$10^{-5}M$ antagonized responses to both serotonin and acetylcholine greater than 30-fold (three experiments).

## Discussion

5,8-ADT has a complex profile of effects as demonstrated by the data presented. The apparent conflict in results between the effect on spontaneous motor activity and the effect on Sidman avoidance is rationalized on the basis of the differences in responses measured and the species used. Whether or not 5,8-ADT is psychotomimetic in man like its open-chain analog, 2,5-dimethoxyamphetamine, cannot be answered by these studies. The fact that both 2-AT and 5,8-ADT release norepinephrine from adrenergic nerve terminals and that 5,8-ADT has some direct α-adrenergic stimulatory activity in the anesthetized dog is consistent with the results from the Sidman avoidance studies. Phentolamine blocks direct α effects, while cocaine inhibits indirect acting agents. The contention of hallucinogenic activity for 2-AT based on the Sidman avoidance studies is consistent with the results on serotonin receptors. The effect observed with psychotomimetics related to LSD and mescaline can be blocked by serotonin antagonists. 2-AT exhibits agonistic effects at low concentrations. The psychotomimetic effects of DOM, 2,5-dimethoxy-4-methylamphetamine, are also blocked by serotonin antagonists.[23] Pharmacological studies on 2-AT and 5,8-ADT are continuing in order to understand the mechanisms by which they cause their varied effects.

Acknowledgments. The authors wish to acknowledge the following sources of financial support: Ortho Research Foundation Grant (C. F. B.), NDEA Title IV Fellowship (D. E. N.), Salsbury Foundation Fellowship (D. B. R.), NIH Grant GM15991 (D. C. D.), and Washington Heart Association (D. C. D.).

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# Isoquinolines. 3.[1] 3-Aminoisoquinoline Derivatives with Central Nervous System Depressant Activity†

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A series of novel 3-amino-4-arylisoquinoline (2) and 3-amino-4-benzylisoquinoline derivatives 3 was synthesized and evaluated primarily for their CNS effects. The method used for the synthesis of the 3-aminoisoquinolines 2 and 3 involved the alkylation of the appropriate α-cyano-*o*-tolunitrile 4 followed by an acid-catalyzed cyclization to yield the 4-aryl- or 4-benzyl-substituted isoquinolines 2 and 3. Two compounds in this series, 3-amino-4-(*p*-aminophenyl)isoquinoline (10) and 4-(*p*-acetamidophenyl)-3-aminoisoquinoline (11), were shown to have similar and marked central nervous system activity, characterized by general CNS depression and anticonvulsant activity.

In the course of a routine pharmacological screen of 3-amino-4-methylisoquinoline (1) primarily prepared as a potential antimalarial drug,[2] we observed considerable CNS activity in the primary mouse screen.[3] In order to assess the effect of 4-phenyl and 4-benzyl substitution in this series, the novel 3-amino-4-phenylisoquinoline (2) and 3-amino-4-benzylisoquinoline (3) derivatives were synthesized and evaluated. This communication presents the synthesis and pharmacological evaluation of this series of compounds.

**Chemistry.** The method used for the synthesis of the 3-aminoisoquinolines reported in Tables II and III involved the alkylation of α-cyano-*o*-tolunitrile (4) followed by

†Presented in part at the 163rd National Meeting of the American Chemical Society, Boston, Mass., 1972.

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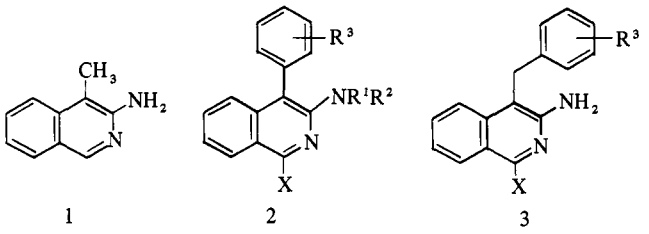
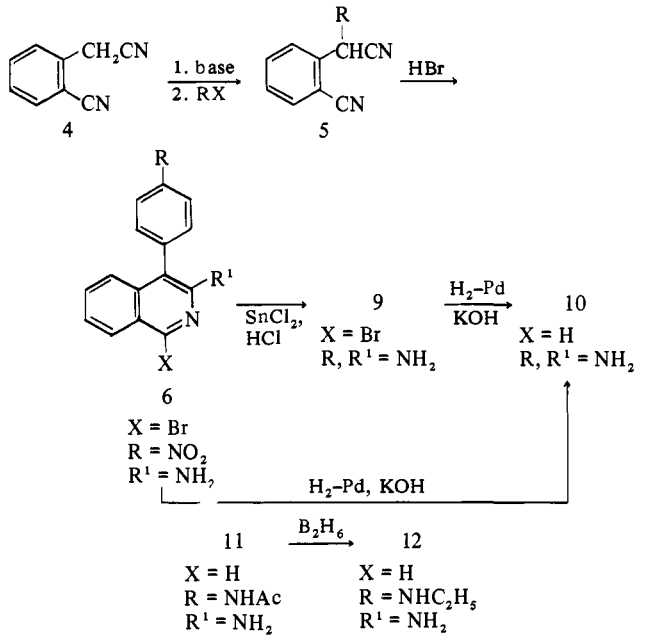
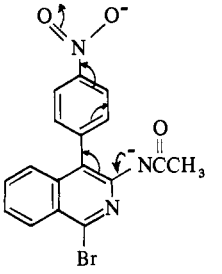


