# 1-Halobenzyl-1*H*-indazole-3-carboxylic acids. A New Class of Antispermatogenic Agents

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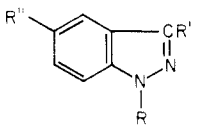
The synthesis of a series of halogenated 1-benzylindazole-3-carboxylic acids and related derivatives is described. These compounds were studied for their effect on testicular weight and on the inhibition of spermatogenesis. Many of the derivatives, but in particular 1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carboxylic acid (**11**), 1-(2,4-dibromobenzyl)-1*H*-indazole-3-carboxylic acid (**13**), 1-(4-chloro-2-methylbenzyl)-1*H*-indazole-3-carboxylic acid (**27**), and their glycerol esters, showed potent antispermatogenic activity.

A pharmacological examination of 1-*p*-chlorobenzyl-1*H*-indazole-3-carboxylic acid[1-4] has shown it to have a specific antispermatogenic activity. The interest, both theoretical and practical, in this type of activity, and the relative lack of sufficiently potent and selective antispermatogenic drugs,[5] encouraged us to synthesize and test numerous 1*H*-indazole-3-carboxylic acids and derivatives.

In this paper, the preparation and the antispermatogenic activity of a series of new compounds with the general formula I are reported. While R'' is represented only by

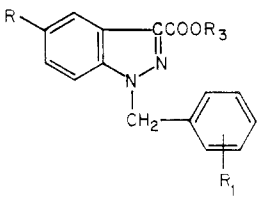


I

H, Cl, OH, $CH_3$, and $OCH_3$, R and R' may be represented by various substituents as illustrated in Table I and in the text.

## Results and Discussion

All the most active compounds belong to a series with the general formula Ia. As far as the substituents R in



Ia

the indazole nucleus are concerned, only 5-hydroxy, 5-methoxy, 5-chloro, and 5-methyl derivatives were considered, all of which proved to be inactive. The presence of a substituted benzyl group in position 1 is essential for activity. However, not all substituents $R_1$ confer activity to the substance; as a matter of fact, it seems necessary that $R_1$ should be either a halogen or a methyl group. The position of the halogen does not seem to have much influence (for instance, compounds **1–3** are approximately equiactive), whereas the position of the methyl group is decisive since only the ortho derivative is active. The activity increases in the case of two substituents $R_1$ in positions ortho and para, both when they are halogens (as, for instance, in compound **11**, which is among the most active derivatives) and when one is a halogen and the other a methyl (as in compound **27**). If the two substituents are in different positions, the activity is considerably diminished (compare, for instance, compounds **10** and **12** with compound **11**). Also a third substituent lowers the activity. No activity has been found in 1-unsubstituted 1*H*-indazole-3-carboxylic acid or in acids substituted in position 1 with haloaryl or with haloaroyl groups.

As far as substituents $R_3$ are concerned, the activity is present when $R_3$ stands for a hydrogen or a residue of ethylene glycol or glycerol. Other esters are inactive, whereas some activity is still retained in amides corresponding to an active acid (as, for instance, in compound **41**) and in compounds not included in general formula Ia, but which are derived from substances with general formula I in which R', instead of a carboxylic group, is represented by an acetic residue (as, for instance, in compound **42**). However, the activity disappears in the homologous propionic acid.

No activity is shown by the nitrile corresponding to compound **1** or by compounds having other groups in position 3, such as amino, a thiol, an acetyl, or a hydroxymethyl group. Also the tetrahydroindazole corresponding to compound **1** is inactive.

Since the known antispermatogenic agent 2,3-dihydro-2-(1-naphthyl)quinazolinone[6] (U-29409) can be chemically correlated to *N*-(1-naphthylmethyl)anthranilic acid, this new compound and the analogous *N*-(*p*-chlorobenzyl)anthranilic acid were also tested as simplified models of our active series. Neither of the compounds showed any activity. Indole analogues of our active indazole derivatives, for instance, 1-*p*-chlorobenzylindole-3-carboxylic acid, 1-*p*-chlorobenzyl-2-methylindole-3-carboxylic acid, and 1-*p*-chlorobenzylindole-2-carboxylic acid, were also found to be devoid of antispermatogenic activity.

A discussion of the general pharmacological properties of these derivatives is beyond the scope of this paper, also on account of the fact that a detailed study of the first derivative to be found active, i.e., compound **1**, has already been published.[1-4] We shall consequently make only a few comments. From the histological point of view, the antispermatogenic activity of all the active derivatives is similar. The testis showed disorganization of the seminiferous tubules consisting mainly of lesion and loss of spermatocytes and spermatids, whereas the spermatogonia and interstitial tissue appeared to be normal. It should also be stressed that without exception all the compounds listed as active did not reduce body weight at doses having antispermatogenic effects. In contrast to most antispermatogenic agents described in the literature,[7-9] the derivatives of this series have the interesting characteristic of being active after a single administration. This explains why compound Win 18446, which is one of the two reference substances used in this study, has been found to be inactive up to the dose of 400 mg/kg po. It should be noted that this product, which proved to be an effective antispermatogenic drug also in humans, has been described to produce an antispermatogenic effect in rats only after a 3-week daily treatment at doses starting from 63 mg/kg

po.[10] The second reference compound used in our studies was U 29409, belonging to the group of drugs which are active after a single administration.[6] Under our experimental conditions, U 29409 proved to be less effective than the more potent derivatives of this series.

On the basis of the available data it can be concluded that this new class of compounds is of interest for different reasons. In the first place, some of them have proved to be much more active than the first member of the series, whereas results obtained from the highest doses studied have shown no increase in the toxicity. Consequently, these compounds are potentially useful for birth control. Secondly, since they have the ability to act after a single administration they are particularly interesting for the physiological study of the male reproductive system. This property, together with the specific antispermatogenic effect, opens new possibilities for this type of study. It should also be pointed out that this is the first time that such an effect has been described in this chemical class for which a completely new field of chemical research has therefore been opened.

