With regard to the intermolecular bond, however, the three compounds differed entirely. In I and II, the hydrogen bonds were arranged alternately; they included the 2- and 4-pyrimidinetrione carbonyl groups (I) and the 4-pyrimidinetrione and 3-oxocyclohexenyl carbonyl groups (II). In III, hydrogen bonds generated cyclic structures, analogous to the adenine–thymine configuration in the Watson–Crick model of DNA, which included the 4- and 6-pyrimidinetrione carbonyl groups (Table III), and the molecules formed two linear interlacing chains. The structure's tenseness explains the high melting temperature (>290°) of III.

Concerning the preparation of II and III, the chemical oxidation of I, using *tert*-butyl chromate and the *in vivo* biochemical oxidation by six human subjects, gave exclusively the ketonic derivative II. On the other hand, UV irradiation of I gave III and not II. The results were unambiguous; the compounds were purified by fractional sublimation at 220° and then at 280°. Thus, detection of 0.5% of III in II and vice versa was very easy. Conflicting results (4) were probably due to the use of poorly purified materials.

A yield of oxidation products with different structures as a result of the different experimental conditions may be explained by the reaction mechanisms: a radical reaction for III and a chemical or biochemical mechanism, which implies steric hindrance with regard to enzyme or reactant, for II.

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Synthesis and Hypotensive Activity of a Series of 2-Substituted 5,6-Dimethoxyindazoles

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Received July 28, 1977, from the *Research and Development Department, Norwich-Eaton Pharmaceuticals Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication October 18, 1977.

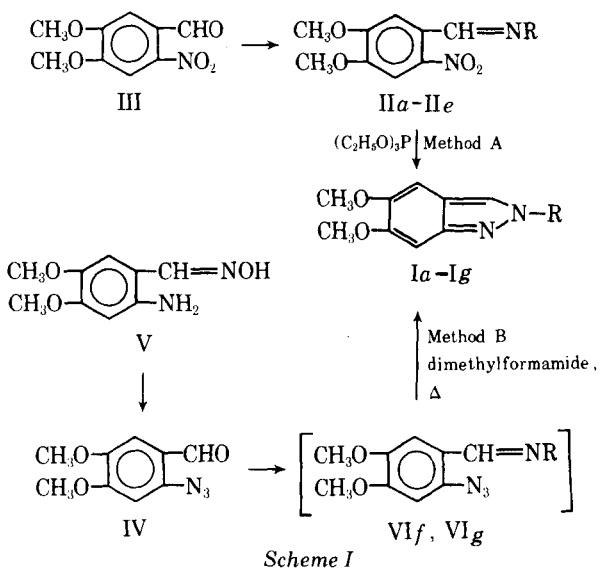
Abstract □ The synthesis and hypotensive activity in the dog of a series of 2-substituted 5,6-dimethoxyindazoles are reported. Structure–activity relationships for this class of compounds are discussed. Indazoles containing the diethylaminoethyl, 3-pyridyl, and hydroxyethyl functions in the 2-position were the most effective in lowering blood pressure for the longest times (>270 min).

Keyphrases □ Indazoles, substituted—synthesized, evaluated for hypotensive activity □ Hypotensive activity—various substituted indazoles evaluated □ Structure–activity relationships—various substituted indazoles evaluated for hypotensive activity

A limited number of 2-substituted indazoles have been subjected to extensive pharmacological screening (1–4). For example, 2-(2-aminoethyl)indazole showed only weak serotonin-like activity and exhibited no appreciable pharmacological responses in smooth muscle tests (5). This report describes the synthesis and results of a pharmacological evaluation of a series of 2-substituted 5,6-dimethoxyindazoles.

EXPERIMENTAL[1]

Chemistry—The majority of the 2-substituted 5,6-dimethoxyindazoles (Ia–Ie, Table I) were synthesized from *N*-substituted 6-nitroveratrylideneamines (IIa–IIe) in refluxing triethyl phosphite (6). These intermediate Schiff bases were conveniently prepared by the acid-catalyzed condensation of 6-nitroveratraldehyde (III) with primary amines



Scheme I

(Scheme I). However, since Schiff bases containing labile hydrogens are susceptible to alkylation by triethyl phosphite or the triethyl phosphate formed during indazole synthesis[2], an alternative route using nonal-

[1] Melting points were determined on a Mel-Temp apparatus, and those below 230° are corrected. IR spectra were determined as Nujol mulls on a Perkin-Elmer 137-B spectrophotometer and were consistent with the assigned structures.