1 2 3

cyclization with HBr to yield the 4-substituted isoquinolines. (Scheme I). Alkylation or arylation of phenylacetonitriles and α-cyano-*o*-tolunitrile (**4**) has been reported.[2,4-7] It has been shown that for the arylation of phenylacetonitriles activated aromatic halides, such as nitrophenyl halides, are required. However, the condensation of *p*-chlorophenylacetonitriles with *p*-nitrophenyl chloride in methanolic KOH does not yield the expected arylated phenylacetonitrile but results in the formation of 3-phenyl-5-chloroanthranil.[7] When the reaction was carried out in the presence of KOH in pyridine[8] or in an aqueous slurry of NaOH and triethylbenzyl ammonium chloride,[9] the desired phenylacetonitrile was obtained. This method was subsequently used for the arylation of the dinitrile **4**. Cyclization of the appropriately substituted nitrile **5** (Table I) with HBr by methods previously described[2] results in excellent yields of the 3-amino-1-bromo-4-alkyl- (or aryl-) isoquinolines. Similarly, as previously reported for the 4-alkyl series,[2] the labile halogen at the 1 position of the isoquinoline ring constitutes a convenient method for the preparation of a variety of substituted isoquinolines. Acylation of the 3-amino group can be accomplished with acetyl bromide or acetic anhydride in pyridine. 3-Amino-1-bromo-4-(*p*-nitrophenyl)isoquinoline (**6**) can be converted to the pharmacologically active 3-amino-4-(*p*-aminophenyl)-isoquinoline (**10**) in one step by catalytic reduction and hydrogenolysis or in a two-step sequence by first reducing the nitro group on **6** with $SnCl_2$-HCl producing **9** which can then be dehalogenated to **10**. Acetylation of 3-amino-4-(*p*-aminophenyl)isoquinoline (**10**) with 1 mol of acetic anhydride in pyridine acylates the 4′-amino group preferentially to yield compound **11**. This preferential acylation would be expected in light of the difference in basicity of the two amino groups. The chemical shifts in the nmr spectrum of the protons on the 3′ and 5′ positions confirmed the position of the acyl group at the 4′ nitrogen. Acetylation of 3-amino-1-bromo-4-(*p*-nitrophenyl)isoquinoline gave a mixture of 3-mono- (**7**) and 3-diacetamide (**8**). This unexpected diacylation of the amino group on the 3 position can be explained by the presence of the nitrophenyl group at the 4′ position which provides the necessary resonance stabilization for the formation of the anion which is then further acylated (Table II).

Scheme I

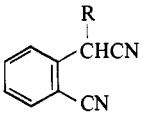




Both the 3-monoacetamide **7** and the diacetamide **8** were converted to the *p*-aminophenylisoquinoline **13** with $SnCl_2$-HCl. The monoacetamide **11** was reduced to the 4′-ethylamino derivative **12** with diborane in a THF solution. The 3-amino-4-benzylisoquinolines shown in Table III were prepared for pharmacological comparison with the active 4-phenylisoquinolines **10** and **11**. 3-Amino-1-bromo-4-(*o*-cyanobenzyl)isoquinoline (**20**) was the sole product isolated when the trinitrile **5e** was cyclized with HBr in benzene-ether solution. A cyclization of the trinitrile **5e** under identical conditions has been reported and resulted in the formation of unidentified products.[7]

**Pharmacology.** Two compounds in this series, 3-amino-4-(*p*-aminophenyl)isoquinoline (**10**) and 4-(*p*-acetamidophenyl)-3-aminoisoquinoline (**11**), were evaluated in the following pharmacological tests: cat cardiovascular screen;

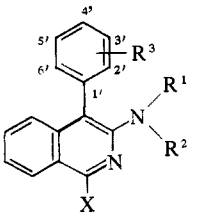
Table I. Aryl-Substituted Acetonitriles



| Compd | R | Mp, °C | Recrystn solvent | Rxn medium | % yield | Formula | Analyses |
|---|---|---|---|---|---|---|---|
| 5a | $-C_6H_4$-*p*-$NO_2$ | 82.5–84 | EtOH | Py-KOH | 69 | $C_{15}H_9N_3O_2$ | C, H, N |
| 5b | $-C_6H_4$-*o*-$NO_2$ | Oil | | Py-KOH | | $C_{15}H_9N_3O_2$ | *a* |
| 5c | $-C_6H_3$-*p*-$NO_2$-*m*-$OCH_3$ | 118–119.5 | $C_6H_6$-hexane | Py-KOH | 63 | $C_{16}H_{11}N_3O_3$ | C, H, N |
| 5d | $-CH_2-C_6H_4$-*p*-$NO_2$ | 172–173 | Toluene | $Me_2CO$-EtOH-KCN | 33 | $C_{16}H_{11}N_3O_2$ | C, H, N |
| 5e | $-CH_2-C_6H_4$-*o*-CN | 110–112[b] | EtOH | $Me_2CO$-EtOH-KCN | 39 | $C_{17}H_{11}N_3$ | |

[a] This compound could not be purified and was converted directly to 14. [b] Lit. mp 114° [S. Gabriel and T. Posner, *Ber.*, 27, 2492 (1894)].