## Experimental Section

**Chemistry.** Melting and boiling points are uncorrected. Ir and uv spectra of all compounds were obtained and absorptions were as expected. Analyses, indicated only by symbols of the elements, gave results within ±0.4% of theoretical values.

Benzyl chlorides were generally used for the reactions and prepared according to the methods described in the literature; when these were not available benzyl bromides were used instead, also prepared according to the methods described in the literature.

All the new 1-substituted 1*H*-indazole-3-carboxylic acids were prepared by two methods. The first one consisted of the direct alkylation of the corresponding 1*H*-indazole-3-carboxylic acids with a benzyl halide in aqueous sodium hydroxide. When this method failed, a second one was used in which the benzylation was carried out on the ethyl ester in an ethanol or dioxane solution. The new ester thus obtained was then hydrolyzed with potassium hydroxide in a 50% ethanol solution.

All the other compounds listed in Table I were prepared either according to conventional procedures for similar derivatives or as described in the text for particular derivatives.

**1-Benzyl-1*H*-indazole-3-carboxylic Acids. Method A. General Procedure.** 1*H*-Indazole-3-carboxylic acid (0.1 mol) was added to a solution of NaOH (0.3 mol) in water (280 ml). The mixture was heated on a steam bath with stirring and then a benzyl halide (0.13 mol) was added dropwise. The mixture was heated and stirred for about 4 h and then acidified with dilute HCl. After standing, the resulting solid was separated and washed with water and then with small portions of ether in a mortar. The product left after removal of the solvent was shaken at room temperature with aqueous NaOH which dissolved unreacted 1*H*-indazole-3-carboxylic acid. The insoluble portion was separated and treated with dilute aqueous NaOH on a steam bath. The warm solution was filtered and then acidified with dilute HCl. After standing, the new precipitate was separated and thoroughly washed with water and dried. The product crystallized from acetic acid alone or, sometimes, with the addition of dioxane.

**Method B. General Procedure.** (a) A sodium ethoxide solution, prepared from Na (0.05 mol) and ethanol (100 ml), was added dropwise with stirring to a solution of 1*H*-indazole-3-carboxylic acid ethyl ester (0.05 mol) and a benzyl halide (0.05 mol) in anhydrous ethanol (150 ml) under reflux. The addition took 3–4 h, the solution being constantly controlled to prevent it from becoming alkaline.

In case of an oily precipitate, this was extracted with ether (or chloroform); the organic phase was washed with 0.25 N NaOH and then with water until neutral and finally dried over anhydrous $Na_2SO_4$. The removal of the solvent usually left a solid residue which could be crystallized; this not being the case, the residue was hydrolyzed without further purification.

(b) A mixture of 1*H*-indazole-3-carboxylic acid ethyl ester (0.1 mol), sodium hydride (0.1 mol as 50% dispersion in oil), and anhydrous dioxane (185 ml) was refluxed for 0.5 h under stirring. A solution of benzyl halide (0.1 mol) in anhydrous dioxane (55 ml) was added dropwise to this mixture. When the addition was over, heating was continued under reflux for 0.5 h and the mixture was then poured into water. The precipitate was extracted with $CHCl_3$ and the organic layer washed with water and dried. After removal of the solvent the residue was weighed and hydrolyzed as such.

A mixture of 1-substituted 1*H*-indazole-3-carboxylic acid ethyl ester (0.1 mol) and KOH (0.2 mol) in 50% ethanol (800 ml) was refluxed for 5 h. The mixture was cooled with ice and water and acidified with 6 N HCl. The precipitate was separated and washed until neutral with water and then several times in a mortar with small portions of ether. After removal of the solvent, the crude product generally crystallized from acetic acid alone or, sometimes, with the addition of dioxane.

**1-Substituted 1*H*-Indazole-3-carboxylic Acid Chlorides. General Procedure.** One part of acid was added in portions to four parts of $SOCl_2$. The mixture was then warmed for 5 min at 80°. $SOCl_2$ in excess was removed under reduced pressure and the residue was crystallized from benzene. The new compounds reported are 1-(*p*-chlorobenzyl)-1*H*-indazole-3-carboxylic acid chloride [mp 165°; yield 86%. Anal. ($C_{15}H_{10}Cl_2N_2O$) C, H, N, Cl] and 1-(*o*-chlorobenzyl)-1*H*-indazole-3-carboxylic acid chloride [mp 167°; yield 80%. Anal. ($C_{15}H_{10}Cl_2N_2O$) C, H].

**1-Substituted 1*H*-Indazole-3-carboxylates. General Procedure.** Methyl and ethyl esters were prepared by refluxing the corresponding acid chlorides with methanol or ethanol in excess.

**2-Hydroxyethyl 1-*p*-Chlorobenzyl-1*H*-indazole-3-carboxylate (33).** This compound was obtained by treating the sodium salt of the acid in DMF with 2-chloroethanol for 4 h at 110°. The product was crystallized from benzene: mp 75°; yield 29%. Anal. ($C_{17}H_{15}ClN_2O_3$) C, H, N.