[2] Cadogan *et al.* (6) showed that 2-nitro-4'-hydroxyazobenzene (A) in refluxing triethyl phosphite gave the ethoxy compound (B), presumably by alkylation of the phenol with triethyl phosphate, the oxidation product of the phosphite.

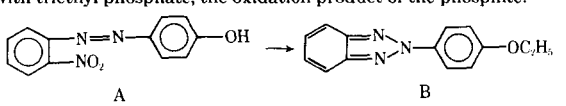


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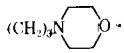
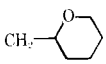
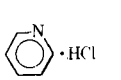
Table I—Physical and Analytical Data for 2-Substituted 5,6-Dimethoxyindazoles

Compound	R	Recrystallization Solvent[a]	Melting Point	Yield, %[b]	Formula	Analysis, %	Calc.	Found
Ia	$N(CH_3)_2$·HCl	A	195–196°	32	$C_{11}H_{15}N_3O_2$·HCl	C	51.26	51.12
						H	6.26	6.12
						N	16.30	16.34
Ib	$(CH_2)_2N(C_2H_5)_2$·2HCl	A	202–204°	30	$C_{15}H_{23}N_3O_2$·2HCl	C	51.43	51.32
						H	7.19	7.28
						N	12.00	11.88
Ic		B	238–239°	22	$C_{16}H_{23}N_3O_3$·2HCl	C	50.80	51.04
						H	6.66	6.85
						N	11.11	10.85
Id		C	95–96°	45	$C_{15}H_{20}N_2O_3$	C	65.20	65.31
						H	7.30	7.08
						N	10.14	9.92
Ie	$CH_2CH_2OCH_3$·HCl	D	160–161°	15	$C_{12}H_{16}N_2O_3$·HCl	C	52.84	53.08
						H	6.28	6.44
						N	10.27	10.37
If		E	243–245°	64	$C_{14}H_{13}N_3O_2$·HCl	C	57.64	57.91
						H	4.84	4.93
						N	14.40	14.35
Ig	CH_2CH_2OH	F	122–124°	57	$C_{11}H_{14}N_2O_3$	C	59.45	59.36
						H	6.35	6.26
						N	12.61	12.57

[a] A = 95% ethanol, B = methanol, C = heptane, D = ethyl acetate, E = 2-ethoxyethanol, and F = toluene. [b] Yield of material from cyclization suitable for biological evaluation (*i.e.*, 95% pure).

kylating conditions was sought. A procedure in which 2-azidobenzylideneamines were thermally decomposed to indazoles was reported by Krbechek and Takimoto (7) and then modified (8). Since this approach required 6-azidoveratraldehyde (IV), a convenient synthesis of large quantities of IV was desired.

Bamberger and Demuth (9) reported the preparation of 2-azidobenzaldehyde by reaction of 2-aminobenzaldoxime with nitrous acid, but attempted utilization of this method for the synthesis of IV from the oxime V (10) gave erratic results. However, addition of an external source of azide ion to the diazotization mixture of V gave the desired azide IV in 70–80% yields (Scheme I).

Acid-catalyzed treatment of IV with amines in dimethylformamide at 90° gave rise to the azides VI*f* and VI*g*, which were not isolated. Conversion to the desired indazoles I*f* and I*g* (Table I) was effected in boiling dimethylformamide (Scheme I). This method, although preferable for the synthesis of indazoles containing labile hydrogens (*e.g.*, I*g*), also was employed for the preparation of I*f*.

N-Substituted 6-Nitroveratrylideneamines (IIa–IIe)—These compounds were synthesized by refluxing equimolar quantities of 6-nitroveratraldehyde and the amine in ethanol for 5 min–1.5 hr, using catalytic amounts of acetic acid. The separated products were suitable for conversion to the desired indazoles. In cases where the product failed to crystallize, the solvent was removed *in vacuo* and the residue was used directly to prepare the indazole.

5,6-Dimethoxy-2-(3-morpholinopropyl)indazole Dihydrochloride (Ic)—A mixture of 127 g (0.376 mole) of 6-nitroveratrylidene-2-(3-morpholinopropyl)amine (IIc) and 144 g (0.867 mole) of triethyl phosphite was refluxed for 4.5 hr. The excess phosphite and phosphates were removed in high vacuum. The residue was dissolved in 300 ml of chloroform. The resulting solution was washed with water, dried, and concentrated to dryness to give the crude indazole.

