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Estradiol Analogs with Conformational Flexibility. 4-(1,2,3,4-Tetrahydro-6-hydroxy-2-naphthyl)-butan-2-ol†

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One approach to the development of nonsteroidal estrogens is through simple analogs of estradiol lacking parts of the C and D rings. We have therefore synthesized and studied the biological activity of 4-(1,2,3,4-tetrahydro-6-hydroxy-2-naphthyl)butan-2-ol (**1**). Horeau[1] has already shown that naphthalene derivatives with side chains extending from C-2, such as methallenestril (**2**), can retain strong estrogenic activity. Our model compound **1** was anticipated to have the conformational flexibility to bind selectively to some estradiol receptors, and possibly to show at least a partial separation of estrogenic and other hormonal (*e.g.*, hypocholesterolemic) activities. Furthermore, the synthetic route developed for **1** would allow the introduction of other substituents with ease, thus making available a variety of bicyclic derivatives for additional study.

The synthesis of **1** was straightforward. The carbomethoxy derivative **4**,[2] obtained from 6-methoxy-1-tetralone (**3**), was cyanoethylated[3] with acrylonitrile in the presence of Triton B to give the cyanoethyl compound **5** in good yield. Hydrolysis of **5** with a mixture of HCl and AcOH resulted in the propionic acid **6**. A modified Wolff-Kischner reduction[4] of the keto acid **6** yielded **7**, which on subsequent treatment with MeLi gave the methyl ketone **8**. This methoxy compound **8** was then demethylated by fusion with pyridine hydrochloride at 205° to give the phenol **9**. The carbonyl group in the side chain was finally reduced with $NaBH_4$ to furnish the de-

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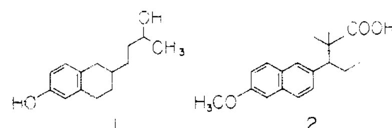
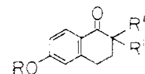
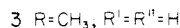
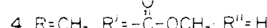
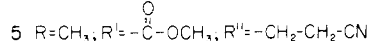
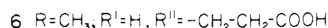
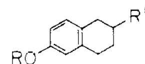
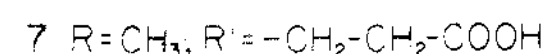
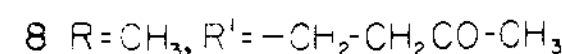
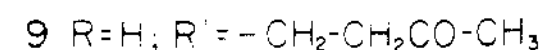

sired alcohol **1**. All the compounds described were racemates, but the final reduction introduced a second asymmetric center into the molecule. There was no obvious steric control of this reaction; this, together with the yield (69%) and the single crystallization of the chromatographically pure product, make it improbable that the compound tested was a single (inactive) diastereoisomer and that the other might be active.

Compound **1** was assayed for both estrogenic and antiestrogenic activity in rats, using uterine weight and vaginal opening as criteria.‡ It was found inactive at doses up to 300 μg/kg body weight. It was subsequently assayed for hypocholesterolemic activity in rats at 7 mg/kg twice daily for 2 days, and the serum cholesterol was determined.§ No significant lowering of the cholesterol level was observed.

Experimental Section#

Methyl 1,2,3,4-tetrahydro-6-methoxy-1-oxo-2-naphthalenecarboxylate (4) was prepared from **3** as described by Juday *et al.*;[5] the physical constants were in agreement with those described by Bachmann and Thomas.[2]

Methyl 2-Cyanoethyl-1,2,3,4-tetrahydro-6-methoxy-1-oxo-2-naphthalenecarboxylate (5). To the carbomethoxy-1-tetralone (**4**) (10 g) in *p*-dioxane (10 ml, free from peroxides) were added acrylonitrile (3 ml) and Triton B (0.9 ml, 40% in MeOH), and the mixt was stirred at ambient temp for 16 hr. The reaction product was isolated** with Et_2O and recrystd from a mixt of Et_2O and petr ether to yield 7.5 g (62%) of **5**: mp 66-69°; 231 (ε 5920) and 280 mμ (15,000). *Anal.* ($C_{16}H_{17}NO_4$) C, H, N.

‡Estrogen and antiestrogen assays were conducted under the direction of Dr. Elva G. Shipley at the Endocrine Laboratories, Madison, Wis.

