Potential Antifertility Agents. 7. Synthesis and Biological Activities of 2-, 3-, and 6-Alkyl-Substituted 4-Aryl-2-methylcyclohexanecarboxylic Acids[1]

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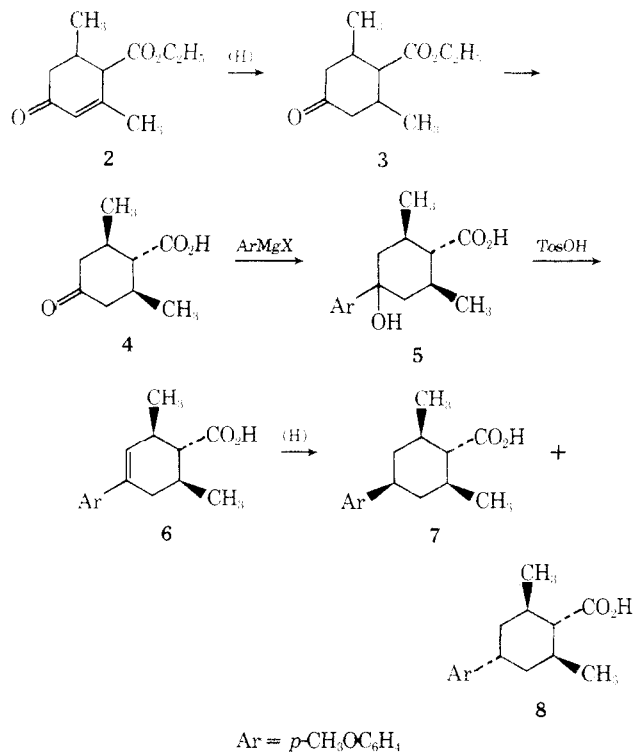
Syntheses and biological activities are reported for 17 of the title compounds. A progressive increase in uterotropic activity was seen with a systematic placement in proper stereochemical orientations of alkyl groups onto the reference compound, racemic 4β-(*p*-methoxyphenyl)-2β-methylcyclohexane-α-carboxylic acid (1). The activity profile was interpreted in terms of an estrogenic receptor model which had been proposed previously to account for biological activities seen with optical antipodes of 1. The most active compounds of the series were 10,000–18,000 times as active as 1 in uterotropic assays and prevented pregnancy in mated mice or rats at 0.1 mg/kg postcoitum.

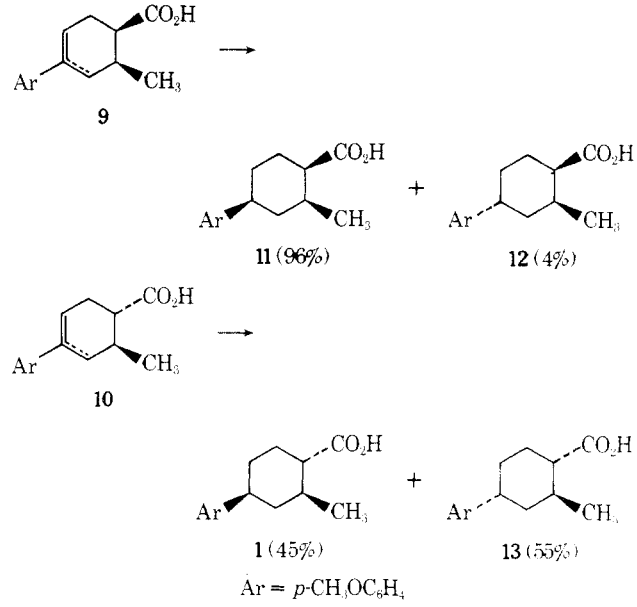
In the previous paper we proposed certain spatial areas on a hypothetical model of an estrogenic receptor site which are involved in the elicitation of a uterotropic response (Figure 1).[1] This model was invoked primarily to explain the approximately equal uterotropic potencies seen with the enantiomers of (±)-4β-(*p*-methoxyphenyl)-2β-methylcyclohexane-α-carboxylic acid (1). We now report the synthesis and biological activities of a number of alkylated derivatives of 1. The relative uterotropic potencies seen with these derivatives lend support to our original hypothesis and additionally give new insights into structural requirements for estrogenic activity.

