for lidocaine. This finding indicates that rigid antiarrhythmic drugs of lower toxicity and without a parallel decrease in activity can be prepared and that further efforts in this direction may result in superior drugs.

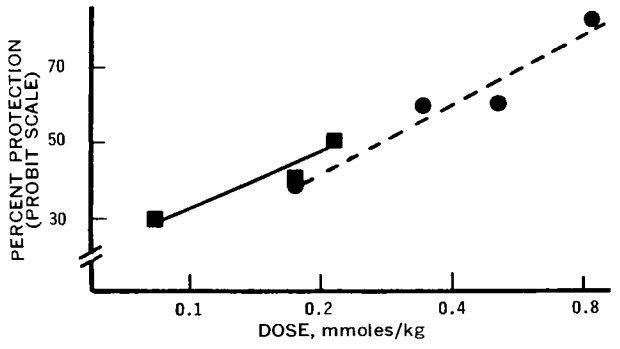


Figure 1—*Percent protection from chloroform-induced arrhythmia in female mice* versus *the administered intraperitoneal dose of II*b(●) *or lidocaine* (■).

[3] The 95% confidence interval.

EXPERIMENTAL[4]

3,7-Dimethylindole—This compound was prepared by modification of a previously reported procedure (17). A solution of 10 g (0.0764 mole) of 7-methylindole and 28 g (0.518 mole) of sodium methoxide in 150 ml of methanol was heated in a 1000-ml Parr bomb at 210–218° for approximately 12 hr and resulted in a pressure of 780 psi. The mixture was cooled to room temperature, and the contents were diluted with methanol and water. The methanol was evaporated under reduced pressure, and the residue was steam distilled to give 7.7 g (69%) of solid, mp 59°. Recrystallization from hexane (charcoal) resulted in white crystals, mp 63° [lit. (17) mp 56°]; NMR ($CDCl_3$): δ 2.33 (d, 3, CH_3 of hetero ring) and 2.43 (s, 3, benzylic H), 6.85–8.05 (m, 5, ArH, NH, and N—CH=).

3-Amino-3-methyloxindoles—The preparation of 3,7-dimethyl-3-ethylaminooxindole is described as a typical reaction. For additional data, see Table I. *N*-Bromosuccinimide (21.4 g, 0.12 mole) was added portionwise over 1 hr to a magnetically stirred solution of 5.8 g (0.04 mole) of 3,7-dimethylindole in 260 ml of purified (11) *tert*-butanol. The mixture was stirred for an additional 2 hr at room temperature. Evaporation of the solvent *in vacuo* at room temperature resulted in a syrupy residue, which was treated with 50 ml of anhydrous ether and filtered to remove the precipitated succinimide. The precipitate was washed well with ether, and the combined filtrate was diluted with additional ether to a vol-

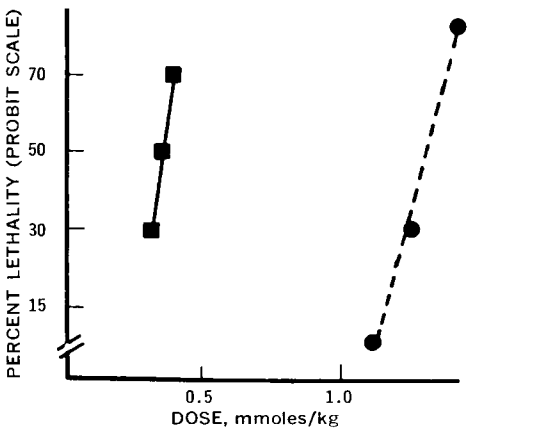


Figure 2—*Percent lethality of II*b (●) *or lidocaine* (■) *in female mice* versus *the intraperitoneal dose.*

[4] Melting points were determined with a Thomas-Hoover melting-point apparatus and are uncorrected. The structures of the compounds were confirmed by their IR and NMR spectra. IR spectra were obtained on a Beckman IR-8 spectrophotometer. NMR spectra were determined on a Varian A-60A spectrometer, using tetramethylsilane as the internal reference. Microanalyses were performed by Dr. Kurt Eder, Geneva, Switzerland, and PCR, Inc., Gainesville, Fla.