Treatment of a methanolic solution of the crude product with methanolic hydrogen chloride gave Ic (Table I).

Similarly prepared were I*a* and I*b* (Table I). For I*d* and I*e* (Table I), synthesized using similar conditions, the crude products were purified by passing a benzene solution of the indazole through a column containing neutral alumina. The purified free base of indazole I*e* was converted to the product with ethereal hydrogen chloride.

6-Azidoveratraldehyde (IV)—To 1290 ml of concentrated hydrochloric acid, cooled at −10°, was added rapidly 252 g (1.29 moles) of V (10). While the reaction mixture was maintained at from 0 to −10°, a solution of 97.7 g (1.41 moles) of sodium nitrite in 400 ml of water was added (30 min required). Then the mixture was stirred at from 0 to −15° for an additional 30 min. While the mixture was maintained below 0°, a solution of 83.5 g (1.28 moles) of sodium azide was added cautiously.

After 15 min of stirring, the mixture was diluted with 3.2 liters of cold water. It then was stirred at from 0 to −10° for 1.5 hr; while being maintained at this temperature, a solution of 3.47 liters of 25% sodium hydroxide was introduced. This mixture was stirred at ambient temperature for 15 hr, and the precipitated product was filtered, washed with water, and dried to constant weight to give 207 g (78.1%) of the azide, mp 112–114°. An analytical sample, mp 116–118°, was prepared by recrystallization from 2-propanol; IR: 4.71 (N_3), 6.01 (C=O), and 6.27 (C=C) μm.

Anal.—Calc. for $C_9H_9N_3O_3$: C, 52.17; H, 4.38; N, 20.28. Found: C, 52.12; H, 4.26; N, 20.29.

5,6-Dimethoxy-2-(3-pyridyl)indazole Hydrochloride (If)—A mixture of IV (62 g, 0.300 mole), acetic acid (1 ml), dimethylformamide (250 ml), and 3-aminopyridine (28.2 g, 0.300 mole) was heated at 90° for 3 hr. The dark solution was refluxed for 1 hr and then allowed to stand overnight at room temperature. The dimethylformamide was removed under reduced pressure, and the residue was poured into water (1500 ml). The aqueous solution was extracted with ethyl acetate (4 × 500 ml).

The ethyl acetate extract was dried over sodium sulfate and concentrated to dryness. The resulting oil was dissolved in methanol, decolorized, and filtered. The methanolic filtrate was treated with methanolic hydrogen chloride to give 56 g (64%) of the crude product, which was recrystallized from ethoxyethanol (2500 ml) to yield 34 g of I*f*, mp 230–238° (Table I).

Compound I*g* was prepared in a similar manner from IV. Removal of the dimethylformamide in high vacuum gave the crude product, which was taken up in ethyl acetate, decolorized, and filtered. Removal of the solvent gave the crude product (Table I).

Pharmacology—Indazoles I*a*–I*g* were evaluated for cardiovascular activity in anesthetized dogs at doses ranging from 10 to 100 mg/kg iv using the CARDAMAP procedure (11). Their hypotensive activity is summarized in Table II.

Compounds I*b*–I*d*, I*f*, and I*g* were most effective in lowering blood

Table II—Hypotensive Activity of 2-Substituted 5,6-Dimethoxyindazoles in Dogs

Compound	Dose, mg/kg iv	Blood Pressure, % Change	Duration, min
Ia	10	−16	25
	100	−25	210
Ib	100	−74	270
Ic	10	−34	210
	100	−59	150
Id	100	−70	15
Ie	100	−25	150
If	10	−25	10
	25	−59	>330
	50	−66	>270
	100	−78	>270
Ig	50	−43	15
	100	−84	>270

pressure, but the duration of action of Ib, If, and Ig was considerably greater than that of the other indazoles.

Regarding structure–activity relationships, increasing the distance between the indazole 2-nitrogen and the tertiary amino group of the side chain increased hypotensive activity (Ia *versus* Ib and If). Furthermore, Id, with the alkoxyalkyl moiety as part of the tetrahydropyran ring, was more active than the straight chain compound Ie, although the duration of action of Id was considerably less. Finally, the carbinol Ig exhibited greater hypotensive activity than its *O*-methyl derivative Ie.