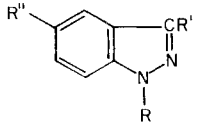
**α-Glyceryl Esters. General Procedure.** α-Glyceryl esters were obtained either directly by heating for 8 h at 160–170° 0.1 mol of the corresponding acid with 0.7 mol of anhydrous glycerol and 1 drop of concentrated HCl or through the intermediate acetonide. In this latter case, 0.1 mol of the indazolecarboxylic acid chloride was stirred at 0° for 6 h with 0.12 mol of acetone–glycerol in anhydrous quinoline (ten parts per part of acetone–glycerol); the temperature was then left for 40 h at 25°. Water and ether were added and several extractions were performed. The solvent was removed; the residue was heated under reduced pressure in order to obtain a complete elimination of quinoline and then dissolved in 5 vol of ether. An equal volume of concentrated HCl was added dropwise in 20–30 min. The mixture was then diluted with water (6 vol of water per volume of concentrated HCl) and extracted with $CHCl_3$. After removal of the solvent, the residue became solid and could be suitably crystallized from benzene.

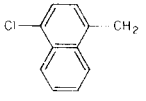
**β-Glyceryl 1-*p*-Chlorobenzyl-1*H*-indazole-3-carboxylate (35).** A solution of 1-(*p*-chlorobenzyl)-1*H*-indazole-3-carboxylic acid chloride (34 g, 0.11 mol) in anhydrous pyridine (26 ml) was added dropwise to a stirred solution of 1,3-benzylideneglycerol (18 g, 0.1 mol) in pyridine (26 ml). The mixture was stirred at room temperature for 48 h and then poured into water. Dilute HCl was added and the precipitate extracted with ether from which the benzylidene derivative crystallized after a while. This substance (20 g), concentrated HCl (0.9 ml), and ethanol (210 ml) were stirred for 30 min at 80°. The mixture was cooled and neutralized with 2 N $Na_2CO_3$ and the solvent was removed at reduced pressure. The oily residue was crystallized from a small volume of benzene: mp 108°; overall yield 68%. Anal. ($C_{18}H_{17}ClN_2O_4$) C, H, N.

**1-*p*-Chlorobenzyl-1*H*-indazole-3-carboxamide (41).** This compound was prepared by three traditional methods, i.e., by reacting the acid chloride with concentrated ammonia, by reacting the ethyl ester with an excess of alcoholic ammonia for 24 h at 160° in a sealed tube, and by treating the corresponding nitrile with NaOH and $H_2O_2$: mp 150°; yield 70%. Anal. ($C_{15}H_{12}ClN_3O$) C, H.

**1-*p*-Chlorophenyl-1*H*-indazole-3-carboxylic Acid (28).** Ethyl phenylglyoxylate (37 g, 0.21 mol) was added to a mixture of *p*-chlorophenylhydrazine (30 g, 0.21 mol) and 90% acetic acid (170 ml) and the solution refluxed for 30 min. Ethyl phenylglyoxylate *p*-chlorophenylhydrazone crystallized on cooling; mp

Table I. Chemical Data and Antispermatogenic Activity of 1-Substituted 1*H*-Indazole Derivatives