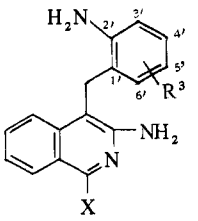
Table II. 3-Amino-4-arylisoquinolines



| Compd | $R^1$ | $R^2$ | $R^3$ | X | Mp, °C | Recrystn solvent | Formula | Analyses |
|---|---|---|---|---|---|---|---|---|
| 6 | H | H | 4′-$NO_2$ | Br | 275–276 dec | Dioxane | $C_{15}H_{10}N_3O_2Br$ | C, H, N, Br |
| 7 | Ac | H | 4′-$NO_2$ | Br | 220–220.5 | Toluene | $C_{17}H_{12}N_3O_3Br$ | C, H, N, Br |
| 8 | Ac | Ac | 4′-$NO_2$ | Br | 195–198[a] | Toluene | $C_{19}H_{14}N_3O_4Br$ | C, H, N, Br |
| 9 | H | H | 4′-$NH_2$ | Br | 175 dec | $CHCl_3$–hexane | $C_{15}H_{12}N_3Br$ | C, H, N, Br |
| 10 | H | H | 4′-$NH_2$ | H | 153–154.5[b] | Toluene | $C_{15}H_{13}N_3$ | C, H, N |
| 11 | H | H | 4′-NHAc | H | 235–237 | EtOH | $C_{17}H_{15}N_3O$ | C, H, N |
| 12 | H | H | 4′-$NHC_2H_5$ | H | 141–143 | MeCN | $C_{17}H_{17}N_3$ | C, H, N |
| 13 | Ac | H | 4′-$NH_2$ | Br | >180 dec | [d] | $C_{17}H_{14}N_3OBr$ | [c] |
| 14 | H | H | 2′-$NO_2$ | Br | 215–216.5 | Dioxane | $C_{15}H_{10}N_3O_2Br$ | C, H, N, Br |
| 15 | H | H | 2′-$NH_2$ | H | 142–143 | Cyclohexane–EtOH | $C_{15}H_{13}N_3$ | C, H, N |

[a]This compound crystallizes as a hydrate, mp 223–224°, from moist MeCN. [b]Dihydrochloride mp 292°. [c]Satisfactory elemental analyses were not obtained. [d]Compound could not be recrystallized.

Table III. 3-Amino-4-benzylisoquinoline Derivatives



| Compd | $R^3$ | X | Mp, °C | Recrystn solvent | Formula | Analyses |
|---|---|---|---|---|---|---|
| 16[a] | H | H | 135.5–137 | EtOH | $C_{16}H_{14}N_2$ | C, H, N |
| 17 | 4′-$NO_2$ | Br | 251–252 dec | | $C_{16}H_{12}N_3O_2Br$ | H, N, Br; C[b] |
| 18 | 4′-$NH_2$ | Br | >170 dec | EtOAc | $C_{16}H_{14}N_3Br$ | C, H, N, Br |
| 19 | 4′-$NH_2$ | H | 153–155 | $C_6H_6$ | $C_{16}H_{15}N_3$ | [c] |
| 20 | 2′-CN | Br | 240–242 | Dioxane | $C_{17}H_{12}N_3Br$ | C, H, N, Br |

[a]This compound was prepared by the catalytic debromination of 3-amino-1-bromo-4-benzylisoquinoline. [b]C: calcd, 53.65; found, 52.54. [c]A satisfactory elemental analysis (C, H, N, Cl) was obtained for the dihydrochloride, mp 249° dec, determined by dta.

Table IV. $ED_{50}$ Values of Some Drugs in the Forced Motor Activity (FMA) Test (Mice)

| Drug | FMA $ED_{50}$ (95% confidence limits), mg/kg, ip administration | Time to peak effect (min post-administration) |
|---|---|---|
| 10 | 37.8 (32.9–43.5) | 30 |
| 11 | 63.3 (47.3–84.7) | 30 |
| Phenobarbital Na | 66.4 (57.7–76.4) | 90 |
| Diphenylhydantoin Na | 48.3 (39.1–59.7) | 120 |

forced motor activity test (ip and oral) in mice; anticonvulsant profile (electroshock, pentylenetetrazol and strychnine) in mice; acetylcholine writhing in mice; and antioxotremorine in mice. In addition, compounds **9**, **10**, **12**, **13**, **15**, and **19** were evaluated in the mouse screen[3] at dose levels up to 100 mg/kg.

**Cat Cardiovascular Screen.** (a) Compound **10** produced a biphasic response (fall followed by a slight rise) at 1.0 mg/kg and a moderate (~40 mm) fall at 5 and 25 mg/kg together with an extreme (~50 bpm) increase in heart rate at all dose levels (1, 5, and 25 mg/kg). These effects were relatively transient. There was slight potentiation in the response to histamine at 1, 5, and 25 mg/kg and inhibition in the response to vagal stimulation at 5 and 25 mg/kg. (b) Compound **11** produced an extreme fall (~60–100 mm) in blood pressure at 1, 5, and 25 mg/kg. Heart rate was moderately decreased (~36 bpm) at 25 mg/kg. These effects were relatively transient except for 25 mg/kg where the effects were sustained. There was inhibition in the responses to DMPP (dimethylphenylpiperazinium) and to vagal stimulation at 5 and 25 mg/kg with modifications in the depressor component of the isoproterenol response at 5 and 25 mg/kg.