Chemistry. The first derivative of 1 chosen for evaluation was the methylated derivative 7 which may be viewed as a "hybrid" of (+)-1 and (−)-1. Synthesis of 7 was achieved as outlined in Scheme I starting with the keto ester 2[2] which we found to be a mixture of cis–trans isomers. Hydrogenation of 2 using a Pd catalyst gave a mixture of isomeric esters 3, from which a single pure keto acid 4 was eventually obtained. This product was converted to the acids 5–8 as indicated. Arguments to support stereochemical assignments of 4–8 are as follows. Dehydration of 5 produced a single (racemic) ene acid 6. This result requires that the methyl groups in 5 (and also in 4 and 6) be cis (since dehydration of 5, which would be expected to occur in two directions, would produce two isomers from a 1,6-*trans*-dimethyl configuration). This assignment is consistent with the result reported by Jacobs, *et al.*, that catalytic reduction with Pt of the keto ester 2 is stereospecific to the extent that both methyl groups in 3 are cis.[3] Catalytic reduction of 6 produced two isomers 7 and 8 in a ratio of about 3 to 1. This result suggests that the carboxyl in 6 is trans relative to the methyls because the opposite configuration for the carboxyl should result in only one saturated isomer upon reduction (*viz.* 1β,2β,4β,6β)† by analogy with the following previous results.[4]

Additionally, treatment of a mixture of 7 and 8 (as methyl esters) with KO-*t*-Bu in refluxing *t*-BuOH resulted in essentially no change in the isomeric composition; a carboxyl cis relative to the cis methyls would be expected to epimerize.[4] Basic hydrolysis of a mixture of 7 and 8 esters resulted in a preferential hydrolysis of the isomer which corresponded to the minor isomer from the catalytic reduction. This result led to our assignment of the stereochemistry of 7 and 8 as shown. Although preferred conformations of both 7 and 8 involve conformers with an equatorial carboxyl, epimer 8 should have a greater population than 7 of a conformer with an axial carboxyl, and it appears to us that hydrolysis should occur easier at an

Scheme I





†*Cf.* ref 1 for explanations of the α and β designations. All unsymmetrical structures discussed herein are racemic unless otherwise noted; for brevity only one enantiomeric form is shown in the schemes and tables. To facilitate discussion, 9, 11, and 12 are depicted in the text in different enantiomeric forms than those shown in Table I.

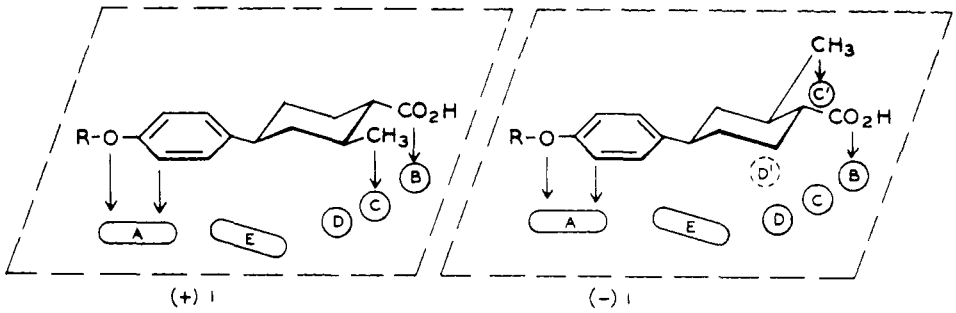
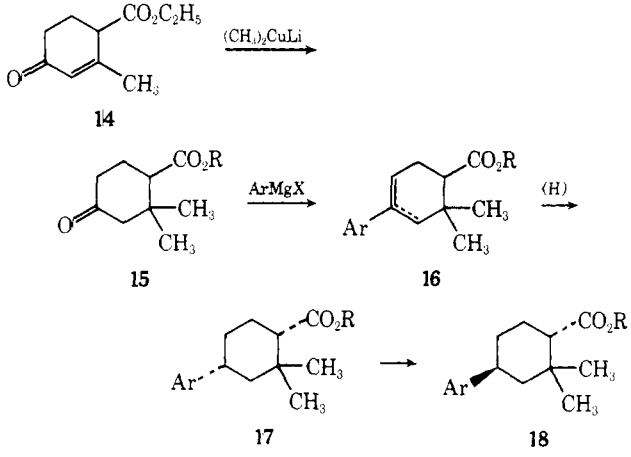


Figure 1. Hypothetical model of estrogenic receptor showing imposition of optical isomers of (±)-1 on proposed areas A-E involved in elicitation of biological response; see ref 1 for further explanation.

