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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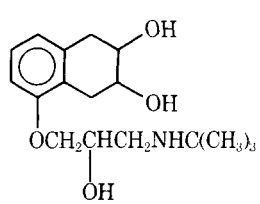
Abstract □ Nadolol was extracted from tablet excipients with an acidic potassium chloride solution. The drug was oxidized with periodic acid, and the resulting aldehyde was reacted with 2,4-dinitrophenylhydrazine to form the corresponding hydrazone. Excess reagent was removed with a cupric chloride solution. 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].



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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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Table I—Reproducibility of Results

Tablet Formulation, mg	*RSD*, %[a]
10	1.11
40	0.64
80	0.86
120	0.46

[a] Ten runs of each tablet formulation.

Fifteen minutes after mixing the last tube on the vortex mixer, add 0.20 ml of sodium arsenite solution with an automatic syringe and mix well on the vortex mixer. Five minutes after mixing the last tube on the vortex mixer, add 1.0 ml of 2,4-dinitrophenylhydrazine solution with a volumetric pipet and mix well on the vortex mixer. After 5 min, add 10.0 ml of chloroform to each tube and mix on the vortex mixer for 5 sec.

To each tube add 10.0 ml of 20% cupric chloride, cover the tubes with plastic caps, and shake mechanically for 15 min. Centrifuge the tubes at about 2000 rpm for 5 min. Aspirate completely and discard the top aqueous layer. If needed, aspirate some of the chloroform layer.

Measure the absorbance of the chloroform solutions of the sample and standard in 1-cm cells at the 352-nm maximum with the chloroform reagent blank in the reference cell. Nadolol in an average tablet is calculated from the concentration and absorbance of a nadolol standard.

RESULTS AND DISCUSSION

Experimental results for the oxidation of nadolol with periodate were given previously (2). The 2,4-dinitrophenylhydrazine reagent was optimized by using excess reagent and a proper hydrochloric acid concentration. When less than 0.5 ml or more than 4.0 ml of acid/100 ml of reagent was used, a lower absorbance was obtained. A novel method for eliminating excess 2,4-dinitrophenylhydrazine from the chloroform extracts with cupric chloride was introduced.

Several concentrations of cupric chloride were tried for the elimination of the excess 2,4-dinitrophenylhydrazine. From 10 to 30% cupric chloride solutions were used successfully. Higher than 30% cupric chloride solutions decomposed the oxidation reagent by producing a small amount of iodine, which slightly lowered the absorbance of the solutions and had an adverse effect on reproducibility. Elimination of 2,4-dinitrophenylhydrazine was rapid and occurred within a few minutes, but 15 min of shaking is recommended for reproducible results.

Nadolol 2,4-dinitrophenylhydrazone was stable in chloroform when shaken with cupric chloride solutions. No change in the absorbance was found when chloroform solutions were shaken with 20% cupric chloride solutions for up to 0.5 hr.

Table II—Nadolol in Tablet Formulations

Tablet Formulation, mg	Nadolol, mg/tablet Run 1	Run 2
10	10.04	9.97
40	40.00	40.20
80	80.20	80.00
120	120.80	120.10

The reaction of oxidized nadolol with 2,4-dinitrophenylhydrazine was completed in less than 1 min. The product was stable in the reaction mixture and in chloroform for at least 60 min.

The volume of sample or standard to be oxidized and then reacted with 2,4-dinitrophenylhydrazine should be 1 ml. When samples of more than 2 ml were used, lower absorbances were obtained. At least 30% methanol also was needed in the reaction mixture for maximum color development.

Various solvents were tried for the extraction of nadolol 2,4-dinitrophenylhydrazone from aqueous solutions. Methylene chloride, chloroform, and ethylene chloride produced the same reagent blank and extracted the color completely. No color was extracted with carbon tetrachloride, ether, ethyl acetate, butyl alcohol, and methyl ethyl ketone. Butyl acetate extracted the color completely, but incomplete destruction of 2,4-dinitrophenylhydrazine was obtained. Toluene extracted about 60% of the color.

Nadolol 2,4-dinitrophenylhydrazone–chloroform solutions had a maximum absorption at 352 nm and obeyed Beer's law at least between 0 and 200 μg of nadolol.

Recovery of nadolol was studied by adding the drug to the appropriate placebo tablet. Recoveries from 10-, 40-, 80-, and 120-mg tablets varied between 99.2 and 101.0%. Reproducibility of results was checked by analysis of each tablet formulation 10 times (Table I).

The method has performed reliably for a number of batches of nadolol tablets and, therefore, can be used for manufacturing control. Results from the analysis of some nadolol tablets are given in Table II.

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New 5-Hydroxy-2-indolecarbohydrazides as Platelet Aggregation Inhibitors in Ethylene Glycol

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Received May 2, 1977, from the *Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain.* Accepted for publication October 27, 1977.

Abstract □ The effect of ethylene glycol on blood platelet aggregation was examined using a previously described method. This method also was used to investigate several derivatives of 2-indolecarbohydrazide *in vitro*. All compounds inhibited platelet aggregation induced by collagen, epinephrine, or adenosine diphosphate at concentrations below 5×10^{-4} *M*.

Keyphrases □ Indolecarbohydrazides, various—effect on human platelet aggregation in ethylene glycol *in vitro* □ Platelet aggregation, human—effect of various indolecarbohydrazides in ethylene glycol *in vitro* □ Structure–activity relationships—effect of various indolecarbohydrazides on human platelet aggregation in ethylene glycol *in vitro*

The role of platelets in the formation of thrombi and arterial occlusions and the effect of adenosine diphosphate (ADP) on platelet aggregation are well documented (1). It has been suggested that the inhibition of platelet aggregation may be more useful than standard anticoagulant therapy (2, 3).

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