**Neurotoxicity and Anticonvulsant Testing.** Table IV summarizes the results for compounds **10** and **11**. Two reference agents are included for comparison. The $ED_{50}$ values for both **10** and **11** are similar to those for phenobarbital sodium and diphenylhydantoin sodium. The time to peak effect for **10** and **11** is less than for either of the reference compounds. Depression of forced motor activity

Table V. Anticonvulsant Profile of Some Drugs at Nonneurotoxic Doses (Mice)

| Drug | Ability to protect against[a] | | |
|---|---|---|---|
| | Strychnine convulsions | Metrazole convulsions | Electroshock convulsions |
| 10 | + | – | +++ |
| 11 | + | + | ++ |
| Phenobarbital Na | – | ++ | ++ |
| Diphenylhydantoin Na | – | – | ++ |

[a](–) not active, (+) slightly active at nonneurotoxic doses, (++) moderately active at nonneurotoxic doses, (+++) markedly active at nonneurotoxic doses.

Table VI. Antioxotremorine and Antiacetylcholine Writhing Effects of Drugs at Nonneurotoxic Doses (Mice)

| Drug | Antitremor[a] | Antiwrithing[a] |
|---|---|---|
| 10 | + | + |
| 11 | ++ | – |
| Phenobarbital Na | + | – |
| Trihexyphenidyl HCl | +++ | – |
| Diphenylhydantoin Na | – | Not tested |

[a](–) not active, (+) slightly active at nonneurotoxic doses, (++) moderately active at nonneurotoxic doses, (+++) markedly active at nonneurotoxic doses.

is a measure of neurotoxicity since the ability of the animal to coordinate and function is impaired. Thus, it appears that both compounds **10** and **11** are neurotoxic at dose levels similar to those at which the reference CNS depressants phenobarbital and diphenylhydantoin are neurotoxic. Table V summarizes the anticonvulsant profile for these drugs. Like phenobarbital and diphenylhydantoin compounds **10** and **11** had good antielectroshock activity at doses that were not neurotoxic. Compound **11** had a slight antimetrazole activity but was not as active in this test as phenobarbital. Both **10** and **11** had slight antistrychnine activity whereas no activity was seen with phenobarbital or diphenylhydantoin. Neither compound **10** nor **11** had oustanding effects in the escape test. Compound **10** decreased the mean reaction time on the hot plate at 28 mg/kg ip but the effect was somewhat erratic and of very short duration. Compound **11** produced only a slight effect on the hot plate response at 50 mg/kg ip.

**Antitremor Testing.** Table VI summarizes the activity of several drugs in the oxotremorine test. Compound **11** had moderate activity that was better than that observed with phenobarbital or diphenylhydantoin but not as active as trihexyphenidyl. However, trihexyphenidyl had a definite anticholinergic effect as measured by the decreases in lacrimation and salivation, whereas compound **11** did not have an effect on these parameters.

**Analgesic-Antiinflammatory Testing.** Since compounds **10** and **11** have CNS-depressant activity and produced slight activity in the hot plate test, they were tested in the acetylcholine writhing test to determine if they had analgesic or antiinflammatory potential. As indicated in Table VI, neither drug produced outstanding effects.

**Summary of Pharmacological Effects of Compounds 10 and 11.** Both compounds **10** and **11** appear to have a similar profile of activity and have marked central nervous system activity. Compound **11** was somewhat different from compound **10** as reflected in more antitremor activity and an apparent increase in spontaneous motor activity.

Compound **12** at 30 mg/kg was active in the primary screen showing general CNS depression and a similar profile of activity as compound **10**. Compounds **9**, **10**, **13**, **15**, and **19** were all inactive in the mouse screen at dose levels up to 100 mg/kg ip. It may thus be concluded that a *p*-aminophenyl group in the 4′ position without any halogen function at the 1 position of such 3-aminoisoquinolines is a prime requirement for CNS activity. None of the compounds reported in this paper were evaluated for their potential antimalarial activity.

