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.

References

(1) J. L. Neumeyer and K. K. Weinhardt, *J. Med. Chem.*, **13**, 999 (1970) (paper 2).
(2) J. L. Neumeyer and K. K. Weinhardt, *ibid.*, **13**, 613 (1970).
(3) S. Irwin, *Psychopharmacologia*, **13**, 222 (1968).
(4) A. C. Cope, H. L. Holmes, and H. O. House, *Org. React.*, 9, 107 (1957).
(5) S. Migano and N. Abe, *J. Org. Chem.*, **36**, 20, 2948 (1971).
(6) S. Gabriel and R. Otto, *Ber.*, **20**, 2222 (1887); S. Gabriel, *ibid.*, **20**, 2499 (1887); S. Gabriel and T. Posner, *ibid.*, **27**, 2492 (1894); G. Eichelbaum, *ibid.*, **21**, 2679 (1888).
(7) F. Johnson and N. Nasutavicus, *J. Org. Chem.*, **27**, 3953 (1962).
(8) R. B. Davis and L. C. Pizzini, *ibid.*, **25**, 1884 (1960).
(9) M. Makosza, *Tetrahedron Lett.*, 673 (1969).

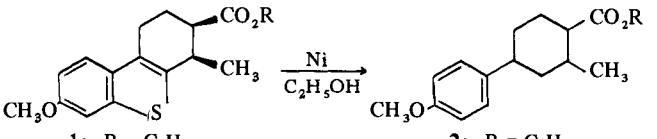
Potential Antifertility Agents. 4. Biological Properties of Diastereoisomeric 4-Aryl-2-methylcyclohexanecarboxylic Acids and Related Compounds[1]

R. R. Crenshaw,* George M. Luke, Thomas A. Jenks, Richard A. Partyka, Gabriel Bialy, and Max E. Bierwagen

Research Division, Bristol Laboratories, Divison of Bristol-Myers Company, Syracuse, New York 13201. *Received January 5, 1973*

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-



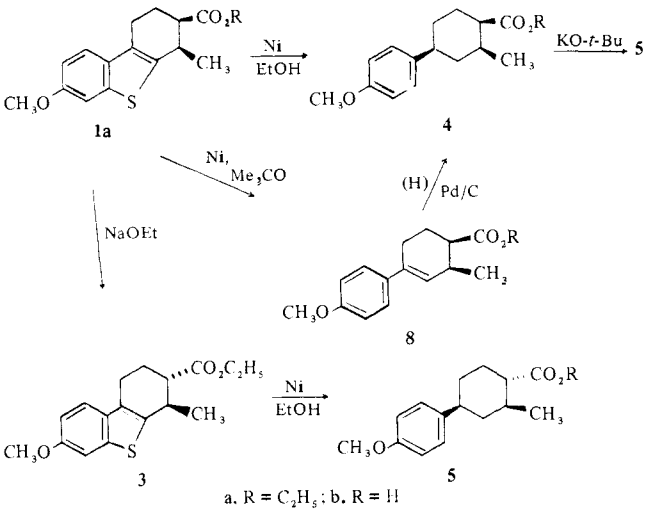
1a, R = C_2H_5
b, R = H

2a, R = C_2H_5
b, R = H

tionally, we made a number of substituent changes in that isomer in which the biological activity resided.

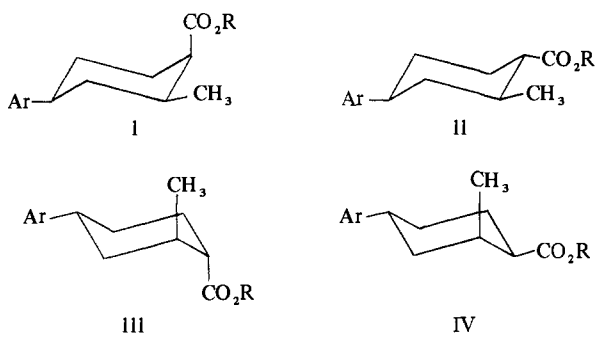
Chemistry. Initial work in defining the composition of **2** is outlined in Scheme I. We considered that **2** was a

Scheme I



mixture of either C-4 or of C-1 epimers. To obtain an indication of which, we repeated the desulfurization of **1a** using Raney nickel containing a lower base content than was used previously. The product contained only the major isomer **4a** of the original mixture **2a** and unchanged starting material **1a**. It thus seemed likely that the second isomer in the original mixture was formed from base-induced epimerization α to the carbonyl and that **2** represented a mixture of C-1 epimers. This was confirmed when the *trans*-dibenzothiophene ester **3** was desulfurized, and the resultant product hydrolyzed to yield, as the major product, an acid **5b** identical with the minor component in the original mixture **2b**. The stereochemistry of **4b** and **5b** was thus indicated as 1,2-cis and 1,2-trans, respectively.

The stereochemical assignment at C-4 was made from a consideration of the preferred conformers I–IV of the four



possible diastereoisomers.† The pure isomer obtained from the desulfurization of **1a** was one of the two possible 1,2-cis-substituted derivatives, *i.e.*, either I or IV. Conformer I, as an ester, should be readily epimerized at C-1 by strong base to II, having all equatorial substituents, whereas similar treatment of an ester of IV would not be expected to result in an appreciable amount of its C-1 epimer III. When we treated the product which had been obtained from desulfurization of **1a** with KO-*t*-Bu, we obtained, in nearly quantitative yield, the same 1,2-trans isomer **5a** which had been isolated from the desulfurization of **3**. Since the epimerization was virtually complete, we thus made structural assignments for **4** and **5** as indicated in Scheme I. Additional work to be discussed below supported these assignments. We choose to depict the structures as shown in Schemes I–IV with the aryl group always to the left of the cyclohexane ring, and, in this orientation, we employ the convention used in steroid nomenclature. For example, **5**, as depicted, is termed the 1α,2β,4β isomer. All structures shown and discussed herein represent racemates.

The 1β,2β,4β isomer **4b** was essentially inactive while all of the biological activity of the mixture **2b** resided in isomer **5b**. Because synthesis of a number of structural variants of **5b** *via* Scheme I was impractical, we sought an alternative route. We considered the sequence outlined in Scheme II as a direct, general synthesis which had potential for permitting easy variation of C-4 substituents and also for providing stereoselectively the four possible 1,2,4-substituted diastereoisomeric racemates **4–7**.

Catalytic reduction of Hagemann's ester **9** gave essentially one isomeric ester **10a** which was hydrolyzed without epimerization to the acid **10b**. Treatment of **10b** with 2 equiv of *p*-methoxyphenylmagnesium bromide produced a mixture of lactone and hydroxy acid which was treated with *p*-toluenesulfonic acid to yield **11b**, a mixture of Δ^3 and Δ^4 isomers. Catalytic reduction of **11b** gave, in over 95% yield, the same acid **4b** which had been obtained from the *cis*-dibenzothiophene derivative 1a. This result defined the stereochemistry of **10** and **11** as 1,2-cis and showed that this sequence **9** → **5** could be used for preparation of a number of relatives of the biologically active 1α,2β,4β isomer.

Examination of models of **11** suggested that catalytic reduction of either the Δ^3 or Δ^4 isomer, with cis addition of hydrogen from the less hindered face, should give rise predominantly to the one isomer **4** which was obtained. On the other hand, inspection of models of the *trans*-Δ^3,Δ^4-ene acid **15b** suggested that both sides were equally accessible to reduction and that the two saturated 1,2-trans isomers, **5** and **6**, should be obtained. Further, it seemed that if the 1α,2β,4α isomer (**6**; conformer III) were obtained, equilibration of its ester in base should relieve the considerable compression forces in III and produce conformer IV which is the remaining diastereoisomer **7**.

We initially tried to obtain the *trans*-ene structure **15** directly by epimerization of the *cis*-ene ester **11c** with KO-*t*-Bu in *t*-BuOH. The equilibrium mixture which resulted was predominantly the trans product **15c**, but pure **15** could not be separated (as ester or acid) from the cis isomer **11**.

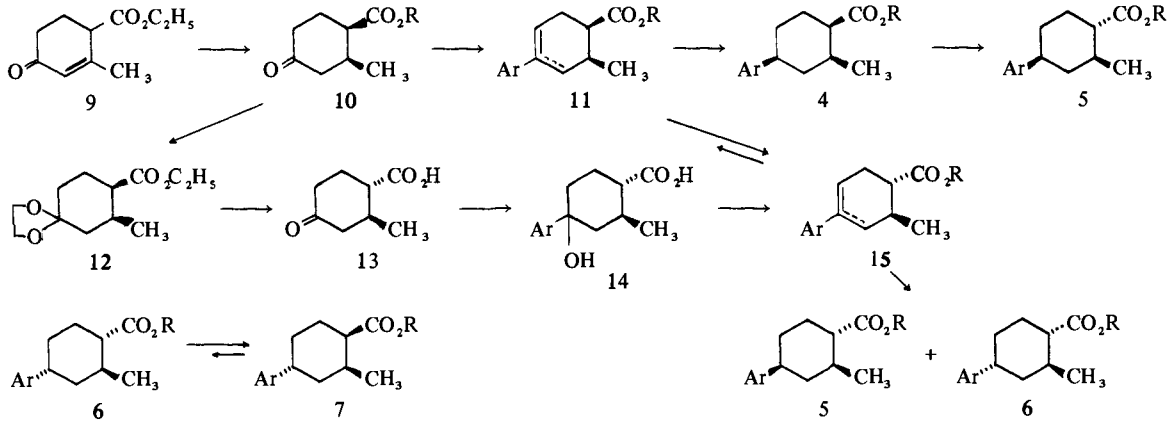
The *trans*-keto acid **13** was prepared *via* epimerization of the *cis*-ethylene ketal **12**. The acid **13** then was elaborated, *via* the hydroxy acid **14**, to the desired ene acid **15b**. Catalytic hydrogenation of **15b** produced the anticipated mixture of **5b** and **6b** in a ratio of 45:55. The isomer **6b** proved very soluble in several solvents relative to **5b** and was obtained in pure form by fractional recrystallization.