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Colorimetric Determination of Nadolol in Tablets

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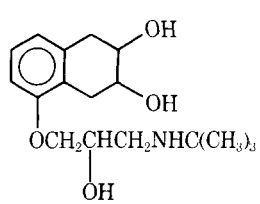
Abstract □ Nadolol was extracted from tablet excipients with an acidic potassium chloride solution. The drug was oxidized with periodic acid, and the resulting aldehyde was reacted with 2,4-dinitrophenylhydrazine to form the corresponding hydrazone. Excess reagent was removed with a cupric chloride solution. The hydrazone was extracted into chloroform, and its absorbance was measured at the 352-nm maximum.

Keyphrases □ Nadolol—colorimetric analysis in tablets □ Colorimetry—analysis, nadolol in tablets □ Antiadrenergic agents—nadolol, colorimetric analysis in tablets

Nadolol [*cis*-5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy] -1,2,3,4- tetrahydro-2,3-naphthalenediol] (I) is a new β-adrenergic blocking agent (1). A fluorometric determination of nadolol in human serum and urine, with references on clinical uses of the drug, was reported previously (2). The present method involves coupling the carbonyl groups of the periodate-oxidized drug with 2,4-dinitrophenylhydrazine to form a highly colored hydrazone. Procedures for the determination of carbonyl compounds as their 2,4-dinitrophenylhydrazones have been reported (3, 4). In the present paper, a novel elimination of the excess 2,4-dinitrophenylhydrazine with a cupric chloride solution is described.

EXPERIMENTAL

Apparatus—Colorimetric measurements were made with a spectrophotometer[1]. Samples were shaken on a heavy-duty shaker[2]. Screw-top test tubes, 150 mm, with plastic caps were washed as described (2). A centrifuge with stainless steel adapters was used[3].



I

Reagents—All chemicals were reagent grade. A 10-ml quantity of 1% sodium metaperiodate[2] was prepared in 0.1 *N* HCl. Sodium arsenite[2] was prepared by dissolution of 0.4 g of the reagent in 9 ml of 0.1 *N* HCl and addition of 1 ml of concentrated hydrochloric acid. Both reagents were prepared daily.

Methanol[2] was purified by refluxing 4 liters with 20 g of 2,4-dinitrophenylhydrazine[4] and 20 ml of hydrochloric acid followed by glass distillation. 2,4-Dinitrophenylhydrazine solution was prepared daily by dissolution of 50 mg of 97% pure reagent in 25 ml of purified methanol containing 0.5 ml of hydrochloric acid. Acidic potassium chloride[5] solution was prepared by dissolution of 50 g of potassium chloride in 950 ml of 0.1 *N* HCl, and 20% cupric chloride[5] was prepared by dissolution of 200 g of cupric chloride dihydrate in distilled water.

Preparation of Standard Assay[6]—Weigh about 40 mg of standard nadolol into a 100-ml volumetric flask. Dissolve and dilute to volume with acidic potassium chloride solution. Dilute 10.0 ml of this solution to 100 ml with acidic potassium chloride.

Assay—*Sample Preparation*—Weigh and finely powder not less than 20 nadolol tablets. Accurately weigh a portion of the powder equivalent to 40 mg of nadolol. Transfer the powdered sample to a 120-ml glass bottle.

Extraction—To each bottle, add 100.0 ml of acidic potassium chloride solution. Cover the bottles with aluminum foil and caps and shake them for 1 hr. Filter the solutions through medium-porosity sintered-glass filters and collect the filtrates in 120-ml glass bottles. Dilute 10.0 ml of the filtrate to 100 ml with acidic potassium chloride solution.

Oxidation and Hydrazone Formation—Pipet 1.0 ml of acidic potassium chloride solution (reagent blank), 1.0 ml of tablet extract, and 1.0 ml of the diluted standard nadolol solution into separate 150-mm, screw-capped, acid-washed test tubes. With an automatic syringe, add 0.10 ml of sodium periodate solution to each tube and mix well on a vortex mixer. Centrifuge the tubes at about 2000 rpm for 2 min.

[1] Beckman DU equipped with a tungsten lamp.
[2] Fisher Scientific Co.
[3] IEP 2741, Scientific Products.
[4] Aldrich.
[5] Mallinckrodt Chemical Works.
[6] Both standard solutions are stable at room temperature for at least 4 weeks.

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