| Compd no. | R | R′ | R″ | Crystn solvent | Mp, °C | Analyses | Formula | Method | Yield, % | Antispermatogenic act.[a] (MED, mg/kg po) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *p*-Cl-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 196 | C, H, Cl | $C_{15}H_{11}ClN_2O_2$ | A | 55 | 400 |
| 2 | *m*-Cl-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 184 | C, H, N | $C_{15}H_{11}ClN_2O_2$ | A | 69 | 400 |
| 3 | *o*-Cl-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 214 dec | C, H, Cl | $C_{15}H_{11}ClN_2O_2$ | A | 68 | 200 |
| 4 | *p*-F-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 194 | F | $C_{15}H_{11}FN_2O_2$ | A | 39 | 377 |
| 5 | *p*-Br-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 197 dec | C, H, N | $C_{15}H_{11}BrN_2O_2$ | A | 61 | 231 |
| 6 | *p*-I-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 211 dec | I | $C_{15}H_{11}IN_2O_2$ | A | 82 | 528 |
| 7 | *m*-$CF_3$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 193 dec | F | $C_{16}H_{11}F_3N_2O_2$ | A | 37 | Inactive |
| 8 | *p*-NC-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 218 dec | C, H, N | $C_{16}H_{11}N_3O_2$ | A | 51 | Inactive |
| 9 | *p*-$C_6H_5SO_2$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 210 | C, H, N | $C_{21}H_{16}N_2O_4S$ | A | 35 | Inactive |
| | | | | | | | | B | 20 | |
| 10 | *m,p*-$Cl_2$-$C_6H_3$-$CH_2$[b] | COOH | H | AcOH | 186 | C, H, N | $C_{15}H_{10}Cl_2N_2O_2$ | A | 58 | 448 |
| 11 | *o,p*-$Cl_2$-$C_6H_3$-$CH_2$[c] | COOH | H | AcOH | 207 | C, H, N | $C_{15}H_{10}Cl_2N_2O_2$ | A | 41 | 56 |
| 12 | *o,o*-$Cl_2$-$C_6H_3$-$CH_2$[b] | COOH | H | AcOH–dioxane | 265 dec | C, H, N | $C_{15}H_{10}Cl_2N_2O_2$ | A | 76 | Inactive |
| 13 | *o,p*-$Br_2$-$C_6H_3$-$CH_2$ | COOH | H | AcOH | 228 dec | C, H, N | $C_{15}H_{10}Br_2N_2O_2$ | A | 63 | 76.5 |
| 14 | 2,4,5-$Cl_3$-$C_6H_2$-$CH_2$ | COOH | H | AcOH–dioxane | 256 dec | C, H, N | $C_{15}H_9Cl_3N_2O_2$ | A | 53 | 248 |
| 15 | *p*-Cl-$C_6H_4$-$CH_2$ | COOH | Cl[d] | AcOH | 210 | C, H, N | $C_{15}H_{10}Cl_2N_2O_2$ | A | 30 | Inactive |
| 16 | *p*-Cl-$C_6H_4$-$CH_2$ | COOH | OH | AcOH | 272 | C, H, N | $C_{15}H_{11}ClN_2O_3$ | *f* | 16 | Inactive |
| 17 | *p*-Cl-$C_6H_4$-$CH_2$ | COOH | $CH_3$[e] | AcOH | 244 | C, H, N | $C_{16}H_{13}ClN_2O_2$ | A | 39 | Inactive |
| 18 | *p*-Cl-$C_6H_4$-$CH_2$ | COOH | $OCH_3$ | AcOH | 212 dec | C, H, N | $C_{16}H_{13}ClN_2O_3$ | A | 27 | Inactive |
| 19 | *p*-$CH_3$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 195 | C, H, N | $C_{16}H_{14}N_2O_2$ | B | 16 | Inactive |
| 20 | *m*-$CH_3$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 162 | C, H, N | $C_{16}H_{14}N_2O_2$ | B | 54 | Inactive |
| 21 | *o*-$CH_3$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 175 | C, H, N | $C_{16}H_{14}N_2O_2$ | B | 60 | 185.5 |
| 22 | *p*-Cl-$C_6H_4$-$COCH_2$ | COOH | H | Ethanol | 220 dec | C, H, N | $C_{16}H_{11}ClN_2O_3$ | B | 65 | Inactive |
| 23 | *m*-$C_6H_5CO$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 214 | C, H, N | $C_{22}H_{16}N_2O_3$ | B | 85 | Inactive |
| 24 | *p*-$CH_3SO_2$-$C_6H_4$-$CH_2$ | COOH | H | AcOH | 219 dec | C, H, N | $C_{16}H_{14}N_2O_4S$ | B | 86 | Inactive |
| 25 |  | COOH | H | AcOH | 225 dec | C, H, N | $C_{19}H_{13}ClN_2O_2$ | B | 75 | Inactive |
| 26 | *o,p*-$(CH_3)_2$-$C_6H_3$-$CH_2$ | COOH | H | Ethanol | 170 dec | C, H, N | $C_{17}H_{16}N_2O_2$ | B | 44 | 101 |
| 27 | *o*-$CH_3$-*p*-Cl-$C_6H_3$-$CH_2$ | COOH | H | AcOH | 220 dec | C, H, N | $C_{16}H_{13}ClN_2O_2$ | B | 51 | 52 |
| 28 | *p*-Cl-$C_6H_4$ | COOH | H | Methanol | 222 | C, H, N | $C_{14}H_9ClN_2O_2$ | *f* | 31[g] | Inactive |
| 29 | *p*-Cl-$C_6H_4$-CO | COOH | H | Ethanol–dioxane | 235 dec | C, H, N | $C_{15}H_9ClN_2O_3$ | *f* | 38 | Inactive |
| 30 | *p*-Cl-$C_6H_4$-$SO_2$ | COOH | H | Methanol–ether | 285 dec | C, H, N | $C_{14}H_9ClN_2O_4S$ | *i* | 41 | Inactive |
| 31 | *p*-Cl-$C_6H_4$-$CH_2$ | $COOCH_3$ | H | Methanol | 161 | Cl | $C_{16}H_{13}ClN_2O_2$ | *f* | 80 | Inactive |
| 32 | *p*-Cl-$C_6H_4$-$CH_2$ | $COOC_2H_5$ | H | Benzene–hexane | 116 | C, H, N | $C_{17}H_{15}ClN_2O_2$ | *f* | 64 | Inactive |
| 33 | *p*-Cl-$C_6H_4$-$CH_2$ | $COOCH_2CH_2OH$ | H | Benzene | 75 | C, H, N | $C_{17}H_{15}ClN_2O_3$ | *f* | 29 | 486.5 |
| 34 | *p*-Cl-$C_6H_4$-$CH_2$ | $COOCH_2CH(OH)CH_2OH$ | H | Benzene | 112 | C, H, N, Cl | $C_{18}H_{17}ClN_2O_4$ | *f* | 50 | 503 |
| 35 | *p*-Cl-$C_6H_4$-$CH_2$ | $COOCH(CH_2OH)_2$ | H | Benzene | 108 | C, H, N | $C_{18}H_{17}ClN_2O_4$ | *f* | 68[g] | 503 |
| 36 | *o*-Cl-$C_6H_4$-$CH_2$ | $COOCH_2CH(OH)CH_2OH$ | H | Benzene | 115 | C, H, N | $C_{18}H_{17}ClN_2O_4$ | *f* | 45 | 503 |
| 37 | *o,p*-$Cl_2$-$C_6H_3$-$CH_2$ | $COOCH_2CH(OH)CH_2OH$ | H | Toluene | 137 | C, H, N | $C_{18}H_{16}Cl_2N_2O_4$ | *f* | 63 | 69 |
| 38 | 2,4,5-$Cl_3$-$C_6H_2$-$CH_2$ | $COOCH_2CH(OH)CH_2OH$ | H | Toluene | 123 | N, Cl | $C_{18}H_{15}Cl_3N_2O_4$ | *f* | 37 | 299.5 |
| 39 | *p*-Cl-$C_6H_4$-$CH_2$ | $COCH_3$ | H | Hexane | 115 | C, H, N | $C_{16}H_{13}ClN_2O$ | *f* | 42 | Inactive |
| 40 | *m*-Cl-$C_6H_4$-$CH_2$ | SH | H | Benzene-pet. ether | 74 | C, H, N, S | $C_{14}H_{11}ClN_2S$ | *f* | 44 | Inactive |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 41 | p-Cl-$C_6H_4$-$CH_2$ | $CONH_2$ | H | Ethanol | 150 | C, H | $C_{15}H_{12}ClN_3O$ | *f* | 70 | 399 |
| 42 | p-Cl-$C_6H_4$-$CH_2$ | $CH_2COOH$ | H | Benzene | 183 dec | C, H, N | $C_{16}H_{13}ClN_2O_2$ | *f* | 52[g] | 419 |
| 43 | p-Cl-$C_6H_4$-$CH_2$ | $CH_2CH_2COOH$ | H | Benzene–hexane | 141 | C, H, N | $C_{17}H_{15}ClN_2O_2$ | *f* | 76 | Inactive |
| 44 | p-Cl-$C_6H_4$-$CH_2$ | H | H | Hexane | 80 | C, H, N | $C_{14}H_{11}ClN_2$ | *j* | 84 | Inactive |
| 45 | p-Cl-$C_6H_4$-$CH_2$ | $CH_3$ | H | Hexane | 66 | C, H, N | $C_{15}H_{13}ClN_2$ | *k* | 47 | Inactive |
| 46 | p-Cl-$C_6H_4$-$CH_2$ | $CH_2OH$ | H | Benzene | 105 | C, H, N | $C_{15}H_{13}ClN_2O$ | *f* | 81 | Inactive |
| 47 | p-Cl-$C_6H_4$-$CH_2$ | $NH_2$ | H | Benzene–hexane | 139[h] | C, H, N | $C_{14}H_{12}ClN_3$ | *f* | 54[g] | Inactive |
| 48 | p-Cl-$C_6H_4$-$CH_2$ | CN | H | Benzene–hexane | 138 | C, H, N | $C_{15}H_{10}ClN_3$ | *f* | 38 | Inactive |
| 49 | p-Cl-$C_6H_4$-$CH_2$ |  | H | Ethyl acetate | 225 dec | C, H, N | $C_{15}H_{11}ClN_6$ | *f* | 67 | Inactive |
| 50 | o,p-$Cl_2$-$C_6H_3$-$CH_2$ |  | H | Ethanol | 205 dec | C, H, N | $C_{15}H_{10}Cl_2N_6$ | *f* | 55 | Inactive |
| 51 | p-Cl-$C_6H_4$-$CH_2CH_2$ |  | H | Ethanol | 181 dec | C, H, N | $C_{16}H_{13}ClN_6$ | *f* | 71 | Inactive |
| 52 | o-$CH_3$-p-Cl-$C_6H_3CH_2$ |  | H | Dioxane | 260 dec | C, H, N | $C_{16}H_{13}ClN_6$ | *f* | 36 | Inactive |