## Experimental Section‡

**α-Cyano-α-(*p*-nitrophenyl)-*o*-tolunitrile (5a).** A ball mill loaded with *ca.* 1 lb of KOH pellets and *ca.* 0.5 l. of pyridine§ was rolled for 2 days. The resulting slurry was transferred into a 5-l., three-necked flask which was equipped with one addition funnel, one thermometer, and one mechanical stirrer. The addition funnel was sealed off with a KOH drying tube. The ball mill was rinsed with portions of fresh pyridine (a total of 300 ml) to make the transfer as complete as possible. The reaction vessel was immersed into an ice–water bath and a solution of 100 g (0.705 mol) of α-cyano-*o*-tolunitrile (**3**) (mp 77–79°) and 143 g (0.709 mol) of *p*-nitrobromobenzene in 1 l. of pyridine was added dropwise over a period of 4 hr. The reaction temperature was kept at 0°. After about two-thirds had been added, the mixture became too thick and was diluted with additional 0.3 l. of pyridine. The reaction mixture was stirred for an additional 1 hr and was then diluted with 2 l. of benzene. The precipitate was collected on a Büchner funnel# and was washed once with benzene. The solid was transferred into an 8-l. beaker and was stirred with *ca.* 3 l. of water. The mixture was neutralized by addition of 600 ml of glacial acetic acid and extracted into 1 l. of benzene. The benzene layer was separated and washed two times with 1 l. of 2% HCl, two times with 1 l. of water, and once with brine. The solution was then stirred with $MgSO_4$ and decolorizing charcoal and filtered, and the solvent was removed. The residual oil was dissolved in 1.5 l. of *ca.* 40° warm ethanol. Slow cooling and scratching caused the product **5a** to crystallize to yield 111 g, mp 81–83°. An additional 16 g, mp 78–80°, was obtained when the mother liquor was concentrated to 0.4 l. The total yield was 127 g, 68.5%. A small sample was recrystallized from 2-propanol, mp 82.5–84°. The compound can form a stable solvate when crystallized from cold ethanol, mp 51–53°.

**α-Cyano-α-(3-methoxy-4-nitrophenyl)-*o*-tolunitrile (5c)** was similarly prepared from **3** (0.0705 mol) and 5-chloro-2-nitroanisole (0.083 mol) to yield 13 g (63%) of product, mp 118–119.5°

**α-Cyano-α-(2-nitrophenyl)-*o*-tolunitrile (5b)** was prepared similarly to **5a** from 3 (0.12 mol) and 25 g (0.124 mol) of *o*-nitrobromobenzene to yield 28.2 g of a dark-brown semisolid which could not be crystallized. The crude material was used in the next step.

**α-Cyano-α-(*p*-nitrobenzyl)-*o*-tolunitrile (5d).** A mixture of 14.2 g (0.1 mol) of 3, 10.0 g of NaCN, 0.15 g of NaI, 100 ml of acetone, and 100 ml of ethanol was stirred at room temperature for 10 min. A solution of 22.0 g (0.102 mol) of *p*-nitrobenzyl bromide in 150 ml of acetone–ethanol (2:1) was added dropwise. The mixture was stirred overnight and the solvent was removed. The residue was treated with 500 ml of water and was made mildly acidic by addition of acetic acid** to give 13.7 g of a material that was insoluble in water and in benzene. Recrystallization from 100 ml of toluene yielded 9.24 g (33%) of 5d, mp 171–172°.

**α-Cyano-2,2′-ethylenebis(benzonitrile) (5e).** A mixture of 350 g (1.79 mol) of α-bromo-*o*-tolunitrile, 100 g (2.05 mol) of NaCN, and 1.0 g of NaI was stirred in a mixture of 0.6 l. of acetone and 0.8 l. of ethanol at room temperature for 24 hr. The inorganic salts were removed by filtration and the filtrate was concentrated to

‡All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. The microanalyses were performed by Galbraith Laboratories, Inc. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Nmr spectra were obtained on a Varian Model A-60 spectrophotometer. Peak positions are reported in terms of parts per million from tetramethylsilane. Ultraviolet absorption spectra were determined on a Beckman DK-1A recording spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 237 spectrometer.

§All the pyridine used was predried over KOH pellets.

#A layer of glass cloth was used instead of filter paper. Glass-fiber filter paper was used on small-scale runs.

**A well-ventilated hood has to be used for the acidification.

near dryness. The residue was treated with 300 ml of $C_6H_5$ and was filtered. Addition of 300 ml of $Et_2O$ yielded 50 g of 5e, mp 107–113°. The mother liquor was diluted with additional 100 ml of $Et_2O$, cooled in an ice bath, and on addition of 150 ml of *n*-hexane caused the precipitation of an additional 130 g of 5e, mp 108–112°. The combined yield was 180 g (39%). Recrystallization from ethanol raised the melting point to 110–112°.

**3-Amino-1-bromo-4-(*p*-nitrophenyl)isoquinoline (6).** A sample of 105 g (0.4 mol) of the dinitrile 5a was dissolved in 850 ml of $C_6H_6$ and 250 ml $Et_2O$ was added. The reaction flask was immersed into an ice bath and anhydrous HBr was bubbled through the cold solution until saturated and was stirred for an additional 1 hr at 0° The orange precipitate was collected on a Büchner funnel and washed extensively with $Et_2O$. The solid was added in portions to 1.2 l. of stirred, saturated $KHCO_3$ solution. The solid was collected, water-washed, and stirred with distilled water, collected, and dried at 95° to yield 128 g (93%) of 6, mp 270° dec. Recrystallization from toluene gave orange crystals, mp 275–276° dec.