§The serum cholesterol levels were determined at the Southwest Foundation for Research and Education by Mr. Armando de la Peña.

#Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded in KBr discs on a Unicam S.P. 200 spectrometer and were compatible with the structures assigned. Neutral aluminum oxide, activity II, was employed for column chromatography. Petroleum ether refers to that fraction boiling from 40-60°. The compounds described are all racemates. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

**The following sequence describes a typical isolation procedure. The reaction mixture was treated with H_2O and extracted with the specified organic solvent. The solvent extract was washed with brine, and dried over Na_2SO_4, and filtered, and the solvent was evapd under reduced pressure on a Büchi rotary evaporator at 60-65°. The residue left in the flask was then purified as described.

1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthalenepropionic Acid (6). Compd **5** (7 g) was added to AcOH (16.7 ml), HCl (33.4 ml), and H_2O (2.2 ml); the mixt was heated at 100° for 4 hr, then cooled, and poured into crushed ice-H_2O. The solid (5.8 g, 97%) was filtered and recrysted from Me_2CO–petr ether: mp 133–135°; λ_{max}^{MeOH} 224 (ϵ 4620) and 272 mμ (14,200). *Anal.* ($C_{14}H_{16}O_4$) C, H.

1,2,3,4-Tetrahydro-6-methoxy-1-naphthalenepropionic Acid (7). Hydrazine hydrate (2.5 ml, 80% soln) was added to a soln of **6** (3 g) and KOH (2.7 g) in 1,2-propanediol (15 ml), the mixt was heated to 120°, and H_2O was distd off. After this distillation ceased, the temperature was gradually raised to 180–190° and was maintained there under reflux for 4 hr. The reaction product was then poured into ice-H_2O, and acidified with HCl. The precipitated acid **7** (1.67 g, 59%) was filtered and recrystd from Me_2CO: mp 122–123°; λ_{max}^{MeOH} 279 (ϵ 2150) and 288 mμ (1970). *Anal.* ($C_{14}H_{18}O_3$) C, H.

4-(1,2,3,4-Tetrahydro-6-methoxy-2-naphthyl)butan-2-one (8). To a stirred soln of **7** (5 g) in anhydrous THF (70 ml) maintained at 10° under N_2 was added a soln of MeLi (50 ml, 1.6 *M* soln in Et_2O), dropwise over a period of 30 min. Stirring was continued for another 1.5 hr, then excess reagent was decompd with ice H_2O and the product isolated with Et_2O.** From the crude reaction product the ketone **8** was purified through a bisulfite addition product and was obtained as an oil (1.75 g, 35%). A 2,4-DNP deriv of **8** was prepd and recrystd from EtOH: mp 106–108°. *Anal.* ($C_{21}H_{24}N_4O_5$) C, H, N.

4-(1,2,3,4-Tetrahydro-6-hydroxy-2-naphthyl)butan-2-one (9). A mixt of **8** (1.18 g) and pyridine hydrochloride (1.5 g) was heated at 205° for 1 hr under N_2, then cooled, and diluted with H_2O. The pptd material was filtered and purified by column chromatography (alumina). The fractions eluted with PhH and PhH–Et_2O (8:2) were combined to give **9** (0.48 g) which was recrystallized from Me_2CO–petr ether: mp 87–89°; λ_{max}^{MeOH} 280 mμ (ϵ 2180). *Anal.* ($C_{14}H_{18}O_2$) C, H.

4-(1,2,3,4-Tetrahydro-6-hydroxy-2-naphthyl)butan-2-ol (1). To a soln of the above ketone **9** (0.262 g) in MeOH (20 ml) was added $NaBH_4$ (0.145 g), and the soln was stirred at room temp for 30 min. The excess reagent was decompd by addition of a few drops of AcOH. Most of the MeOH was evapd under vacuum and the product was isolated** with EtOAc, then purified over a column of alumina. The fractions eluted with PhH–Et_2O (8:2) were combined (0.18 g, 69%) and crystallized once from Et_2O–petr ether, mp 81-84°; λ_{max}^{MeOH} 281 mμ (ϵ 2130). *Anal.* ($C_{14}H_{20}O_2$) C, H.