Treatment of the ester **6c** with KO-*t*-Bu gave a mixture of **6c** and **7c** in a ratio of approximately 40:60. Surprisingly, small amounts of **4c** and **5c** were also present in the equilibration mixture although chromatographically pure **6c** was used as starting material. The isomeric acid **7b** proved least soluble of the four diastereoisomers and was readily isolated pure.

With the availability of isomer **7b** for use as a standard, the catalytic reduction of **11b** was reexamined. The product was found to contain, in addition to **4b** (96% yield), a small amount (4%) of a product identical with **7b** which had been

†The conformations depicted I–IV agree with preferred conformations given by Sicher, *et al.*,[2] for the four diastereoisomeric 4-*tert*-butyl-2-methylcyclohexanecarboxylic acids.

Scheme II



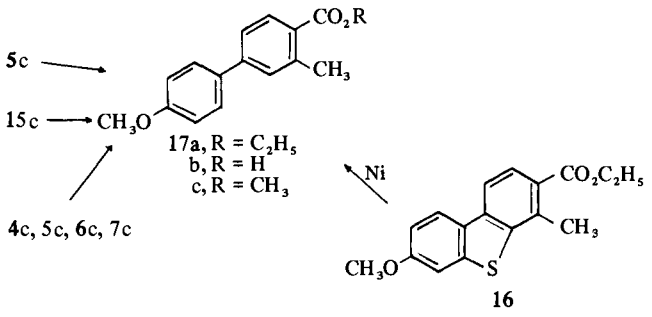
Ar = *p*-$CH_3OC_6H_4$; a, R = C_2H_5; b, R = H; c, R = CH_3

obtained from the epimerization of **6**. This result gave further support to the stereochemical assignments of **4**, **5**, **6**, and **7** as shown. The isomer **7b** proved to have essentially the same biological profile as **5b**, while **6b**, like **4b**, was inactive.

Properties of the four isomeric acids are summarized in Tables I-III. Each isomer has a characteristic melting point which is depressed upon admixture with other isomers. The gross structures are supported by elemental and spectral analyses. Additionally, 100-MHz nmr proton fine structure analyses of the four isomers are consistent with the isomeric and conformational assignments as shown.

Additional evidence in support of the gross structures of the four isomers is seen in Scheme III. Aromatization with palladium of **5c**, **15c**, or of a mixture (**4c**, **5c**, **6c**, **7c**) yields, after hydrolysis, an acid, **17b**, identical with that obtained from the dibenzothiophene **16**.[1]

Scheme III



The four isomers are distinguishable (as esters) on a Craig polyester column with retention times increasing in the order **4c**:**6c**:**5c**:**7c** (*cf.* Experimental Section). Interestingly, a 3% OV-17 silicone glpc column initially used in our work gave esters of **5** and **6** as a single peak. This fact, plus the high solubility of **6b**, resulted in our failure to detect the isomer **6** in our initial desulfurization of **3** (Scheme I). When we reexamined the desulfurized product from **3** on the Craig column, we found both **5a** and **6a** to be present; the **6a** was identical in retention time with **6a** prepared from the acid **6b** produced *via* Scheme II.

Similarly, the OV-17 column gave separation of **11a** and **15a** but not of the individual Δ^3,Δ^4 isomers. The Craig column separated the Δ^3 and Δ^4 isomers of **11a** and of **15a**, but the Δ^4 of **11a** and Δ^4 of **15a** had a common retention time. The same was true for the methyl esters **11c** and **15c**.

The mixture of *cis*-Δ^3- and -Δ^4-ene acids **11b** proved to have biological activity similar to that shown by **5b**. This was considered of sufficient interest to warrant separation of the two double bond isomers. Attempts to obtain a pure isomer from either **11a** or **11b** by physical separation were unsuccessful. We then examined synthetic approaches which would give a pure double bond isomer. We were able to desulfurize the dibenzothiophene ester **1a** to the pure Δ^3 isomer **8a** using Raney nickel in acetone (Scheme I); basic hydrolysis gave the Δ^3 cis acid **8b**. Hydrogenation of **8b** gave the same results obtained from hydrogenation of the Δ^3,Δ^4 mixture **11b**.

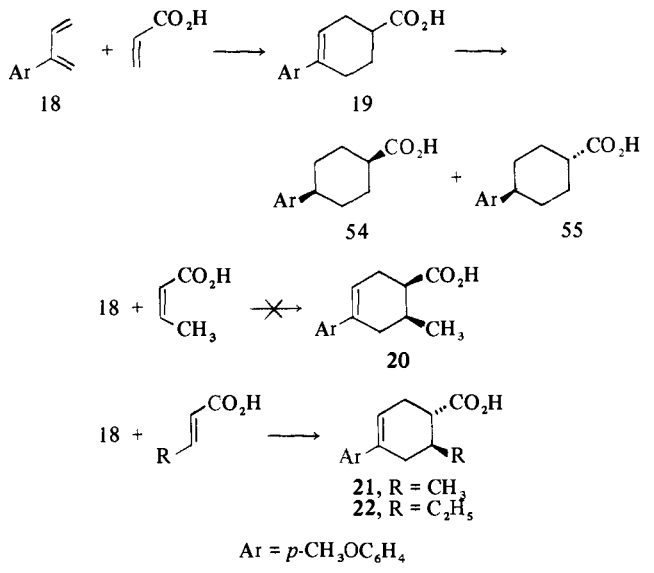
Table I. Biological Properties of the Four Diastereoisomeric 4-(*p*-Methoxyphenyl)-2-methylcyclohexanecarboxylic Acids

Compd	Total dose, μg	Uterine wt increment over control, mg[b]
A. Uterotropic Activities (Rats)[a]		
4b	250	+6.6
	2500	+4.9
5b	62.5	+6.0
	125	+10.4
	200	+19.6
	250	+20.5
	500	+25.8
	2000	+47.4
	2500	+47.3
	12500	+69.8
6b	200	−2.3
	2000	+10.1
7b	200	+12.2
	2000	+33.8
EE	0.1	+11.0
	0.2	+13.9
	1.0	+58.7
	2.0	+66.6

Compd	Dose, mg/kg per day	Serum cholesterol, % change
B. Hypocholesterolemic Activities[a]		
4b	100	−8
5b	10	−78[c]
	5	−49[c]
	2.5	−6
6b	50	−18
	10	−4
7b	10	−82[c]
	5	−52[c]
	2.5	−33[c]
EE	1.0	−66[c]
	0.2	−43[c]

[a] *Cf.* text for description of assays. [b] Vehicle control uteri weighed 20–25 mg. [c] $p < 0.01$ from controls.

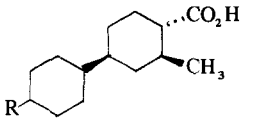
Scheme IV



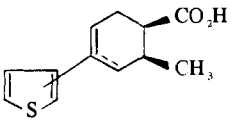
We considered synthesizing the Δ^4 isomer **20** *via* a Diels–Alder reaction (Scheme IV). As a model, acrylic acid and 2-(*p*-methoxyphenyl)-1,3-butadiene (**18**) were condensed according to Buchta and Satzinger.[3] Their yield of **19** (51%) was duplicated. Condensation of **18** and isocrotonic acid under the same conditions, however, produced none of the expected cis Δ^4 acid **20**. Condensation of **18** and crotonic acid afforded a low yield (3%) of the trans Δ^4 acid **21**. A similarly low yield of the 2-ethyl analog **22** was obtained from *trans*-2-pentenoic acid and **18**. As an incidental point, catalytic hydrogenation of **21** yielded **5b** and **6b** in the same proportion obtained from like treatment of **15b**.

A number of relatives of the lead compounds were prepared (Tables II and III). Compounds bearing no substituent at C-2 (**19** and **37**) were prepared from the appropriate Grignard reagent and 4-ketocyclohexanecarboxylic acid. Compound **19** prepared by this route was identical with **19** prepared by the Diels–Alder reaction (Scheme IV). Catalytic reduction of **19** and **37** gave mixtures of cis and trans isomers (*cf.* Experimental Section). Compounds with a C-2 ethyl substituent were prepared starting from 2-ethyl-4-ketocyclohexanecarboxylic acid and an aryl Grignard reagent. Most of the 2-methyl-4-substituted aryl derivatives listed were prepared according to the sequence **10** → **11** → **4** → **5** (Scheme II) starting with **10** and the appropriate aryl Grignard reagent. Since this route provided easy access to the biologically active 1α,2β,4β isomers, most of the compounds prepared for comparative screening were of this configuration rather than the equally active, but less readily accessible, 1β,2β,4α configuration. Stereochemical assignments were made by analogy with those shown in Scheme II. In general, the 1β,2β,4β isomers showed shorter glpc retention times (as esters) and nmr resonances for the C-2 methyl doublet at lower field than the corresponding 1α,2β,4β epimers. A number of additional derivatives of the parent structures listed in Tables II and III were prepared by standard procedures (*cf.* Experimental Section). Also prepared by standard procedures were the saturated compounds **78–80** and the thiophene derivatives **81** and **82**.

Biology. All biological data are from oral dosing. Acids were administered as aqueous solutions of sodium salts, while other compounds were administered as aqueous solutions or as suspensions in aqueous 0.5% CMC solution. All compounds were initially screened for estrogenic and hypocholesterolemic activities. Results from these initial assays are given in Tables II and III. Compounds not listed in the Tables (**17b**, **78**, **79**, **80**, **81**, and **82**) showed no estrogenic (2500 μg) or hypocholesterolemic (100 mg/kg) activities. More detailed discussion will be presented for some of the compounds chosen for additional biological evaluation.



78, R = H
79, R = H, OH
80, R = O



81, 2-thienyl
82, 3-thienyl

Antifertility Activities. Selected compounds were tested for antifertility activity in mice dosed daily for 9 days over an 11-day period. The dosing regimen involved both pre- and postcoital administrations. Compounds **4b**, **5b**, **6b**, **7b**, **23**, **26**, **27**, **28**, **30**, **32**, **36**, **38**, **40a**, **52**, **53**, **56**, **57**, **58**, **61**, **63**, and **65** were inactive in this assay at 50 mg/kg per day, while compound **11b** showed activity at this dose level. Doses of 200 mg/kg per day of **5b** were required to prevent pregnancy in this assay. Compounds **5b** and **11b** prevented pregnancy in rats dosed on days 1–5 postcoitum at 10 mg/kg per day but not at lower doses. The compounds described were considerably less potent than the benzothiophene **1b**[1] and thus were of minimal interest as antifertility agents.