**3-Amino-4-(*p*-aminophenyl)-1-bromoisoquinoline (9).** The nitro compound 6 (2.8 g, 8.1 mmol) was partially dissolved in a mixture of 100 ml of glacial acetic acid and 15 ml of concentrated HCl. A solution of 11 g of $SnCl_2 \cdot 2H_2O$ in 15 ml of concentrated HCl was added dropwise and the mixture was stirred at room temperature for 4 hr and was then cooled to 0°. The crude product (hydrochloride) was collected by filtration. The solid was partially dissolved in 200 ml of 1% HCl and this slurry was made strongly alkaline with excess 20% NaOH. The product was extracted into 2 × 100 ml of $Et_2O$ and the combined extracts were washed with water and then with brine, dried over $MgSO_4$ and charcoal, and filtered. The filtrate was concentrated to dryness and the residue was dissolved in 30 ml of chloroform. This solution was kept warm while 50 ml of *n*-hexane was added. Slow cooling caused the precipitation of 2.02 g (79%) of pure yellow crystals, mp 175° dec.

**3-Amino-4-(*p*-aminophenyl)isoquinoline (10).** **A. From 6.** A slurry of 2.0 g of 6 in 100 ml of ethanol that contained 1.0 g of KOH was hydrogenated in the presence of 0.2 g of 10% Pd/C for 2 hr. The mixture was filtered and the residue was concentrated to dryness. The residue was treated with water, collected on a Büchner funnel and was washed thoroughly with water, and recrystallized from 10–15 ml of ethanol to yield 0.55 g (40%) of 10, mp 152–153°.

**B. From 9.** A mixture of 27 g of 9, 15 g of KOH, and 1 g of 10% Pd/C in 250 ml of ethanol was hydrogenated on a Parr apparatus for 3 hr. The mixture was filtered yielding a large amount of insoluble material which consisted mainly of product mixed with KBr and catalyst. This solid mixture was treated once with 200 ml of hot ethanol and was filtered. There was still undissolved product which was finally separated from the inorganic material by dissolution in 2% HCl, filtration, and precipitation with NaOH. This material was combined with the evaporation residues of the ethanolic solutions. The mixture was treated with water and the solid was collected on a Büchner funnel, was washed thoroughly with water, and dried in a vacuum oven at 50° to give 18.7 g (92.5%) of 10, mp 151–153°. An analytical sample was prepared by recyrstallization from MeOH, mp 153–154°. The dihydrochloride mp 292 ± 1° (determined by dta) was prepared. *Anal.* ($C_{17}H_{15}N_3O \cdot 2HCl$) C, H, N, Cl.

**4-(*p*-Acetamidophenyl)-3-aminoisoquinoline (11).** A sample of 19 g (0.081 mol) of the diaminoisoquinoline 10 was dissolved in 100 ml of pyridine. A solution of 8 g (0.087 mol) of $Ac_2O$ in 20 ml of pyridine was added over a period of 10 min and the mixture was stirred for an additional 10 min at 20–25°. The reaction was slightly exothermic and some external cooling was necessary. When the solution was diluted to a volume of *ca.* 550 ml, there was slow crystallization of the acetamide 11. An additional 100 ml of water was added and the crystallized product was collected, water-washed, and dried to yield 15.3 g, mp 232–235°. An additional 2.1 g, mp 232–235°, of 11 was obtained when the mother liquor was diluted further with 400 ml of water and stored at 5° for several days. The total yield of 11 was 17.4 g (77.5%). Recrystallization from *ca.* 350 ml of EtOH yielded 12.3 g of purified material, mp 234–236°. An analytical sample was prepared from toluene–ethanol (8:1), mp 235.5–237°. In the nmr spectrum of 11 the aromatic protons α to the acetamido group are further downfield (δ 6.97 ppm). Acetylation at the 4′ position is also in accordance with the expected difference in the basicity of the two amino groups in 10.

**3-Amino-4-[*p*-(ethylamino)phenyl]isoquinoline (12).** A slurry of 4.1 g (14.8 mmol) of the monoacetamide 11 in 100 ml of THF was stirred with 40 ml of 1 *M* diborane in THF at 0–5° for 30 min and then at room temperature for 12 hr. Acetone (30 ml) was added followed by 100 ml of 2% HCl. The mixture was concentrated to *ca.* 70 ml and was then poured into 300 ml of saturated $KHCO_3$ solution. The yellow solid was collected, washed with water, and dried for 1 hr at 95°. The dark brown product was recrystallized from 25 ml of ethanol to yield 1.1 g of 12, mp 136–140°. An additional 0.3 g, mp 132–136°, was isolated on concentrating the mother liquor. Both fractions were combined and recrystallized from *n*-hexane to yield 12, 1.03 g, mp 138–142°. An analytical sample was prepared from $CH_3CN$, mp 141–143°.

**Acetylation of 3-Amino-1-bromo-4-(*p*-nitrophenyl)isoquinoline (6).** A slurry of 19.5 g (56 mmol) of 6 in a mixture of 250 ml of benzene and 5 g of pyridine was stirred with 7.2 g (58 mmol) of acetyl bromide at room temperature overnight. The mixture was then heated to reflux for *ca.* 30 min. After cooling the solid was collected and treated first with 200 ml of 0.5% HCl and then with water. Drying at 100° yielded 10.9 g of an orange solid, melting point range 210–260°. This material found to be a mixture of unreacted 6 and the monoacetamide 7. Fractional recrystallizations yielded 5.6 g of pure 7. The filtrate was concentrated to *ca.* 30 ml to give 7.5 g of a yellow solid, mp 189–205°. Recrystallization from toluene yielded pure 8, mp 195–198°.