Table III—Effect of Lidocaine and Eight 3-Amino-3-methyloxindoles against Chloroform-Induced Arrhythmias in Mice

Compound	Dose, mmole/kg	Protected/Number Tested	Mean Heart Rate ± *SE*
Saline	—[a]	6/50	592 ± 29
Lidocaine hydrochloride	0.173	4/10	348 ± 44
Lidocaine hydrochloride	0.346	9/10	245 ± 22
II*a*	0.346	2/10	530 ± 62
II*b*	0.346	7/10	250 ± 64
II*c*	0.346	5/10	404 ± 80
II*d*	0.346	5/10	402 ± 99
II*e*	0.346	2/10	473 ± 60
II*f*	0.346	6/10	276 ± 66
II*g*	0.346	5/10	294 ± 58
II*h*	0.346	3/10	331 ± 40

[a] For each 10 g of body weight, 0.1 ml of saline was injected.

Table IV—ED_{50}, LD_{50}, and Therapeutic Indexes for Lidocaine and II*b* in Mice

Compound	ED_{50} (95% Confidence Interval)[a]	LD_{50} (95% Confidence Interval)[a]	Therapeutic Index
Lidocaine hydrochloride	0.22 (0.11–0.42)	0.45 (0.43–0.47)	2.0
II*b*	0.27 (0.15–0.49)	1.7 (1.5–1.9)	6.3

[a] Millimoles per kilogram.

ume of 250 ml. A solution of 33% ethylamine in absolute ethanol (20 ml) was added, and the resulting mixture was stored at room temperature for 1 week. The precipitate of ethylamine hydrobromide was filtered and washed with ether, and the ethereal solution was evaporated to a residue.

The residue was dissolved in 200 ml of 70% aqueous acetic acid and hydrogenated with 2 g of 10% Pd/C catalyst overnight on a Parr apparatus (initial hydrogen pressure = 55 psi). About 1.8 kg (4 lb) of hydrogen was absorbed. The catalyst was filtered on a sintered-glass funnel, and the solution was evaporated *in vacuo*. The semicrystalline residue was treated with 100 ml of 10% hydrochloric acid, and the mixture was diluted with water to 500 ml to dissolve all solids. This solution was then extracted twice with 100- and 75-ml portions of ether, respectively.

The aqueous phase was neutralized with solid sodium bicarbonate until the pH of the solution was 8–9. A white solid separated which was extracted once with 100 ml of chloroform and twice with 50-ml portions of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate. Evaporation of the dried solution gave 7.25 g (89%) of cream-colored solid, mp 138–139° with softening at 130°. Recrystallization from hexane with charcoal[5] treatment afforded 5.5 g of white product, mp 140–141°. Workup of the mother liquors resulted in a second crop of 0.77 g, mp 139.5–140.5°. The total recrystallized material amounted to 6.27 g.

Pharmacology—The antiarrhythmic screening procedure used was a modification of that described by Lawson (13). Female albino Swiss Webster mice, 20–25 g, were obtained from a local dealer[6] and given 5 days to recover from shipping stresses before use. Food and water were supplied *ad libitum*. The screening procedure consisted of injecting the animals intraperitoneally with the experimental compounds or the standard, lidocaine hydrochloride[7], dissolved in 0.1 *N* HCl. The concentration of each preparation was such that a volume of 0.1 ml was injected for each 10 g of body weight.

After injection, each mouse was placed in a chloroform-saturated atmosphere and observed. As soon as respiratory arrest occurred, usually within 1–2 min, the animal was removed from the chloroform atmosphere and an ECG tracing was obtained[8] (lead II). Ventricular rhythms above 300 beats/min were considered arrhythmic; those below 300 were considered rhythmic or protected from the chloroform-induced arrhythmia. Lethality was determined at 3 hr following intraperitoneal injection of lidocaine hydrochloride or the test compound.

The ED_{50} and LD_{50} were calculated by the method of Litchfield and Wilcoxon (14).

[5] Norit.

[6] Laboratory Supply, Indianapolis, Ind.

[7] Lidocaine hydrochloride, Pfaltz and Bauer, Flushing, N.Y.

[8] Preamplifier, MK III; amplifier, CA-200; recorder, DMP-4A; all from E & M Instrument Co., Houston, Tex.

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