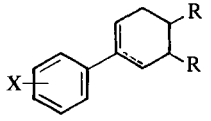
Estrogenic Activities. Estrogenicity was determined by uterine weight responses in immature 21-day-old mice or rats (five animals per group). The animals were dosed daily for 3 days and were autopsied on day 4; doses listed represent total amount of drug administered over the 3-day period. All data are based on averages from at least five animals per group at each dose level and were statistically significant at confidence limits of 95% or greater when the increase in uterine weight from treated animals was 10 mg or more over vehicle controls. Table I lists comparative dose-response data for the diastereoisomers **4b**, **5b**, **6b**, and **7b** while Tables II and III list data for other compounds in the series. Although all compounds listed in Tables II and III were tested for estrogenicity at two dose levels (250 and 2500 μg), only the 2500-μg data are given to indicate the magnitude of the response. More detailed discussion will be devoted to **5b**.

Compound **5b** is considerably less estrogenic in mice than in rats. In the immature mouse uterine weight assay, a true dose-response curve could not be achieved; doses of 5–10 mg of **5b** had equivalent estrogenic potency with 0.1 μg of ethinyl estradiol (EE). Unlike **5b**, a good dose response in mice was seen with **11b**. Doses of 500 and 2000 μg of **11b** were equivalent to doses of 0.1 and 0.3 μg, respectively, of EE.

The weak estrogenic potency of **5b** in mice was confirmed by the observation that 2.5 mg/day of **5b** for a period of 1 week was unable to maintain vaginal cornification without leucocytic infiltration in mice spayed during estrus. When other mice were treated 1 week after spaying, 5 mg/day of **5b** elicited cornification by the fifth day but was unable to prevent leucocytic infiltration. Full cornification without leucocytic infiltration was accomplished with 1 μg of EE in both of these models.

In the rat, a good dose-response curve in the uterotropic assay was achieved with **5b** (Table I). However, the compound is still very weak compared with EE. A relative potency estimate is difficult to obtain because of nonparallelism of the dose-response curves. At the 2500-μg

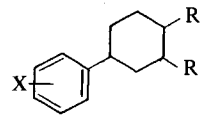
Table II. 2-Substituted 4-Aryl-Δ^3- (Δ^4-) cyclohexenecarboxylic Acids and Derivatives[a]



Compd	Configuration, 1,2-	X	R	R	Mp or bp (mm), °C	Recrystn solvent[b]	Formula	Analyses[c]	Uterotropic activity, rats[d]	Serum cholesterol assay, rats[e]	
										Dose, mg/kg	% change
8b[f]	Cis	*p*-CH_3O	CH_3	CO_2H	174–175.5	A	$C_{15}H_{18}O_3$	C, H	+37.7	2.5	−27
11b	Cis	*p*-CH_3O	CH_3	CO_2H	143–149	A	$C_{15}H_{18}O_3$	C, H	+41.4	2.5	−50
15a	Trans	*p*-CH_3O	CH_3	CO_2Et	137 (0.1)		$C_{17}H_{22}O_3$	C, H	+39.4	5	−23
15b	Trans	*p*-CH_3O	CH_3	CO_2H	163–166.5	A	$C_{15}H_{18}O_3$	C, H	+42.3	10	−37
19		*p*-CH_3O	H	CO_2H	203–204[g]	B	$C_{14}H_{16}O_3$		+7.4	100	−42
21[h]	Trans	*p*-CH_3O	CH_3	CO_2H	172–173.5	A	$C_{15}H_{18}O_3$	C, H			
23	Trans	*p*-CH_3O	C_2H_5	CO_2H	99–103	A	$C_{16}H_{20}O_3$	C, H	+32.3	25	−57
24	Cis	H	CH_3	CO_2H	135–140	A	$C_{14}H_{16}O_2$	C, H	+18.4	5	−26
25	Cis	*p*-HO	CH_3	CO_2H	155–161	A	$C_{14}H_{16}O_3$	C, H	+40.6	2.5	−30
26	Cis	*p*-CH_3O	CH_3	CH_2OH	161–164 (0.15)		$C_{15}H_{20}O_2$	C, H	+43.0	2.5	−46
27	Cis	*p*-HO	CH_3	CH_2OH	139–142	A, C	$C_{14}H_{18}O_2$	C, H	+40.0	10	−78
28	Cis	*p*-CH_3O	CH_3	$CONH_2$	172–174	A	$C_{15}H_{19}NO_2$	C, H, N	+23.1	25	−25
29	Cis	*p*-CH_3O	CH_3	CN	53–55	A, B	$C_{15}H_{17}NO$	C, H, N	0	100	0
30	Mixture	*p*-CH_3O	CH_3	CN_4H	195–197	A	$C_{15}H_{18}N_4O$	C, H, N	0	100	−13
31	Cis	*p*-CH_3O	CH_3	CH_2NH_2	192–197	*i*	$C_{15}H_{21}NO$·HCl	C, H, Cl; N[j]	+46.4	5	−44
32	Cis	*m*-CH_3O	CH_3	CO_2H	108–115	A	$C_{15}H_{18}O_3$	C, H	0	100	−15
33	Cis	*o*-CH_3O	CH_3	CO_2H	178–192	A	$C_{15}H_{18}O_3$	C, H	+1.2	100	0
34	Cis	*p*-Cl	CH_3	CO_2H	184–189	B	$C_{14}H_{15}ClO_2$	C, H, Cl	0	100	+66
35	Trans	*p*-Cl	CH_3	CO_2H	148–150	B	$C_{14}H_{15}ClO_2$	C, H, Cl	0	100	+63
36	Cis[k]	*m*-Cl	CH_3	CO_2H	85–100	A	$C_{14}H_{15}ClO_2$	C, H, Cl	+3.9	100	−9
37		*p*-Cl	H	CO_2H	222.5–224	B	$C_{13}H_{13}ClO_2$	C, H	+1.9	100	+28
38	Cis	*m*-CH_3	CH_3	CO_2H	115–128	B	$C_{15}H_{18}O_2$	C, H	+11.0	100	−11
39	Cis	*m*-CF_3	CH_3	CO_2H	89–92	A	$C_{15}H_{15}F_3O_2$	C, H	+4.5	100	−44
40a	Cis	*p*-CH_3S	CH_3	CO_2Et	41–45	B	$C_{17}H_{22}O_2S$	C, H, S	+10	100	+12
40b	Cis	*p*-CH_3S	CH_3	CO_2H	180–182	B	$C_{15}H_{18}O_2S$	C, H, S	+2.1	100	+8
41	Cis	*p*-$CH_3S(O)$	CH_3	CO_2H	204–209	A	$C_{15}H_{18}O_3S$	H, S; C[l]	0	10	+16
42	Cis	*p*-$CH_3S(O_2)$	CH_3	CO_2H	222–225	B	$C_{15}H_{18}O_4S$	C, H, S	0	100	−15
43	Cis	*p*-F	CH_3	CO_2H	140–146	A	$C_{14}H_{15}FO_2$	C, H	+7.9	100	+5
44	Cis	*p*-F_3CO	CH_3	CO_2H	156–159	A	$C_{15}H_{15}F_3O_3$	C, H	0	100	+7
45a	Cis	*p*-$EtO_2CC(Me_2)O$	CH_3	CO_2Et	Oil		$C_{22}H_{30}O_5$	C, H	0	100	−26
45b	Cis	*p*-$HO_2CC(Me_2)O$	CH_3	CO_2H	170–172	A	$C_{18}H_{22}O_5$	C, H	0	100	−3

[a] All compounds were mixtures of Δ^3 and Δ^4 isomers unless otherwise noted. [b] A, MeCN; B, EtOH; C, H_2O. [c] Analytical results obtained for the elements listed were within ±0.4% of the theoretical values unless otherwise indicated. [d] Increment (mg) over control uteri at 2500 μg total dose of test compound; *cf.* text for description of assay and Table I for control values. [e] *Cf.* text for description of assay. [f] Δ^3 isomer. [g] Lit. mp 202–204° (ref 3). [h] Δ^4 isomer. [i] Purified by distillation of the free amine [bp 138–139° (0.05 mm)] prior to preparation of the hydrochloride salt; final purification of the salt was by titration under Me_2CO. [j] N: calcd, 5.23; found, 6.06. [k] Nmr and glpc indicated presence of about 20% trans (Δ^3,Δ^4). [l] C: calcd, 64.73; found, 64.27.