**3-Acetamido-4-(*p*-aminophenyl)-1-bromoisoquinoline (13).** A slurry of 3.5 g (8.2 mmol) of acetamide 8 and 12 g of $SnCl_2 \cdot 2H_2O$ in a mixture of 35 ml of glacial AcOH and 15 ml of concentrated HCl was stirred at room temperature for 3 days. $Et_2O$ (50 ml) was added and the solid was collected and dissolved in 50 ml of water and the resulting slurry was treated with excess of 20% NaOH. The product was extracted into 2 × 200 ml of $C_6H_6$ and once into chloroform. The combined organic layers were washed with $H_2O$ and brine and were dried over $MgSO_4$. Filtration and removal of the solvents resulted in the isolation of 1.75 g of fine crystals of 13.

**3-Amino-1-bromo-4-(*o*-nitrophenyl)isoquinoline (14).** The total amount (28 g) of the impure α-cyano-α-(2-nitrophenyl)-*o*-tolunitrile (5b) was dissolved in a mixture of 400 ml of $C_6H_6$ and 100 ml of $Et_2O$. The solution was cooled to 10° and anhydrous HBr was bubbled through the mixture for approximately 3 hr. Stirring was continued for an additional 2 hr at 5–10°. The reaction mixture was poured into a solution of 300–400 g of KOH in 1.5 l. of ice–water. The organic layer was diluted with 0.5 l. of $C_6H_6$ and the layers were separated. The insoluble material dispersed in the organic layer was removed by filtration and discarded. The aqueous layer was washed once with 0.3 l. of $C_6H_6$. The combined organic phase was washed with water and brine and then dried over $MgSO_4$. Filtration and concentration to 100 ml yielded 13.8 g of a solid. When this solid was treated with 200 ml of hot toluene and filtered, 5.3 g of insoluble material, mp 215–230° dec, was isolated. From the filtrate a product crystallized, mp 204–213°, which on further recrystallization from 25 ml of dioxane yielded 2.72 g of pure 14, mp 215–216.5°

**3-Amino-4-(*o*-aminophenyl)isoquinoline (15).** A slurry of 2.5 g of 14 and of 10% Pd/C in a solution of 1 g of KOH in 150 ml of EtOH was hydrogenated for 3 hr. The mixture was filtered and concentrated to near dryness. The residue was treated with 50 ml of water and the product was extracted into five small portions of $CH_2Cl_2$. The combined extracts were washed with water, dried over $MgSO_4$, and filtered. The solvent was removed and the residue was recrystallized from 100 ml of 5% EtOH in cyclohexane to yield 1.28 g of 15, mp 142–143°.

**3-Amino-4-benzylisoquinoline (16).** This compound was prepared by the catalytic debromination of 3-amino-1-bromo-4-benzylisoquinoline. The crude product was recrystallized from EtOH (pale yellow needles) and from cyclohexane.

**3-Amino-1-bromo-4-(*p*-nitrobenzyl)isoquinoline (17).** A sample of 8.5 g of the dinitrile 5d was incompletely dissolved in a mixture of 250 ml of $C_6H_6$, 250 ml of toluene, and 100 ml of $Et_2O$. Anhydrous HBr was bubbled through the stirred and cooled (*ca.* 10°) reaction mixture. When the saturation point was reached, the rate of bubbling of HBr was decreased and kept at a low rate for an additional 5 hr. The mixture was then stirred at 15–20° overnight without further HBr addition. The next morning an additional 250 ml of ether was added and the yellow solid was collected on a Büchner funnel. The product was washed with $Et_2O$ and then stirred with excess of $KHCO_3$ solution. The solid was collected and was stirred twice with fresh water and dried to give 11.7 g of 17, mp 251–252° dec.

**3-Amino-4-(*p*-aminobenzyl)-1-bromoisoquinoline (18).** The nitro compound 17 (3.0 g, 8.4 mmol) was added to a mixture of 100 ml of glacial acetic acid and 5 ml of concentrated HCl, followed by 12.0 g of $SnCl_2 \cdot 2H_2O$. This mixture was stirred at room tem-

perature overnight, 200 ml of water was added, and the yellow, slightly cloudy solution was added to 1 l. of cold 20% NaOH. The product was extracted twice with $C_6H_6$ and once with EtOAc. The combined extracts were washed with water and brine and concentrated to dryness to yield 2.42 g (80%) of **18**, mp >170° dec.

**3-Amino-4-(*p*-aminobenzyl)isoquinoline (19).** A slurry of 0.5 g of 10% Pd/C in water was combined with a slurry of 6.6 g (0.0185 mol) of **18** in 200 ml of EtOH. The mixture was hydrogenated for 2 hr after 4.0 g of KOH had been added. The mixture which contained some undissolved product mixed with the catalyst was filtered and the filtrate was concentrated to dryness. The product-catalyst mixture was treated with 100 ml of 2 *N* HCl and the catalyst was removed by filtration. The filtrate was used to dissolve the residue of the ethanolic filtrate. The product was precipitated with excess $Na_2CO_3$, collected, washed thoroughly with water, and dried *in vacuo* at 55° to give 4.4 g (96%) of **19**, mp 150–154°. Recrystallization from 75 ml of $C_6H_6$ afforded 2.77 g of pure **19**, mp 153–155°.