[a] Inactive when given in a single dose and judged according to the criteria specified in the section entitled assay of antispermatogenic activity. [b] The benzyl chlorides were prepared according to the method described in ref 15. [c] Both 2,4-dichlorobenzyl chloride, prepared according to ref 15, and 2,4-dichlorobenzyl bromide, prepared according to ref 16, were used. [d] 5-Chloro-1*H*-indazole-3-carboxylic acid was prepared according to the method described for 1*H*-indazole-3-carboxylic acid in ref 17. [e] 5-Methyl-1*H*-indazole-3-carboxylic acid was prepared according to the method described for 5-methoxy-1*H*-indazole-3-carboxylic acid in ref 18. [f] See Experimental Section. [g] Overall yield. [h] U.S. Patent 3 725 431 (April 3, 1973) gives mp 134–135°. [i] Prepared according to the method described for compound **29**. [j] Prepared from *p*-chlorobenzyl iodide and the Ag salt of 1*H*-indazole. [k] Prepared from *p*-chlorobenzyl chloride and 3-methyl-1*H*-indazole.

100° (from ethanol); yield 67%. Anal. ($C_{16}H_{15}ClN_2O_2$) C, H, N.

A solution of this compound (20 g, 0.066 mol) in dichloromethane (80 ml) was added to a stirred mixture of lead tetraacetate (33 g) and dichloromethane (140 ml), keeping the temperature between 0 and 10°. The mixture was heated for 15 min at 20–25°; water and dilute HCl were then added with the temperature kept below 25°. The organic layer was separated and the solvent evaporated. [α-(*p*-Chlorophenylazo)-α-ethoxycarbonylbenzyl] acetate crystallized from hexane: mp 89°; yield 92%. Anal. ($C_{18}H_{17}ClN_2O_4$) C, H, N.

Boron trifluoride etherate (135 ml) was added dropwise to a stirred solution of the above described compound (25 g, 0.07 mol) in ether (360 ml), keeping the temperature at 0°. The mixture was refluxed for 20 min, poured into water, and stirred until complete evaporation of ether has occurred. Ethyl 1-(*p*-chlorophenyl)-1*H*-indazole-3-carboxylate was separated and crystallized from ethanol: mp 126°; yield 77%. Anal. ($C_{16}H_{13}ClN_2O_2$) C, H, N.

A mixture of this ester (18 g, 0.06 mol), NaOH (18 g, 0.45 mol), and water (600 ml) was refluxed for 3 h. The cooled solution was acidified with 6 N HCl. The precipitate was crystallized from methanol: mp 222°; yield 67%. Anal. ($C_{14}H_9ClN_2O_2$) C, H, N.