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Ornithine Analogs as Potential Ornithine Decarboxylase Inhibitors 1. N-Substituted Ornithine Derivatives

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Polyamines such as spermidine, spermine, and putrescine are found to be present at higher concentrations in mammalian tissues of organs with high rates of RNA synthesis.[1] They are also present in high concentrations in regenerating rat liver[2,3] and during certain growth phases of the chick embryo.[4,5] Jänne and Raina[6a] and Russell and Snyder[6b] found that ornithine decarboxylase, the enzyme that forms putrescine from ornithine, showed a marked increase in activity in regenerating rat liver. Within 1 hr after partial hepatectomy, ornithine decarboxylase activity was tripled. In addition, some rapidly growing tumors have been found to have ornithine decarboxylase activities far greater than that of nonmalignant tissues.[7,8] A recent study[9] showed that ornithine decarboxylase activity in the rat ovary was stimulated by luteinizing hormone.

It was of interest to attempt to find inhibitors of ornithine decarboxylase in order to better understand the role of this enzyme and the polyamines resulting from its action in rapidly growing tissues. A survey of the literature failed to uncover any reports of inhibitors of ornithine decarboxylase. Thus synthetic studies were undertaken to modify the ornithine molecule in order to investigate whether these modifications would result in enzyme inhibitors. Table I summarizes the chemical data on those compounds synthesized.

N^ϵ-Phenyl-*dl*-ornithine (**1**) was synthesized *via* the following sequence: 3-anilinopropanol was treated with BzCl to yield *N*-benzoyl-3-anilinopropanol. The benzoylation was conducted in a 2-phase system so as to benzoylate the amino group. The iodo group was introduced after chlorination with $SOCl_2$ by using NaI in boiling acetone. Reaction of the I derivative with formamido malonate yielded diethyl *N*-phenyl-*N*-benzoyl-3-aminopropyl formamidomalonate that was hydrolyzed to the desired amino acid.

β-Methylornithine hydrochloride (**2**) was prepared *via* reductive cyclization of diethyl 2-(2-cyano-1-methylethyl)malonate over Raney Ni, formation of 4-methylpiperidine-2,3-dione 3-phenylhydrazone using $NaNO_2$ and $PhNH_2$, reduction over Raney Ni of the hydrazone to the amine and acid hydrolysis to the desired amino acid.

N^α-Methyl-N^ϵ-tosyl-*l*-ornithine (**4**) was synthesized *via* N^α-benzyl-N^ϵ-tosyl-*l*-ornithine,[14] that was methylated with formic acid-CH_2O, and hydrogenated over Pd/C to yield **4**.

Initially, these compounds with the exception of **3** were evaluated for their effects on ornithine decarboxylase activity in soluble supernatant preparations of livers from rats 3 hr after partial heptatectomy. The level of activity at this time is elevated about 8–10 times above basal activity.[8] Enzyme activity was determined by the formation of $[^{14}C]CO_2$ from $[1\text{-}^{14}C]$ornithine and was assayed under conditions in which activity was linear with time and enzyme concentration. The compounds were preincubated with the enzyme preparation for 10 min before the addition of $[^{14}C]$ornithine. They were tested in concns of 10^{-4} *M*, 10^{-5} *M*, and 10^{-6} *M*. No inhibition of enzymatic activity was obtained with any of the compounds tested.

In addition to evaluation of their ability to inhibit ornithine decarboxylase, **1**, **4**, **5**, **6**, and **7** were evaluated for anticancer activity in mice with L-1210 leukemia at a dose level of 400 mg/kg, and all were inactive. At this dose, only α-methyl-[*p*-(*N*-morpholino)phenyl]-*dl*-alanine showed any toxicity (4/6 survivors).

The antimicrobial activities of these ornithine derivatives were evaluated by an *in vitro* screen using the paper disk-agar plate method. Each disk was impregnated with 0.5 mg of test compound and laid on sensitivity agar plates streaked with dilute cultures of the test organism. Rings of inhibition after 24 hr were measured. Eight organisms were used for this test: *Staphylococcus albus, Escherichia coli, Serratia marcescens, Klebsiella aerobacter, Micrococcus luteus, Sacharomyces cerevisiae, Penicillium notatum,* and *Sporobolomyces salmoncolor.* None of