Table III. 2-Substituted 4-Arylcyclohexanecarboxylic Acids and Derivatives



C mpd	Configuration,[a] (±)-1,2,4	X	R	R	Mp or bp (mm), °C	Recrystn solvent[b]	Formula	Analyses[c]	Uterotropic activity, rats[d]	Serum cholesterol assay, rats[e] Dose, mg/kg	% change
4a	β,β,β	*p*-CH_3O	CH_3	CO_2Et	132–143 (0.05)		$C_{17}H_{24}O_3$	C, H			
4b	β,β,β	*p*-CH_3O	CH_3	CO_2H	98–99.5	A	$C_{15}H_{20}O_3$	C, H	+4.9	100	−8
5a	α,β,β	*p*-CH_3O	CH_3	CO_2Et	137–142 (0.05)		$C_{17}H_{24}O_3$	C, H	+39.5	5	−30
5b	α,β,β	*p*-CH_3O	CH_3	CO_2H	138–139.5	B	$C_{15}H_{20}O_3$	C, H	+47.3	5	−49
6b	α,β,α	*p*-CH_3O	CH_3	CO_2H	92–93.5	B	$C_{15}H_{20}O_3$	C, H	+10.1[f]	50	−18
7b	β,β,α	*p*-CH_3O	CH_3	CO_2H	154–155.5	B	$C_{15}H_{20}O_3$	C, H	+33.8[f]	2.5	−33
46	β,β,β[g]	H	CH_3	CO_2H	100–106	A	$C_{14}H_{18}O_2$	C, H	+0.7	100	−30
47	α,β,β	H	CH_3	CO_2H	138–139[h]	A	$C_{14}H_{18}O_2$	C, H	+28.1	10	−35
48	β,β,β	*p*-HO	CH_3	CO_2H	164–165	A	$C_{14}H_{18}O_3$	C, H	0		
49	α,β,β	*p*-HO	CH_3	CO_2H	181–183	D, E	$C_{14}H_{18}O_3$	C, H	+36.3	5[i]	−45
50	β,β,β	*p*-CH_3O	CH_3	CH_2OH	79–81	A	$C_{15}H_{22}O_2$	C, H	−2.0	100	−12
51	β,β,β	*p*-HO	CH_3	CH_2OH	134–136.5	A	$C_{14}H_{20}O_2$	C, H	−0.5	100	−7
52	α,β,β	*p*-CH_3O	CH_3	CH_2OH	140–145 (0.1)		$C_{15}H_{22}O_2$	C, H	+59.6	5	−36
53	α,β,β	*p*-HO	CH_3	CH_2OH	127–129	B, C	$C_{14}H_{20}O_2$	C, H	+39.6	10	−66
54	Cis	*p*-CH_3O	H	CO_2H	105–108	B	$C_{14}H_{18}O_3$	C, H	+4.6	100	−14
55	Trans	*p*-CH_3O	H	CO_2H	219.5–222[j]	B	$C_{14}H_{18}O_3$	C, H	+3.9	50	−30
56	Trans	*p*-Cl	H	CO_2H	252–254	B	$C_{13}H_{15}ClO_2$	C, H	−5.6	100	−8
57	α,β,β	*p*-CH_3O	CH_3	$CONH_2$	185–186	A	$C_{15}H_{21}NO_2$	C, H, N	+5.1	100	−10
58	α,β,β	*p*-CH_3O	CH_3	CN	55–57	F	$C_{15}H_{19}NO$	C, H, N	+3.2	100	−2
59	α,β,β	*p*-CH_3O	CH_3	CN_4H	201–202.5	A	$C_{15}H_{20}N_4O$	C, H; N[k]	+1.8	100	−23
60	α,β,β	*p*-CH_3O	CH_3	CH_2NH_2 HCl	183–185	F	$C_{15}H_{23}NO \cdot HCl$	C, H, N	+37.8	5	−31
61	α,β,α[l]	*p*-CH_3O	C_2H_5	CO_2H	93–96	A	$C_{16}H_{22}O_3$	C, H[m]	+11.6	50	−37
62	*n*	*p*-CH_3O	C_2H_5	CO_2H	Oil		$C_{16}H_{22}O_3$	C, H	+48.0	5	−13
63	β,β,β	*m*-CH_3O	CH_3	CO_2H	132–132.5	A	$C_{15}H_{20}O_3$	C, H[m]	+2.8	100	−21
64	α,β,β	*m*-CH_3O	CH_3	CO_2H	113–114	A	$C_{15}H_{20}O_3$	C, H[m]	+7.4	50	−7
65	β,β,β	*o*-CH_3O	CH_3	CO_2H	101–103.5	A	$C_{15}H_{20}O_3$	C, H	−5.1	100	−13
66	α,β,β	*o*-CH_3O	CH_3	CO_2H	130.5–133	A	$C_{15}H_{20}O_3$	C, H	+5.3	100	−9
67	β,β,β	*p*-Cl	CH_3	CO_2H	101.5–103	A	$C_{14}H_{17}ClO_2$	C, H, Cl	*o*	100	+17
68	α,β,β	*p*-Cl	CH_3	CO_2H	160–162	A	$C_{14}H_{17}ClO_2$	C, H, Cl	*o*	200	+34
69	β,β,β[g]	*m*-Cl	CH_3	CO_2H	77–87	A	$C_{14}H_{17}ClO_2$	C, H	+2.5	72	−16
70	α,β,β	*m*-Cl	CH_3	CO_2H	140–141.5	G	$C_{14}H_{17}ClO_2$	C, H, Cl	−1.6	100	−23
71	β,β,β	*m*-CH_3	CH_3	CO_2H	88–90.5	A	$C_{15}H_{20}O_2$	C, H	+16.5	100	+4
72	α,β,β	*m*-CH_3	CH_3	CO_2H	123–124.5	A	$C_{15}H_{20}O_2$	C, H	+2.1	100	+2
73	β,β,β	*m*-CF_3	CH_3	CO_2H	112–113.5	A	$C_{15}H_{17}F_3O_2$	C, H	−1.4	100	−26
74	α,β,β	*m*-CF_3	CH_3	CO_2H	158–160	A	$C_{15}H_{17}F_3O_2$	*p*	−0.9	100[i]	49
75	β,β,β[g]	*p*-F	CH_3	CO_2H	106–112	A	$C_{14}H_{17}FO_2$	*q*	0	90	−2
76	α,β,β	*p*-F	CH_3	CO_2H	144–146.5	A	$C_{14}H_{17}FO_2$	C, H	0	100	−7
77	α,β,β	*p*-F_3CO	CH_3	CO_2H	145–147	A	$C_{15}H_{17}F_3O_3$	C, H	0	70	−15

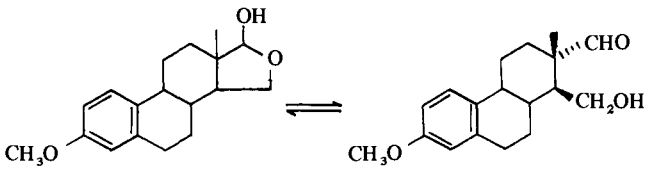
[a] *Cf.* text for explanation of stereochemical designations. [b] A, MeCN; B, EtOH; C, H_2O; D, EtOAc; E, Skellysolve B; F, MeOH; G, AcOH. [c–e] *Cf.* corresponding footnotes, Table II. [f] Value obtained from 2000 μg total dose. [g] Nmr and glpc indicated the presence of 10–15% of the 1α epimer. [h] Lit. mp 140–141° [C. Chuang, J. Chu, and Y. Kao, *Chem. Ber.*, **73b**, 1347 (1940)] and for an isomer of unspecified stereochemistry prepared from an alternative synthesis. [i] Lowest dose tested. [j] Lit. mp 220.5–222° (no isomer designation; *cf.* ref 24). [k] N: calcd, 20.57; found, 21.49. [l] Contains ≤10% C_4 epimer. [m] Analyses on sodium salt. [n] Approximately 1:1 mixture of 1α,2β,4β and 1α,2β,4α epimers. [o] Not tested in rats; inactive in mice at 1000 μg total dose. [p] C: calcd, 62.93; found, 64.01. [q] C: calcd, 71.16; found, 71.87.

dose, **5b** has uterotropic activity nearly equivalent with 1 μg of EE. Thus, at this dose level it has approximately 0.04% the estrogenicity of EE.

Hypocholesterolemic Activities. Hypocholesterolemic assays entailed daily dosing for **4** days of normocholesterolemic male rats (five rats per group). Compounds that lowered serum cholesterol by at least 20% were considered active. In general, values listed for active compounds in Tables II and III represent minimal effective doses and are based on data statistically significant at the 95-99% confidence levels.

Active compounds in this series afford a favorable separation between uterotropic and cholesterol lowering properties when compared with EE. For example, compound **11b** gives comparable cholesterol lowering activity at 5 mg/kg (−64%) with EE at 1 mg/kg (−66%), while possessing less than 1/2500 the uterotropic activity of EE (*cf.* 2500 μg of **11b** *vs.* 1 μg of EE). Using the rationale of Goldkamp, *et al.*,[4] to compare hypocholesterolemic-estrogenic ratios, this represents a 500-fold gain in efficacy relative to EE.

It is noteworthy that the 16-oxa steroid **83**, which has



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structural resemblances with **5b**, has been reported to have a high separation between hypocholesterolemic and uterotropic activities.[5]

Goldkamp, *et al.*,[4] reported a favorable lipodiatic-estrogenic ratio for 3-desoxy-4-methyl-substituted estrone derivatives. Since the meta position in our series of compounds corresponds to the 4 position of steroidal estrogens (*vide infra*), we made the *m*-methyl derivatives **38** and **72** as well as a number of additional substituent changes with the aim of achieving an even greater separation between cholesterol-lowering and uterotropic properties than seen with **11b** and **5b**. The *m*-methyl derivatives were devoid of both activities at the dose levels tested. A more favorable separation was seen, however, with the *m*-CF_3 compounds **39** and **74** which showed no uterotropic activity at the 2500-μg dose.

Interestingly, the *p*-chloro derivatives **34**, **35**, **67**, and **68** significantly elevated serum cholesterol levels. This activity was also seen at 50 mg/kg with **34** (+22%) and **35** (+24%).

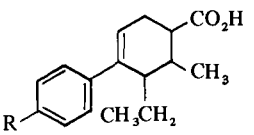
Gonadotropin Inhibitory Activities. Weights of testes, ventral prostate, and seminal vesicles were routinely determined on male rats from the hypocholesterolemic assays as an indication of gonadotropin inhibitory activity. In general, active compounds showed nonsignificant lowering of accessory sex organs at the minimal effective hypocholesterolemic dose and marginal to significant depression at twice the minimal dose. Compound **5b** appeared to be more potent than others with respect to its effect on accessory sex glands in rats utilized in this assay.

The gonadotropin inhibitory activity of **5b** was examined in 28-day-old and in mature male rats at daily doses of 2.5-100 mg/kg for periods of **4**, 7, and **14** days. Absolute decreases in organ weights were recorded for the testes, seminal vesicles, and ventral prostate. Effects were directly proportional to the dose and the duration of treatment. The accessory sex glands were reduced to a considerably greater extent than the testes. Reductions of accessory sex glands were apparent at 2.5 mg/kg whereas reductions in testicular weights occurred at 10 mg/kg and above. It is postulated that the effect on the ventral prostate and the seminal vesicles is indirect and that it reflects the reduction in the secretion of testicular androgens. The inhibition of androgen secretion is an indirect measure of gonadotropin inhibition. This is further reflected in human chorionic gonadotropin (HCG) treated normal male rats in which **5b** failed to counteract the known stimulatory effect of HCG on the secretion of androgens and the consequent increase in seminal vesicles and ventral prostate weights.