**4,5,6,7-Tetrahydro-1-(*p*-chlorobenzyl)-1*H*-indazole-3-carboxylic Acid.** Ethyl 4,5,6,7-tetrahydro-1*H*-indazole-3-carboxylate[11] (27 g, 0.14 mol) and, immediately afterward, *p*-chlorobenzyl chloride (32 g, 0.2 mol) were slowly added to a solution of sodium ethoxide prepared from Na (3.14 g, 0.14 mol) and ethanol (270 ml). The mixture was refluxed for about 1.5 h; then, after addition of a solution of KOH (18 g) in water (18 ml), it is refluxed for another hour. The mixture was cooled, filtered, and concentrated and the residue treated with water and ether. The alkaline solution was acidified with dilute HCl, and the precipitate was extracted with ether. After removal of the solvent, the residue was refluxed for 1.5 h with ethanol (300 ml) containing HCl (9 g). The solution was concentrated, neutralized, and extracted several times with ether. The solvent was removed and the residue (20 g) heated under stirring at 80–85° with a solution of KOH (6.6 g) in water (120 ml) for about 40 min until the solution was complete. The solution is then acidified and the precipitate was crystallized from ethanol: mp 170°; yield 30%. Anal. ($C_{15}H_{15}ClN_2O_2$) C, H, N.

**1-*p*-Chlorobenzyl-3-hydroxymethyl-1*H*-indazole (46).** Ethyl 1-*p*-chlorobenzyl-1*H*-indazole-3-carboxylate (31 g, 0.1 mol) dissolved in a mixture of THF (75 ml) and ether (75 ml) was added dropwise to a solution of $LiAlH_4$ (9.2 g, 0.24 mol) in ether (140 ml). The solution was refluxed for 3 h, cooled, and carefully decomposed with ethyl acetate (5 ml), water (17 ml), and 2 N NaOH (8 ml). The precipitate was filtered and the filtrate dried and evaporated. The residue was distilled under reduced pressure: bp 208° (0.6 mm). 1-*p*-Chlorobenzyl-3-hydroxymethyl-1*H*-indazole which was thus obtained solidified and crystallized from benzene: mp 105°; yield 90%. Anal. ($C_{15}H_{13}ClN_2O$) C, H, N.

**(1-*p*-Chlorobenzyl-1*H*-indazol-3-yl)acetic Acid (42).** The previous product was added dropwise under stirring to an equal weight of $SOCl_2$. After standing for 1 h at room temperature the mixture was carefully treated with ice. 1-*p*-Chlorobenzyl-3-chloromethyl-1*H*-indazole precipitated as a solid which was crystallized from ethanol: mp 94°; yield 76%. Anal. ($C_{15}H_{12}Cl_2N_2$) C, H.

A solution of this product (20 g, 0.069 mol) in 2-methoxyethanol (100 ml) was added dropwise under stirring to a solution of KCN (11 g) in water (33 ml) kept at 90°. Heating was continued for another hour and the mixture was then poured into water. The reaction product was extracted with ether which was then dried and removed. The residue was heated with a solution of KOH (17 g) in 50% ethanol (170 ml) for 3 h. The mixture was then filtered and concentrated under reduced pressure. The residue was treated with water and ether and the aqueous layer acidified with $CH_3COOH$ and extracted with $CHCl_3$. After removal of the solvent the residue solidified by heating with benzene. (1-*p*-Chlorobenzyl-1*H*-indazol-3-yl)acetic acid so obtained was recrystallized from benzene: mp 183° dec; yield 52%. Anal. ($C_{16}H_{13}ClN_2O_2$) C, H, N.

**(1-*p*-Chlorobenzyl-1*H*-indazol-3-yl)propionic Acid (43).** 1-*p*-Chlorobenzyl-3-chloromethyl-1*H*-indazole (20 g, 0.069 mol) was dissolved in ethanol (40 ml) and the solution added dropwise

to a solution of diethyl malonate (11 g, 0.069 mol) in ethanol containing 1 equiv of sodium methoxide prepared from sodium (1.6 g, 0.069 mol) and ethanol (50 ml). The solution was then refluxed for 2 h and concentrated under reduced pressure. The residue was treated with water and extracted with ether. After removal of the solvent the new residue was added to a warm solution of KOH (9.6 g) in water (30 ml) and heated for 3 h; the solution was cooled and acidified with HCl. The mixture was thoroughly extracted with $CHCl_3$, which was then dried and removed. The residue (12 g) was heated under reduced pressure at 170° for 0.5 h. It is then treated with dilute NaOH and charcoal, filtered, and acidified with 6 N HCl. The precipitate was extracted with $CHCl_3$ and crystallized from benzene–hexane to give (1-*p*-chlorobenzyl-1*H*-indazol-3-yl)propionic acid: mp 141°; overall yield 49%. Anal. ($C_{17}H_{15}ClN_2O_2$) C, H, N.

**1-*p*-Chlorophenyl-1*H*-indazole-3-sulfonic Acid.** A solution of sodium 1-*p*-chlorophenyl-3-mercapto-1*H*-indazole[12] (25 g, 0.09 mol) in water (150 ml) was added dropwise in 30 min to a solution of concentrated HCl (100 ml) in water (300 ml) previously saturated with chlorine. The solution was stirred and chlorine bubbled through it for a further 30 min; the precipitate was extracted with ether, which was then removed leaving as solid residue 1-*p*-chlorophenyl-1*H*-indazole-3-sulfonyl chloride which crystallized from benzene–hexane and melted at 103°. This chloride (8 g) was refluxed in water (160 ml) until the solution was complete (about 16 h). Charcoal was added and the solution was filtered and then evaporated to dryness under reduced pressure. Treatment of the residue with benzene and then with anhydrous ethanol, removing the solvent each time, changed it into a crystalline mass, which was purified from a small volume of benzene. 1-*p*-Chlorophenyl-1*H*-indazole-3-sulfonic acid melted at 198° dec. It contains one molecule of water: overall yield 21%. Anal. ($C_{13}H_9ClN_2O_3S \cdot H_2O$) C, H, N, S.