**3-Amino-1-bromo-4-(*o*-cyanobenzyl)isoquinoline (20).** A sample of 5 g of **5e** was dissolved in 80 ml of $C_6H_6$; 10 ml of ether and anhydrous HBr was bubbled into the reaction mixture at *ca.* 15°. The precipitate which formed was collected, washed with $Et_2O$, and stirred first with $NaHCO_3$ solution and then with $H_2O$. Recrystallization from dioxane yielded 1.95 g of **20**.

**Cat Cardiovascular Screen.** Compounds **10** and **11** were tested intravenously at 5, 10, and 25 mg/kg in anesthetized mongrel cats. Blood pressure was recorded *via* the left carotid artery. Respiration and heart rate were also recorded. Control responses to vagal stimulation, nictitating membrane contraction, and doses of acetylcholine, norepinephrine, histamine, isoproterenol, and dimethylphenylpiperazinium (DMPP) were obtained. The drugs were then administered *via* the femoral vein and the above challenges repeated.

**General CNS Testing.** A log dose *vs.* response curve was generated, using the inability of trained animals to walk a rotating wooden rod (28 mm diameter, at 20 rpm) for 1 min as the response criterion. The animals were also tested at the same time for their reaction to enclosure in hand of the technician. Six 18–22-g nonfasted albino mice were used per group, and dosing was done intraperitoneally. The animals were tested at 0, 15, 30, 60, 90, 120, 150, and 180 min postinjection. The time postinjection for the peak effect was determined, and the quantal response at that dose was used to plot the curve. A graphical $ED_{50}$ was estimated. This dose and peak effect time were then used for subsequent work in the antistrychnine, antimetrazole, antielectroshock, and antioxotremorine tests. In these tests, the experimental drug was injected intraperitoneally into groups of six 18–22-g nonfasted albino mice; after the time to peak effect, strychnine sulfate (1.1 mg/kg sc), pentylenetetrazole (metrazol) (70 mg/kg sc), oxotremorine (350 mcg/kg sc), or a 50-A, 0.2-sec shock was administered to each animal. The animals were observed for 30 min for tonic extensor seizures in the antistrychnine test and for clonic seizures in the antimetrazole test. In the antielectroshock test, the animals were observed for hind-limb tonic extensor seizures. In the antioxotremorine test, tremors were subjectively rated per animals on a scale of 0–3 and the total response for the entire group was calculated and compared to that of a control group.

In each test a group of animals receiving 0.9% saline, 5 mg/kg, was run simultaneously with the experimental groups. In the acetylcholine writhing test, mice were injected orally with the experimental agent and, after an elapse of the peak effect time, challenged with a 7.0 mg/kg ip injection of acetylcholine bromide. The number of animals in the experimental group that elicited a writhing response within 2 min post the challenge was compared to that of a control group receiving 0.9% saline.

**Acknowledgments.** We wish to thank Dr. L. S. Harris, University of North Carolina School of Medicine, for the initial pharmacological studies and useful discussions.

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# Potential Antifertility Agents. 4. Biological Properties of Diastereoisomeric 4-Aryl-2-methylcyclohexanecarboxylic Acids and Related Compounds[1]

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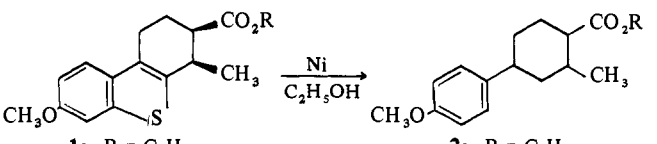
Syntheses and biological activities are reported for the four (±) diastereoisomers of 4-(*p*-methoxyphenyl)-2-methylcyclohexanecarboxylic acid and for 75 related compounds. Antifertility, estrogenic, hypocholesterolemic, and gonadotropin inhibitory activities are discussed. Biological activity resided in the (±)-1α,2β,4β and (±)-1β,2β,4α isomers. Evidence is presented that the activity in these isomers is owing to their stereochemical relationship with steroidal estrogens. One of the compounds (**5b**) which showed an unexpectedly large separation between uterotropic and gonadotropin inhibitory activities is discussed more extensively and is suggested for possible utility in the treatment of prostatic cancer and benign prostatic hypertrophy.

While investigating structure-activity correlations in a series of pregnancy-inhibiting dibenzothiophenecarboxylic acids,[1] we desulfurized the ester **1a** with Raney nickel. The product obtained, **2a**, was determined by glpc to be an 80/20 mixture of two isomers, the isomeric ratio of which did not change upon hydrolysis to the acids **2b**. For expediency, biological tests were made on the mixture of acids **2b** to determine whether any of the activities shown by the parent acid **1b**[1] were retained. Initial assays, in mice, showed the mixture **2b** to possess none of the antifertility or estrogenic properties of **1b**. Additional evaluation revealed that **2b** lowered serum cholesterol levels and also produced pronounced effects on male accessory sex organs of rats. In view of the apparent lack of estrogenicity, these activities were of considerable interest and prompted us to define the isomeric composition of **2** and to obtain in pure form the four possible (±) diastereoisomers of structure **2**. Addi-




**1a**, R = $C_2H_5$
b, R = H

**2a**, R = $C_2H_5$
b, R = H