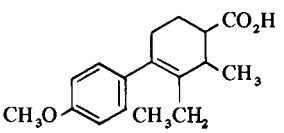
In terms of its estrogenicity, **5b** depressed the weight of the testes and the accessory glands with greater efficiency than did EE. In the young adult rat (175 g) treated for 4 days, 20 mg/kg of **5b** produced suppression somewhat greater than 1 mg/kg of EE. In immature 28-day-old rats the effect of 1 mg/kg of EE could be duplicated by 20-30 mg/kg of **5b**. Thus, in the rat one sees a 100-200-fold gain in inhibitory efficiency compared to EE.

Compound **5b** was tested for effects on prostatic fluid flow induced with pilocarpine in cystopreputiostomized mongrel male dogs. In these animals prostatic fluid uncontaminated by urine could easily be collected. Doses of 50 mg/day subcutaneously for 1 week reduced the prostatic flow by 25-50%. Doses of 100 mg/day for 8-10 days simply exaggerated this effect. EE at 1 mg/day also produced a reduction in fluid emission in this test.

Structure-Activity Correlations. At the beginning of our work, we recognized structural similarities between our compounds and the known compounds **84-87**.[6,7] These



84, R = H
85, R = HO
86, R = CH_3O



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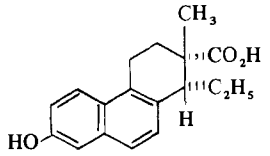
latter compounds are generally viewed as seco steroids with respect to rings B and D. In both rats and mice they exhibit uterotropic activity comparable to or greater than EE. The nearly complete loss of uterotropic activity seen with removal of the ethyl group (**8b**, **11b**) was not unexpected. However, the relative potency retained in respect to the cholesterol lowering and gonadotropin inhibitory components of estrogenic response was surprising.

Because of structural similarities with the known potent estrogenic compounds **84-87**, we adopted as an initial hypothesis that active compounds in our series were simulating steroids in biological activity, with carbons 1, 2, and 4 of the cyclohexane ring corresponding to carbons 13, **14**, and 9, respectively, of the steroidal skeleton. Evidence to support this follows.

(i) In the assays mentioned, essentially the same biological profile was seen with the cis compounds **8b** and **11b** and the isomers **5b** and **7b**. Stereochemical assignments are incomplete for compounds **84-87**. The stereochemistry of doisynolic acids, however, is well known.[8] Carbons 1, 2, and 4 of our compounds correspond to carbons 2, 1, and 12, respectively, of the doisynolic acids, and in this regard the biological activity of compounds in this series correlates with relative activities of the doisynolic acids. For example, appropriate enantiomers of active compounds **8b** and **5b** correspond in stereochemistry to the active *l-cis*-bisdehydrodoisynolic (**88**) and *d-trans*-doisynolic (**89**) acids, respectively. Similarly, the enantiomer of **7b** as drawn in Scheme II (*i.e.*, the 1α,2α,4β isomer) corresponds in configuration to the

active enantiomer **90** of *dl-cis*-doisynolic acid A.[8b] Additionally, the inactive isomer **4b** correlates with the inactive *d-cis*-lumidoisynolic acid (**91**).



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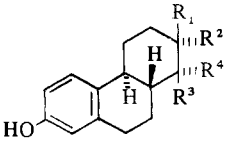

89, $R^1 = CH_3$; $R^2 = CO_2H$; $R^3 = C_2H_5$; $R^4 = H$
90, $R^1 = CH_3$; $R^2 = CO_2H$; $R^3 = H$; $R^4 = C_2H_5$
91, $R^1 = CO_2H$; $R^2 = CH_3$; $R^3 = C_2H_5$; $R^4 = H$

(ii) The para position of the aryl ring in our series and in compounds **84–87** is assumed to correspond to the C-3 position in steroidal estrogens. In a study employing use of the metabolic inhibitor SKF-525A, Yard and coworkers[6] concluded that biological activity of **84** was dependent upon *in vivo* para hydroxylation of **84** to **85**. In a similar study, we showed that the estrogenic response seen with the unsubstituted compound **47** is blocked by administration of SKF-525A with **47**.

(iii) In addition to stereochemical correlations cited above, substituent changes *vs.* biological activity generally follow the pattern seen with doisynolic acids and with estrogenic steroids.[9] For example, in the active configurations, carboxylic derivatives such as CH_2OH (**26, 27, 52, 53**) and CH_2NH_2 (**31, 60**) retain activity. Similarly, phenolic derivatives (**25, 27, 49, 53**) are generally as active as their parent methyl ethers, and unsubstituted aryl derivatives (**24, 47**) are active. Blocking the para position with fluorine (**43, 76**) or other groups abolishes activity. Loss of activity results from reduction of the aromatic ring, with or without a para oxygen function, to a cyclohexane derivative (**78, 79, 80**).

(iv) Compound **5b** was inactive in lowering serum cholesterol in immature (21-day-old) rats. Based on the reports[10,11] that estrogens are not effective in lowering serum cholesterol in immature rats, or in mature hypophysectomized rats, it may be presumed that the hypocholesterolemic effect of **5b** is due to its structural similarities with estrogens and that this effect is mediated *via* the pituitary.

Comment. Structure-activity relationships in this series and various pharmacological studies mentioned have led to our hypothesis that the biological profile of these compounds results from their interactions with steroidal receptors, with carbons 1, 2, and 4 of the cyclohexane moiety corresponding to carbons 13, 14, and 9 of the steroidal skeleton. Being "abbreviated" steroidal structures, they may be eliciting a narrowed and more selective type biological response by interacting preferentially with steroidal receptors which are less stringent in agonist structural requirements than other receptors. For example, compound **5b** may be interacting preferentially with estrogenic receptors in the pituitary relative to estrogenic receptors in the uterus and vagina. A number of workers have demonstrated the existence of estrogenic receptors in the uterus and vagina[12,13] and more recently in the pituitary[14,15] and hypothalamus.[16,17] Recently, Notides[14] has described similarities and differences between uterine and pituitary estrogenic receptors.

The profile of biological activity shown by **5b** suggests its possible utility for the treatment of androgen-dependent benign prostatic hypertrophy (BPH) and prostatic cancer. It has been estimated that 65% of American men over the age of 60 years suffer with BPH and partial success has been reported in using estrogens for this condition.[18] Estrogens appear to be the most useful type of drug therapy for prostatic cancer. Clinical use of estrogens in these areas is controversial, however, because of an increased risk of thromboembolic complications.[19,20] A compound such as **5b** which appears to have a much greater dissociation between uterotropic and gonadotropin inhibitory activities than presently used potent estrogens hopefully would show less liability toward thromboembolic and cardiovascular complications.

Experimental Section

Melting points (capillary) and boiling points are uncorrected. All compounds had consistent ir and nmr spectra for assigned structures. Routine nmr analyses were obtained using a Varian A-60 spectrometer. Nmr data for compounds **4b, 5b, 6b, 7b, 13b,** and **21** were obtained on a Varian HA-100 spectrometer. Chemical shifts for multiplets represent the center of the pattern. Raney nickel used was from W. R. Grace & Co. Where elemental analyses are indicated by symbols of the elements, analytical results were within ±0.4% of the theoretical values.

(±)-4β-(*p*-Methoxyphenyl)-2β-methylcyclohexane-β-carboxylic Acid (4b) from 1a. A solution of ethyl *cis*-7-methoxy-4-methyl-1,2,3,4-tetrahydrodibenzothiophene-3-carboxylate[1] (**1a**, 9.25 g, 0.031 mol) in THF (100 ml) and absolute EtOH (100 ml) containing Raney Ni (12 tbs, washed three times with EtOH) was stirred at reflux for 17 hr. Separation of the Ni and evaporation of the solvent left a mixture of 1a and **4a**; distillation gave **4a**, bp 132–143° (0.05 mm) (5.50 g, 66%). In an earlier experiment, quantitative desulfurization of **1a** was obtained by refluxing 1a in absolute EtOH using Raney Ni which had been washed only once; the product, however, was a mixture of **4a** (80%) and its C-1 epimer 5a (20%).

A solution of **4a** (5.14 g, 0.019 mol) in AcOH (70 ml) and concentrated aqueous HCl (70 ml) was refluxed for 4 hr. The solution was cooled and diluted with H_2O and the precipitate collected by filtration; recrystallization (MeCN) gave **4b** (2.30 g, 47%): mp 98–99.5°; nmr ($CDCl_3$) δ 1.11 (d, 3, C_2-CH_3).

(±)-4β-(*p*-Methoxyphenyl)-2β-methylcyclohexane-α-carboxylic Acid (5b) from 3. A solution of ethyl *trans*-7-methoxy-4-methyl-1,2,3,4-tetrahydrodibenzothiophene-3-carboxylate (3, 4.80 g, 0.016 mol) in THF (70 ml) and absolute EtOH (70 ml) containing Raney Ni (8 tsp, washed twice with absolute EtOH) was stirred under reflux for 18 hr and then worked up as above, yield 3.93 g (91%), bp 137–142° (0.05 mm), of esters 5a and 6a. A solution (3.90 g, 0.014 mol) in EtOH (27 ml) and H_2O (5.4 ml) containing KOH (1.04 g, 0.019 mol) was refluxed for 6 hr. The cooled solution was diluted with H_2O and extracted with Et_2O. Evaporation of the dried Et_2O solution gave 3.10 g. Two recrystallizations (MeCN, EtOH) gave 5b (1.80 g, 52%): mp 138–139.5°; nmr ($CDCl_3$) δ 0.91 (d, 3, C_2-CH_3). Fischer esterification of this acid gave pure ethyl ester 5a with boiling point identical with that of the mixture of 5a and **6a**.

cis-4-(*p*-Methoxyphenyl)-2-methyl-Δ³-cyclohexanecarboxylic Acid (8b). A solution of the ester 1a (13.2 g, 0.043 mol) in Me_2CO (900 ml) containing Raney Ni (12 tsp, washed twice with Me_2CO) was stirred at reflux for 3.5 hr. Work-up as above gave a completely desulfurized product which was a 4:1 mixture of 8a and 4a. (This result proved difficult to reproduce using different lots of Raney Ni or even the same lot which had been standing for several weeks after the experiment described. Subsequent experiments generally gave a much lower percentage of the unsaturated ester 8a relative to **4a** and/or unchanged 1a.) The crude mixture (0.043 mol) was hydrolyzed by refluxing in EtOH (150 ml) and H_2O (30 ml) containing KOH (3.29 g, 0.06 mol) for 17 hr. Work-up as described above gave product which was triturated under MeCN (40 ml) to yield 7.75 g of a mixture of acids 8b and 4b. Three recrystallizations (EtOH, MeCN twice) gave pure **8b** (4.04 g, 38%): mp 174–175.5°; nmr ($CDCl_3$ plus DMSO-d_6) δ 1.05 (d, 3, C_2-CH_3), 5.92 [d (showing long-range coupling), 1, C_3 vinylic proton]; glpc‡ retention time (methyl ester) 24.6 min.