**5-(1-*p*-Chlorobenzyl-1*H*-indazol-3-yl)tetrazole (49).** 3-Cyano-1*H*-indazole[13] (17 g, 0.12 mol) and *p*-chlorobenzyl chloride (19.2 g, 0.12 mol) were added to a solution of sodium methoxide prepared from sodium (2.7 g, 0.12 mol) and ethanol (170 ml). After refluxing for 3 h the solution was cooled and kept overnight in a refrigerator. The precipitate was collected and crystallized from benzene–hexane. 1-*p*-Chlorobenzyl-3-cyano-1*H*-indazole (**48**) which was thus obtained melted at 138°: yield 38%. Anal. ($C_{15}H_{10}ClN_3$) C, H, N.

This product (11.7 g, 0.044 mol), $NaN_3$ (3.6 g, 0.055 mol), $NH_4Cl$ (3.4 g, 0.064 mol), and DMF (81 ml) were heated with stirring for 24 h at 118°. The solvent was removed under reduced pressure and the residue was treated with dilute NaOH until definitely alkaline. The mixture was treated with charcoal, filtered, and acidified with 3 N HCl. The precipitate which was formed was crystallized first from ethanol and then from ethyl acetate: mp 225° dec; yield 67%. Anal. ($C_{15}H_{11}ClN_6$) C, H, N.

Tetrazoles **50**, **51**, and **52** of Table I were similarly prepared.

**1-(*p*-Chlorobenzyl)-5-hydroxy-1*H*-indazole-3-carboxylic Acid (16).** 1-(*p*-Chlorobenzyl)-5-methoxy-1*H*-indazole-3-carboxylic acid (2 g, 0.0063 mol) in acetic acid (10 ml) and 48% HBr (10 ml) was refluxed for 3 h. The reaction mixture was poured into water; the precipitate was filtered, washed with water, and treated with 2 N NaOH on a steam bath. The filtered alkaline solution was acidified with 6 N HCl; the new precipitate was collected, washed, and crystallized from acetic acid: mp 272°; yield 16%. Anal. ($C_{15}H_{11}ClN_2O_3$) C, H, N.

**1-(*p*-Chlorobenzoyl)-1*H*-indazole-3-carboxylic Acid (29).** A mixture of 1*H*-indazole-3-carboxylic acid (10 g, 0.062 mol), *p*-chlorobenzoyl chloride (12 g, 0.069 mol), and anhydrous pyridine (60 ml) was heated under reflux for 5 h. The mixture was cooled and poured into water and the precipitate (*p*-chlorobenzoic acid) separated. The solution was cooled and acidified with 6 N HCl. The solid precipitated was washed with water and crystallized from 4:1 ethanol–dioxane: mp 235°; yield 38%. Anal. ($C_{15}H_9ClN_2O_3$) C, H, N.

**3-Amino-1-*p*-chlorobenzyl-1*H*-indazole (47).** A solution of $NaN_3$ (4.7 g, 0.072 mol) in water (15 ml) was added dropwise to a stirred suspension of 1-*p*-chlorobenzyl-1*H*-indazole-3-carboxylic acid chloride (20 g, 0.065 mol) in acetone (200 ml) cooled with iced water. The precipitate, which consisted of 1-*p*-chlorobenzyl-1*H*-indazole-3-carboxyazide, was washed with water and dried in a desiccator: mp 125°. This product (6 g) was carefully added in portions to a boiling solution of benzyl alcohol (2.7 g) in toluene (40 ml). Decomposition was completed in a few minutes. The solution was cooled and diluted with hexane, and the precipitated benzyl (1-*p*-chlorobenzyl-1*H*-indazol-3-yl)carbamate was crystallized from benzene–hexane: mp 112°; yield 66%. Anal. ($C_{22}H_{18}ClN_3O_2$) C, H, N.

The previous product (20 g) was dissolved in a solution of KOH (100 g) in ethanol (410 ml), heated under reflux for 8 h, and then poured into water. The precipitate was extracted with ether, which was dried and evaporated. The residue was crystallized from benzene–hexane: mp 139°[14]; overall yield 54%. Anal. ($C_{14}H_{12}ClN_3$) C, H, N.

***N*-(1-Naphthylmethyl)anthranilic Acid.** To a solution of anthranilic acid (13.7 g, 0.1 mol) in 0.5 N NaOH (0.12 mol), heated under vigorous stirring at 50°, 1-chloromethylnaphthalene (17.8 g, 0.1 mol) was added in portions; the mixture was heated at 70° for 4 h and acidified with concentrated HCl. The precipitate was crystallized from acetic acid: mp 213°; yield 36%. Anal. ($C_{18}H_{15}NO_2$) C, H.

**Assay of Antispermatogenic Activity.** The study of the antispermatogenic activity was conducted on Long–Evans male rats weighing 90–150 g and ranging in age from 34 to 45 days. Treatments were performed only once by gavage; the drugs were suspended in an 0.5% solution of methylcellulose which was administered at a volume of 10 ml/kg; control rats received the same amount of the vehicle alone. Five days later, the animals were weighed and sacrificed by ether asphyxia and their testes were removed and weighed. When the weight of these organs was significantly reduced with respect to the controls, they were fixed in Bouin's fluid, embedded in paraffin wax, cut in 6-$\mu$ thick sections, and stained with hematoxylin and eosin for microscopic examination. The antispermatogenic activity of the drugs was assessed on the basis of a statistically significant weight reduction of the testes and their histological picture.

To graduate the potency of the antispermatogenic activity, the minimal effective dose (MED) was determined as follows. Each compound was administered at a dose equimolecular to 400 mg/kg po of compound **1**. If this dose was active, half of it was administered; the same procedure was continued until the inactive dose was found; the dose immediately above this one was considered to be the MED. The compounds referred to as inactive did not produce any antispermatogenic effect at the highest dose tested. At least ten animals were used for each dose. The significance of the results was assessed by means of Student's *t* test on the basis of the testicular weight. All the derivatives which were found to be active up to doses equimolecular to 400 mg/kg po of compound **1** are reported in Table I with their MED's. All the derivatives which were found to be inactive up to doses equimolecular to 400 mg/kg po of compound **1** are also listed in Table I. The reference compounds were Win 18446 [*N*,*N*'-bis(dichloroacetyl)-1,8-octamethylenediamine] and U 29409 [2,3-dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone]. The MED's were >400 mg/kg po for Win 18446 and 400 mg/kg po for U 29409.

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# (Vinylaryloxy)acetic Acids. A New Class of Diuretic Agents. 3.[2,3] [(2-Nitro-1-alkenyl)aryloxy]acetic Acids

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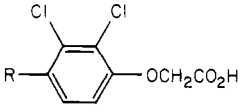
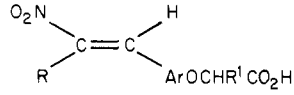
A series of [(2-nitro-1-alkenyl)aryloxy]acetic acids was synthesized and tested in dogs for saluretic and diuretic activity. A number of these compounds exhibit a high order of activity on iv or po administration; representative of these is (*E*)-[2,3-dichloro-4-(2-nitropropenyl)phenoxy]acetic acid (**5**). The most highly active compounds are qualitatively similar in action to [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid (ethacrynic acid) in causing a prompt increase in the excretion of water and of sodium and chloride ions in approximately equimolar amounts but are three to five times as potent. Potassium ion excretion is increased but less markedly than sodium excretion.

Earlier papers in this series have demonstrated that the 2-methylenebutyryl group of ethacrynic acid[1] (**1**), [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid, can be replaced by diacylvinyl (as in **2**)[2] or acylvinyl (as in **3**)[3] to give potent diuretic–saluretic agents which further resemble **1** in their saluretic and diuretic profile.

This paper describes the synthesis and renotropic properties of a related series of [(2-nitro-1-alkenyl)-phenoxy]acetic acids of general structure I which like the aforementioned compounds incorporate a double bond activated toward nucleophilic attack, in this case by a conjugated nitro group.





I

1, R = $C_2H_5CCO-$ ($CH_2$)

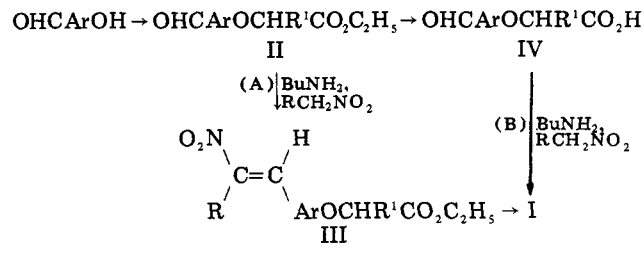
2, R = $CH_3CO$ / $CH_3CO$ >C=CH-

3, R = $CH_3COCH=CH-$

**Chemistry.** The [(2-nitro-1-alkenyl)aryloxy]acetic acids prepared for this study are listed in Table I. The key step in their preparation was a modified Knoevenagel reaction[4] in which an aromatic aldehyde was made to react with butylamine to produce a Schiff base which then reacted with a nitroalkane, presumably in an addition–elimination sequence, to yield the nitroalkene. In method A-1 (see Scheme I), an ethyl formylaryloxyacetate (II) was condensed with a nitroalkane in this manner to yield an ethyl (2-nitro-1-alkenyl)aryloxyacetate (III) which was isolated, purified, and hydrolyzed in acid to the product I (see Table III). In the "one-pot" method A-2, the isolation and purification of the intermediate III were eliminated. In method B, formylaryloxyacetic acid (IV) was condensed with a nitroalkane in the modified Knoevenagel reaction to yield I.

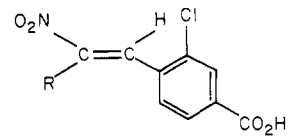
Only one of the two possible geometric isomers of nitroalkenes I and III was isolated in each of the condensation reactions. The disubstituted olefinic products (I, R = H), **10**, **14**, **20**, and **25** may be assigned the trans (*E*) configuration on steric grounds and because of the magnitude of coupling constants ($J$ = 14 Hz) of the vinyl protons of **10** and **25**. Interestingly, the vinyl protons of **14** and **20** are magnetically equivalent giving rise to sharp singlets at $\delta$ 8.10 and 8.18, respectively.

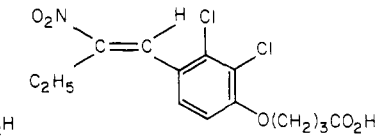
Scheme I




The trisubstituted olefinic products (I, R = alkyl) may tentatively be assigned the *E* configuration (vinyl H cis to $NO_2$) on the following basis. The chemical shifts of the vinyl protons $\beta$ to the nitro group in the disubstituted olefins lie in the range $\delta$ 7.99–8.18. The shifts of the corresponding protons in the trisubstituted olefins lie in the same downfield range: $\delta$ 8.01–8.12. The reasonable assumption is that these protons likewise are located cis to the nitro group.

Two benzoic acid derivatives (**34** and **35**) and a 4-phenoxybutyric acid derivative (**37**) were prepared to determine the effect on biological activity of shortening and lengthening the carboxylic acid chain. Acids **34** and **35** were prepared from 3-chloro-4-formylbenzoic acid (**33**) and **37** from 4-(2,3-dichloro-4-formylphenoxy)butyric acid (**36**) by method B. For the preparation of **33** and **36**, see the Experimental Section.





**34**, R = $CH_3$
**35**, R = $C_2H_5$

**37**

**Saluretic–Diuretic Effects and Structure–Activity Relationships.** Compounds **4**–**26**, **34**, **35**, and **37** were