cis-2-Methyl-4-ketocyclohexanecarboxylic Acid (10b). A solution of Hagemann's ester (9, 405.0 g, 2.22 mol) in absolute EtOH (810 ml) and 3 *N* aqueous HCl (90 ml) containing 5% Pd/C (14.4 g) was shaken under an initial hydrogen pressure of 50 psi for 2.5 hr;

‡Glass column, 6 ft × 4 mm i.d., packed with 5% butane-1,4-diol succinate polyester on 100–120 Gas-Chrom Q (Applied Science Laboratories, Inc.) at 200° isothermally, flow rate of 100 ml of He/min.

uptake corresponded to about 1 equiv of hydrogen. The catalyst was removed by filtration and the filtrate was concentrated at 40° (15 mm) to about 400 g. The product was dissolved in Et_2O and the solution was washed twice with water (2 × 300 ml) and then with brine. Drying (Na_2SO_4) and evaporation gave 360 g which distilled to yield 301.2 g (74%): bp 83–85° (0.35 mm); glpc[§] indicated 95% of the cis ester **10a** (retention time, 9.4 min) and 5% of the trans ester **13a** (retention time, 8.8 min). *Anal.* ($C_{10}H_{16}O_3$) C, H.

A solution of the product (301.2 g, 1.64 mol) in MeOH (860 ml) was diluted with a solution of KOH (133 g, 2.37 mol) in H_2O (860 ml) with ice cooling. The resultant solution was stirred at 25° for 2 hr. Most of the alcohol was removed at 38° (15 mm); the concentrated solution was extracted with CH_2Cl_2 (250 ml) and then was treated with aqueous 6 *N* HCl (360 ml) with ice cooling. The acid was extracted with CH_2Cl_2 (3 × 250 ml); combined CH_2Cl_2 extracts were washed with H_2O (70 ml) and then with brine solution. Drying (Na_2SO_4), evaporation, and distillation of residue gave 238.1 g (93%), bp 128–131° (0.05 mm), of the cis acid **10b** containing 5% of the trans **13b**: nmr ($CDCl_3$) δ 1.05 (d, 3, J = 6 Hz, C_2-CH_3); trace db seen at δ 1.09 for trans isomer). *Anal.* ($C_8H_{12}O_3$) C, H.

trans-2-Methyl-4-ketocyclohexanecarboxylic Acid (13). A solution in C_6H_6 (200 ml) of the *cis*-keto ester **10a** (29.73 g, 0.162 mol), ethylene glycol (10.05 g, 0.162 mol), and TosOH·H_2O (3.00 g, 0.0158 mol) was heated under reflux with a Dean-Stark trap for 15 hr. The cooled solution, after dilution with Et_2O, was washed in succession with aqueous $NaHCO_3$ (once), H_2O (six times), and brine solution. Removal of the Et_2O and distillation of the residue gave the *cis*-ethylene ketal **12** (23.08 g, 63%), bp 93–94° (0.15 mm). *Anal.* ($C_{12}H_{20}O_4$) C, H. Evidence for the cis structure for **12** was obtained when an aliquot was hydrolyzed back to **10a** having the same (95/5) cis/trans ratio as starting material.

A solution of **12** (99.0 g, 0.43 mol) in *t*-BuOH (1200 ml) containing KO-*t*-Bu (from 6.50 g, 0.17 g-atom of K) was heated at reflux for 20 hr. The solution was concentrated to about 150 ml and then was partitioned between ice-water and Et_2O. The organic layer was washed with H_2O (three times) and brine and then dried. Evaporation gave the trans isomer of **12** (92.5 g, 93%); the product was indistinguishable from the cis isomer **12** in several glpc systems tried. The product (92.0 g, 0.40 mol) was refluxed in AcOH (260 ml) and concentrated HCl (260 ml) for 19 hr. The solution was concentrated at 15 mm to a syrup which was dissolved in Et_2O. The solution was extracted with aqueous 3 *N* NaOH (1 × 125 ml, 2 × 50 ml); combined basic extracts were acidified (6 *N* HCl) with cooling and then extracted several times with Et_2O. Combined Et_2O extracts were washed with brine, dried, and evaporated to a residue; distillation gave 32.0 g [51%, bp 140–146° (0.15 mm)] of the trans acid containing 12% of the cis isomer **10b**. Recrystallization (125 ml of methylcyclohexane plus 35 ml of toluene) gave pure **13**: mp 91–94°; nmr ($CDCl_3$) δ 1.09 (d, 3, J = 5.8 Hz, C_2-CH_3). *Anal.* ($C_8H_{12}O_3$) C, H.

trans-2-Ethyl-4-ketocyclohexanecarboxylic Acid. This compound was prepared starting from 2-ethoxy-1,3-butadiene and ethyl *trans*-2-pentenoate according to Buchta and Satzinger,[3] whose yields were duplicated: bp 134–140° (0.15–0.25 mm); homogeneous by glpc; melting point of an aliquot recrystallized from methylcyclohexane–benzene was 106–109° (lit.[3] 110–112°). These authors make no assignment of stereochemistry. Our assignment as trans was made because nmr analysis of the starting ethyl 2-pentenoate (prepared by Fischer esterification of 2-pentenoic acid[21]) showed trans coupling and it was assumed that the usual stereochemical course of the Diels–Alder reaction was followed. This assignment was substantiated by results obtained from the reduction of **23** (*vide infra*).

cis-4-(*p*-Methoxyphenyl)-2-methyl-Δ³-(Δ⁴-)cyclohexenecarboxylic Acid (11b). A Grignard solution, prepared from *p*-bromoanisole (73.5 g, 0.39 mol) and Mg (9.54 g, 0.39 g-atom) in THF (390 ml), was added (over 5 min) to a solution of the keto acid **10b** (30.0 g, 0.19 mol) in THF (420 ml) with ice cooling. The mixture was stirred at reflux for 17 hr. Aqueous 3 *N* HCl (135 ml) was added slowly with cooling. Most of the THF was removed at 15 mm and the residue was partitioned between H_2O and Et_2O. The organic phase was washed with H_2O (twice), dried (Na_2SO_4), and evaporated to leave 67.0 g of a mixture of hydroxy acid and lactone. The product was refluxed for 2 hr in toluene (600 ml), containing *p*-TosOH·H_2O (3.5 g, 0.018 mol), with provision for azeotropic removal of H_2O. The cooled solution was diluted with Et_2O and then was washed in succession with H_2O (three times) and aqueous 5% Na_2CO_3 (five times). Combined base extracts were acidified with aqueous 3 *N* HCl. The precipitated acid was extracted into fresh Et_2O and the solution washed, dried, and evaporated to leave 31.0 g (65% based on **10b**) of **11b**: glpc[‡] (after CH_2N_2 treatment) indicated a mixture of 64% Δ⁴ (retention time, 23.4 min) and 36% Δ³ (retention time, 24.6 min); recrystallization (MeCN) gave needles, mp 143–149°, of unchanged isomeric composition. (The glpc assignments were made using **8b** as reference standard.) Similarly prepared from the appropriate Grignard reagents and **10b** were (% yield) **24** (69), **32** (87), **33** (70), **34** (73), **36** (75), **38** (30), **39** (67), **40b** (37), **43** (65), and **44** (60).

Similarly prepared from the appropriate Grignard reagents and 4-ketocyclohexanecarboxylic acid[22] were **19** (75%) and **37** (79%). Compound **19** by this route was identical with **19** prepared from **18** and acrylic acid.[3]

Similarly prepared from 2-bromothiophene and **10b** was **81**: purified through its methyl ester [bp 128–138° (0.3 mm)]; basic hydrolysis gave the acid **81** (28%), mp 148–152° (MeCN). *Anal.* ($C_{12}H_{14}O_2S$) C, H, S. Similarly prepared from 3-bromothiophene and **10b** was **82**: purified by chromatography (silica gel, $PhCH_3$) of methyl ester; basic hydrolysis gave **82** (4%), mp 159–163° (MeCN); low yield was owing to difficulty in preparation of Grignard reagent.[23] *Anal.* ($C_{12}H_{14}O_2S$) S; calcd: C, 64.85; H, 6.35; found: C, 64.01; H, 6.31.

(±)-4β-(*p*-Methoxyphenyl)-2β-methylcyclohexane-β-carboxylic Acid (4b) from 11b. A solution of **11b** (38.10 g) in EtOH (1200 ml, heating to 40° required for dissolution) containing 5% Pd/C (3.20 g) was shaken under an initial hydrogen pressure of 50 psi for 3 hr (1 equiv of H_2 consumed). Removal of the catalyst and evaporation gave a quantitative yield of a mixture of **4b** (96%) and **7b** (4%) (detection by glpc,[‡] after CH_2N_2 treatment; retention times, 12.9 and 20.1 min for **4b** and **7b**, respectively); recrystallization (MeCN) gave pure **4b**, identical with **4b** prepared from **1a**.

Similarly prepared in high yields from the appropriate ene acids were **46**, **63**, 65, and **71**. Similarly prepared using 5% Pd(S)/C (Engelhard Industries, Inc.) were the chloro compounds **67** and **69**; these reduction products contained dechlorinated products which were readily removed by recrystallization. Reduction of the thio compound **40a** using 5% Pd(S)/C failed.

(±)-4β-(Methoxyphenyl)-2β-methylcyclohexane-α-carboxylic Acid (5b) from 4b. A solution of **4c** (40.5 g, 0.155 mol; prepared by treatment of **4b** in Et_2O with CH_2N_2) in *t*-BuOH (400 ml) containing KO-*t*-Bu (from K, 2.12 g, 0.054 g-atom) was refluxed for 16 hr. The solution was concentrated to about 50 ml, then poured onto ice-water, and extracted into Et_2O. The Et_2O was washed with H_2O (three times), dried, and evaporated to 32.2 g (80%); glpc[‡] indicated >95% epimerization to the 1α isomer **5c** (retention time, 16.1 min). The product was hydrolyzed with KOH as described above to yield the acid **5b**, identical with **5b** prepared from **3**. Similarly prepared in comparable yields from the appropriate 1β,2β,4β isomers were **47**, **64**, **66**, **68**, **70**, **72**, **74**, **76**, and **77**.

trans-4-(*p*-Methoxyphenyl)-2-methyl-Δ³-(Δ⁴-)cyclohexenecarboxylic Acid (15b). The *trans*-keto acid **13b** (10.0 g, 0.06 mol) was condensed with 2 equiv of *p*-methoxyphenylmagnesium bromide as described above; yield of hydroxy acid **14** was 15.0 g (88%). An aliquot was recrystallized (MeCN) to give an analytical sample, mp 127–132°. *Anal.* ($C_{15}H_{20}O_4$) C, H. The product (12.8 g) dehydrated with TosOH·H_2O as described above to yield **15b** (10.8 g, 85%); recrystallization (MeCN) gave mp 163–166.5°; glpc[‡] (after CH_2N_2 treatment) indicated a mixture of 38% Δ³ (retention time, 20.9 min) and 62% Δ⁴ (retention time, 23.4 min). The ethyl ester **15a** was prepared by Fischer esterification; yield, 80%; bp 137° (0.1 mm). Similarly prepared from **13b** and *p*-chlorophenylmagnesium bromide was **35** (77% yield).

(±)-4α-(*p*-Methoxyphenyl)-2β-methyl-α-carboxylic Acid (6b). The acid **15b** (18 g) was dissolved in EtOH (600 ml) at 55°. Two grams of 5% Pd/C was added and the warm solution was shaken under 50 psi of H_2 for 24 hr. Usual work-up gave a mixture (18 g) of 55% **6b** and 45% **5b** (detection by glpc[‡] after CH_2N_2 treatment of an aliquot; retention times were 15.3 and 16.1 min for **6c** and **5c**, respectively). Recrystallization (MeCN, 45 ml) gave 5.9 g of **5b**. The mother liquor was concentrated to 20 ml, cooled to −10°, and seeded with **5b** to yield additional **5b**; this procedure was repeated twice. Evaporation of the remaining mother liquor left 11.3 g containing less than 10% **5b**; recrystallization from EtOH (12 ml, −10°) gave 2.00 g (11%) of **6b**, mp 87.5–91°, containing no detectable **5b** by glpc; additional recrystallization (EtOH) gave **6b** of constant mp 92–93.5°, nmr ($CDCl_3$) δ 1.11 (d, 3, C_2-CH_3).

(±)-4α-(*p*-Methoxyphenyl)-2β-methyl-β-carboxylic Acid (7b). A solution of **6c** (4.90 g, 0.0187 mol; prepared by treatment of pure

[§]Glass column, 6 ft × 4 mm i.d., packed with 3% OV-17 on 100–120 Gas-Chrom Q (Applied Science Laboratories, Inc.), programmed from 100° at 4°/min, flow rate of 100 ml of He/min.

6b with CH_2N_2) in *t*-BuOH (45 ml) containing KO-*t*-Bu (from K, 0.32 g, 0.008 g-atom) was refluxed for 13.5 hr. Work-up as described above gave a product (4.50 g) containing **6c** and **7c** in a ratio of approximately 40:60 (glpc‡; retention times, 6c, 15.3 min, and 7c, 20.1 min); also present were smaller amounts (10–15% total) of **4c** and **5c**.

The product (4.50 g) was hydrolyzed in aqueous ethanolic KOH (16-hr reflux) as described above for **5b**: crude yield, 3.41 g (81%); recrystallization (MeCN) gave **7b** of 95% purity; additional recrystallizations (MeCN, EtOH) gave pure **7b** of constant mp 154–155.5°; nmr ($CDCl_3$) δ 1.08 (d, 3, C_2-CH_3).

4-(*p*-Methoxyphenyl)-2-methylbenzoic Acid (17b). A. From 16. Ethyl 7-methoxy-4-methyldibenzothiophene-3-carboxylate[1] (**16**, 5.0 g) was treated with Raney Ni as described above for **3**. The crude product **17a** was hydrolyzed with base as described previously to yield the acid **17b** (2.9 g, 72%), mp 203–206°. *Anal.* ($C_{15}H_{14}O_3$) C, H.

B. From 5c. The ester **5c** (0.50 g, 0.002 mol) was refluxed in *p*-cymene (10 ml) containing 10% Pd/C for 24 hr. Work-up gave 0.42 g (86%) of **17c**; hydrolysis gave the acid **17b**, identical with **17b** from **16**. Identical results were obtained from aromatization of **15c** or a mixture of **4c**, **5c**, **6c**, and **7c**.

***trans*-4-(*p*-Methoxyphenyl)-2-methyl-Δ⁴-cyclohexenecarboxylic Acid (21).** Condensation of 2-(*p*-methoxyphenyl)-1,3-butadiene (**18**, 8.8 g) and crotonic acid (4.74 g) according to the procedure described for reaction of **18** with acrylic acid[3] gave **21** (0.43 g, 3%): mp 173–173.5° (MeCN); nmr ($CDCl_3$) δ 1.13 (d, 3, C_2-CH_3), 5.98 (m, 1, C_5 vinylic proton); glpc‡ retention time, 23.4 min.

Similarly prepared starting from **18** and *trans*-2-pentenoic acid[21] was the 2-ethyl relative (**22**; yield 4%) as an amorphous solid containing a trace of the Δ³ isomer; identified by glpc (CH_3 ester) using **23** (CH_3 ester) as standard.

***trans*-2-Ethyl-4-(*p*-methoxyphenyl)-Δ³-(Δ⁴-) cyclohexenecarboxylic Acid (23).** This compound was prepared from *p*-methoxyphenylmagnesium bromide and *trans*-2-ethyl-4-ketocyclohexanecarboxylic acid by the method described above for **11b**: yield of **23**, 81%; mp 99–103° (MeCN); glpc‡ (after CH_2N_2) indicated a mixture of 55% Δ³ (retention time, 28.8 min) and 45% Δ⁴ (retention time, 32.7 min), the latter isomer being identical in retention time with that from **22**.

Catalytic reduction of **23** gave two isomers (61 and C_4 epimer) in approximately the same ratios as seen in the analogous reduction of **15b**. Fractional recrystallization (MeCN) gave a 6% yield of the isomer of lower retention time, assigned as 4α,2β,1α (**61**), mp 93–96°, containing <10% of the 4β epimer. The 4β epimer was not obtained pure.

4-(*p*-Methoxyphenyl)cyclohexanecarboxylic Acid (54 and 55). Hydrogenation of ethyl 4-(*p*-methoxyphenyl)-Δ³-cyclohexenecarboxylate (19.0 g, 0.073 mol) in EtOH (300 ml) containing 5% Pd/C (1.5 g) at 50 psi gave a mixture of cis (74%) and trans (26%) isomers. The mixture was hydrolyzed to the acids from which fractional crystallization (EtOH) yielded the cis isomer **54** (mp 105–108° containing <10% trans) and the trans isomer **55**, mp 219.5–222° (lit.[24] 220.5–222°; no isomeric designation).

***trans*-4-(*p*-Chlorophenyl)cyclohexanecarboxylic Acid (56).** Catalytic hydrogenation of ethyl 4-(*p*-chlorophenyl)-Δ³-cyclohexenecarboxylate using 5% Pd(S)/C (Engelhard Industries, Inc.) in EtOH gave a mixture of approximately 3:1 cis:trans 4-(*p*-chlorophenyl)-cyclohexanecarboxylate containing about 25% ethyl 4-phenylcyclohexanecarboxylate in the same cis:trans ratio. The mixture was treated with KO-*t*-Bu in *t*-BuOH as described above to yield a mixture of ethyl and *tert*-butyl esters of predominately trans isomers. The pure trans acid **56** (yield, 32%) was obtained after basic hydrolysis and recrystallization of the product from EtOH.

***cis*-4-(*p*-Hydroxyphenyl)-2-methyl-Δ³-(Δ⁴-) cyclohexenecarboxylic Acid (25) and Related Phenols.** A solution of **11b** (2.50 g, 0.01 mol) in CH_2Cl_2 (100 ml) was treated at −50° with a solution of BBr_3 (5.08 g, 0.02 mol) in CH_2Cl_2 (25 ml). The mixture was stirred at 25° for 2 hr and then poured onto ice. The product extracted into $CHCl_3$; usual work-up gave a residue which was recrystallized (MeCN) to yield **25** (1.85 g, 79%), mp 155–161°, assigned as the cis structure because prolonged esterification of an aliquot with CH_2N_2 gave a small amount of the *cis*-**11c**.

Similarly prepared (% yield) were **27** (36) from **26**; **48** (20) from **4b**; **49** (46) from **5b**; and **53** (25) from **52**.

Alcohols and Amines. The following compounds (% yield) were prepared by reduction of the indicated starting materials using $LiAlH_4$ in Et_2O followed by the usual work-up: **26** (80) from **11c**; **31** (59) from **29**; **52** (76) from **5c**; **60** (90) from **58**.

Alcohol **50** (yield, 60%) was prepared from *cis*-3-hydroxymethyl-7-methoxy-4-methyl-1,2,3,4-tetrahydrodibenzothiophene[1] according to the procedure described above for desulfurization of **3**. Similarly prepared from *cis*-7-hydroxy-3-hydroxymethyl-4-methyl-1,2,3,4-tetrahydrodibenzothiophene was alcohol **51** (73%).

Tetrazoles 30 and 59. These compounds were prepared from acids **11b** and **5b**, respectively, following standard procedures:[25] amides, **28** (70% yield), **57** (62); nitriles, **29** (75), **58** (90); tetrazoles, **30** (13), **59** (35). The tetrazole **30** was judged from the nmr pattern of C_2-CH_3 doublets to be a mixture of cis/trans isomers. By contrast, the nmr of the tetrazole **59** showed a clean C_2-CH_3 doublet ($CDCl_3$, δ 0.94).

***cis*-2-Methyl-4-[*p*-(α-methyl-α-carboxy)ethoxyphenyl]-Δ³-(Δ⁴-) cyclohexenecarboxylic Acid (45b).** A solution of **25** ethyl ester (7.36 g, 0.028 mol) plus 1 equiv of NaH in DMF (100 ml) was alkylated with ethyl 2-bromoisobutyrate (6.08 g, 0.031 mol) at 80° for 16 hr. Work-up and chromatography of the product on acid-washed alumina (elution with 95:5 $PhCH_3$–Et_2O) gave the diester **45a** (3.08 g, 29%). Basic hydrolysis as described above gave the diacid **45b** (77%).

(±)-4β-(4-Ketocyclohexyl)-2β-methylcyclohexane-α-carboxylic Acid (80). A solution of **49** (4.03 g) in AcOH (100 ml) containing prereduced PtO_2 (0.8 g) was shaken under 50 psi of H_2 for 30 min; excess over 1 equiv of H_2 was still being consumed when the reduction was stopped. Usual work-up gave a mixture of **79** and **78**. The mixture was treated with CH_2N_2 and the product chromatographed on silica gel; fractions eluted with $PhCH_3$ contained 1.62 g of the methyl ester of the norhydroxy compound **78**; fractions eluted with 95:5 $PhCH_3$–Et_2O contained 2.19 g of the methyl ester of **79**. Basic hydrolysis of each fraction yielded the acids: **78**, mp 108–110° [*Anal.* ($C_{14}H_{24}O_2$) C, H]; **79**, mp 126–137° (mixture of epimeric hydroxyl compounds (*Anal.* Calcd for $C_{14}H_{24}O_3$: C, 69.97; H, 10.07. Found: C, 70.89; H, 10.54).

Oxidation of **79** with Jones reagent in Me_2CO at 20° gave **80** (yield, 67%), mp 163–165.5° (MeCN). *Anal.* ($C_{14}H_{22}O_3$) C, H.

Oxidation of ethyl-*cis*-2-methyl-4-(*p*-methylthiophenyl)-Δ³-(Δ⁴-) cyclohexenecarboxylic Acid (40a). A. Sulfoxide. The ester **40a** was treated with 1 equiv of 0.25 *M* $NaIO_4$ in aqueous MeOH[26] for 2 hr at 25°. Work-up yielded the sulfoxide ester which was hydrolyzed in aqueous ethanolic KOH to yield the acid **41** (yield, 42% from **40a**).

B. Sulfone. Treatment of **40a** with a 10 molar excess of the same reagent for 6 hr at 75° gave the sulfone ester which was recrystallized (aqueous EtOH) and then hydrolyzed to the acid **42** (yield 27% from **40a**).

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References

(1) R. R. Crenshaw, G. M. Luke T. A. Jenks, G. Bialy, and M. E. Bierwagen, *J. Med. Chem.*, 15, 1162 (1972) (paper 3).
(2) J. Sicher, M. Tichy, and F. Sipos, *Tetrahedron Lett.*, 1393 (1966).
(3) E. Buchta and G. Satzinger, *Chem. Ber.*, 92, 449 (1959).
(4) A. H. Goldkamp, W. M. Hoehn, R. A. Mikulec, E. F. Nutting, and D. L. Cook, *J. Med. Chem.*, 8, 409 (1965).
(5) J. S. Baran, *ibid.*, 10, 1039 (1967).
(6) A. S. Yard, L. P. Juhasz, and R. M. Grimes, *J. Pharmacol. Exp. Ther.*, 167, 105 (1969).
(7) A. H. Nathan and J. A. Hogg, *J. Amer. Chem. Soc.*, 78, 6163 (1956).
(8) (a) *Cf.* L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, pp 487–494; (b) J. Iriarte and P. Crabbé, *Chem. Commun.*, 1110 (1972).
(9) K. Miescher, *Recent Progr. Horm. Res.*, 3, 47 (1948).
(10) K. Uchida, M. Kadowaki, and T. Miyake, *Endocrinology*, 76, 766 (1965).
(11) M. Steinberg, S. Tolksdorf, and A. S. Gordon, *ibid.*, 81, 340 (1967).
(12) E. V. Jensen, T. Suzuki, T. Kawashima, W. E. Stumpf, P. W. Jungblut, and E. R. DeSombre, *Proc. Nat. Acad. Sci. U. S.*, 59, 632 (1968), and references cited therein.
(13) S. R. Henderson and D. S. Schalch, *Amer. J. Obstet. Gynecol.*, 112, 762 (1972).

(14) A. C. Notides, *Endocrinology*, 87, 987 (1970).
(15) J. Kato and C. A. Villee, *ibid.*, 80, 1133 (1967).
(16) A. J. Eisenfeld and J. Axelrod, *Biochem. Pharmacol.*, 16, 1781 (1967).
(17) N. T. Phuong, G. Sauer, and S. Rapoport, *Acta Biol. Med. Ger.*, 28, 379 (1972).
(18) H. J. Roberts, *J. Amer. Geriat. Soc.*, 14, 657 (1966).
(19) J. C. Bailar and D. P. Byar, *Cancer*, 26, 257 (1970).
(20) C. E. Blackard and G. T. Mellinger, *Postgrad. Med.*, 51, 140 (1972).
(21) K. Auwers, *Justus Liebigs Ann. Chem.*, 432, 46 (1923).
(22) W. H. Perkins, *J. Chem. Soc.*, 85, 424 (1904).
(23) S. O. Lawesson, *Ark. Kemi*, 11, 337 (1957).
(24) W. S. Johnson, C. D. Gutsche, and R. D. Offenhauer, *J. Amer. Chem. Soc.*, 68, 1648 (1946).
(25) R. R. Crenshaw, G. M. Luke, and G. Bialy, *J. Med. Chem.*, 15, 1179 (1972).
(26) N. J. Leonard and C. R. Johnson, *J. Org. Chem.*, 27, 282 (1962).

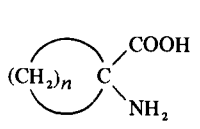
2-Aminoadamantane-2-carboxylic Acid, a Rigid, Achiral, Tricyclic α-Amino Acid with Transport Inhibitory Properties[1,†]

H. T. Nagasawa,* J. A. Elberling, and F. N. Shirota

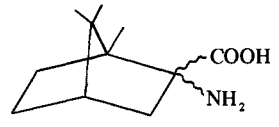
Laboratory for Cancer Research, Minneapolis Veterans Hospital, and the Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55417. *Received February 20, 1973*

2-Aminoadamantane-2-carboxylic acid, a geminally functionalized, achiral, tricycloaliphatic aminocarboxylic acid, was synthesized from adamantan-2-one *via* the Bucherer–Lieb hydantoin procedure. The adamantylamino acid inhibited the transport of L-leucine and L-methionine into Ehrlich ascites cells *in vitro* and, on a molar basis, was a better inhibitor in this system than cycloleucine. The theoretical structural requirements for transport by synthetic aliphatic amino acids proposed by Tager and Christensen appear to apply also to transport inhibition.

1-Aminocyclopropanecarboxylic acid (**1a**), a geminally functionalized cycloaliphatic aminocarboxylic acid, occurs naturally in the cowberry[2] and in perry pears and cider apples.[3] Ring homologs of **1a**, *viz.*, **1b-f**, as well as the bicyclic **2**, have been synthesized[4,5] and tested in a number of tumor systems,[6] but only 1-aminocyclopentanecarboxylic acid (cycloleucine, **1c**) showed sufficient antitumor activity to reach clinical trials.[7] A nitro-substituted **1c**, *viz.*, 1-amino-2-nitrocyclopentanecarboxylic acid, which showed unusual plant growth regulating properties, has been isolated from the fermentation culture filtrates of *Aspergillus wentii*.[8]



1a, *n* = 2
b, *n* = 3
c, *n* = 4
d, *n* = 5
e, *n* = 6
f, *n* = 7



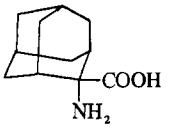
2

3

2-Amino-2-bornanecarboxylic acid (**2**)[5] represents a system where the geminal amino and carboxyl groups are anchored to a rigid, naturally occurring bicyclic terpene. A similar rigid system obtains in the isomeric 2-aminobicyclo-[2.2.1]heptane-2-carboxylic acids (**3**) synthesized by Christensen, *et al.*[9]

Cycloaliphatic α-amino acids such as represented by cycloleucine (**1c**) and the bicyclic **3** are neither metabolized *in vivo* nor incorporated into tissue proteins[10,11] but are actively transported by the transport system serving for the natural amino acids with apolar side chains.[9] They competitively inhibit the uptake of valine, leucine, and methionine into Ehrlich ascites tumor cells, this property constituting a possible mechanism of action for the antineoplastic activity of **1c**.[12]

The theoretical structural requirements for transport by these synthetic aliphatic amino acid analogs have been summarized recently by Tager and Christensen[13] to be (a) side chain bulk in all dimensions for minimal interaction with other transport systems, (b) maximally apolar side chains to promote high affinity for the transport system, (c) a tertiary α-carbon atom to impart resistance to catabolism, and (d) sufficient water solubility. Criteria a, b, and c appear to be fulfilled by the tricyclic 2-aminoadamantane-2-carboxylic acid (**4**),‡ the title compound, a rigid, achiral α-amino acid, and the expected zwitterionic character of **4** would appear to fulfill criterion d.



4

Chemistry. α-Amino acids having an adamantane skeleton as an integral part of the molecule have not yet been reported.[1] The Strecker synthesis to **4** was initially attempted starting from adamantan-2-one (**5a**) *via* its cyanohydrin and the corresponding aminonitrile, but hydrolysis of the latter to **4** could not be effected without extensive by-product formation, and this route was abandoned in favor of the Bucherer–Lieb synthesis as outlined in Scheme I. Although elevated temperatures and pressures were necessary to effect condensation to the spirohydantoin **6a** and similar high temperatures were required to hydrolyze **6a** to **4** (see Experimental Section), the overall yield of **4** from adamantan-2-one (**5a**) was 81%.

The electron-impact (EI) mass spectrum of **4** displayed a prominent $(M - CO_2H)^+$ fragmentation peak at *m/e* 150, with a molecular ion at *m/e* 195 of only feeble intensity as

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‡Suggested trivial name: adamantanine.