Scheme II



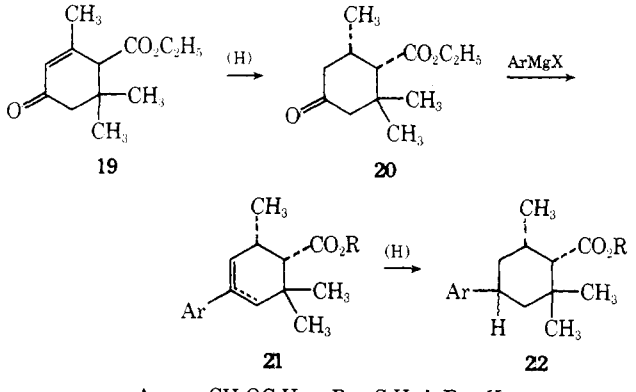
Ar = *p*-$CH_3OC_6H_4$; **a**, R = C_2H_5; **b**, R = H

axial carboxyl than an equatorial carboxyl flanked by two equatorial methyl groups.

The biological activity seen with **7** prompted us to extend our studies to the 2,2-dimethyl derivatives **16b** and **18b** (Scheme II). Earlier work had indicated that either area C or D on the receptor model (Figure 1) could be occupied for estrogenic response,[1,4] but no indication is available as to whether these areas can be occupied simultaneously. The 2,2-dimethyl acid **18b** seemed capable of answering this question since it may be viewed as a "hybrid" of the biologically active diastereomers **1** and **12** (*cf.* **12** as depicted in Table I). The requisite keto acid **15b** proved readily accessible starting from Hagemann's ester **14** and lithium dimethylcuprate.[5,6] This provided a more direct route to **15b** than a previously reported synthesis.[7] Condensation of **15b** with the Grignard reagent followed by dehydration of the intermediate hydroxy acid resulted in **16b**, a 3:1 mixture of Δ^4,Δ^3 isomers. Hydrogenation of **16** (acid or ester) produced an 80/20 mixture of cis-trans isomers from which the pure cis acid **17b** was obtained. Epimerization of the cis ester **17a** resulted in a 20/80 cis-trans mixture of esters. Hydrolysis of this mixture gave an acid enriched still further in the trans isomer from which the pure trans acid **18b** was readily obtained. It is noteworthy that the preferential hydrolysis observed is consistent with our isomeric assignments since it is generally accepted that equatorial esters (preferred conformer in **18a**) saponify faster than axial esters (probable preferred conformer in **17a**).

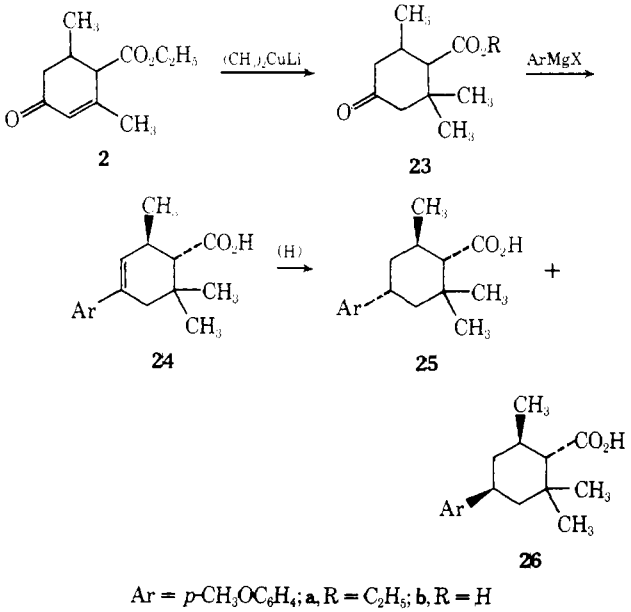
Schemes III and IV represent a continuation of a systemic placement of methyl groups on the parent structure **1**. Catalytic hydrogenation of the ester **19**[8] gave essentially

Scheme III



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

Scheme IV

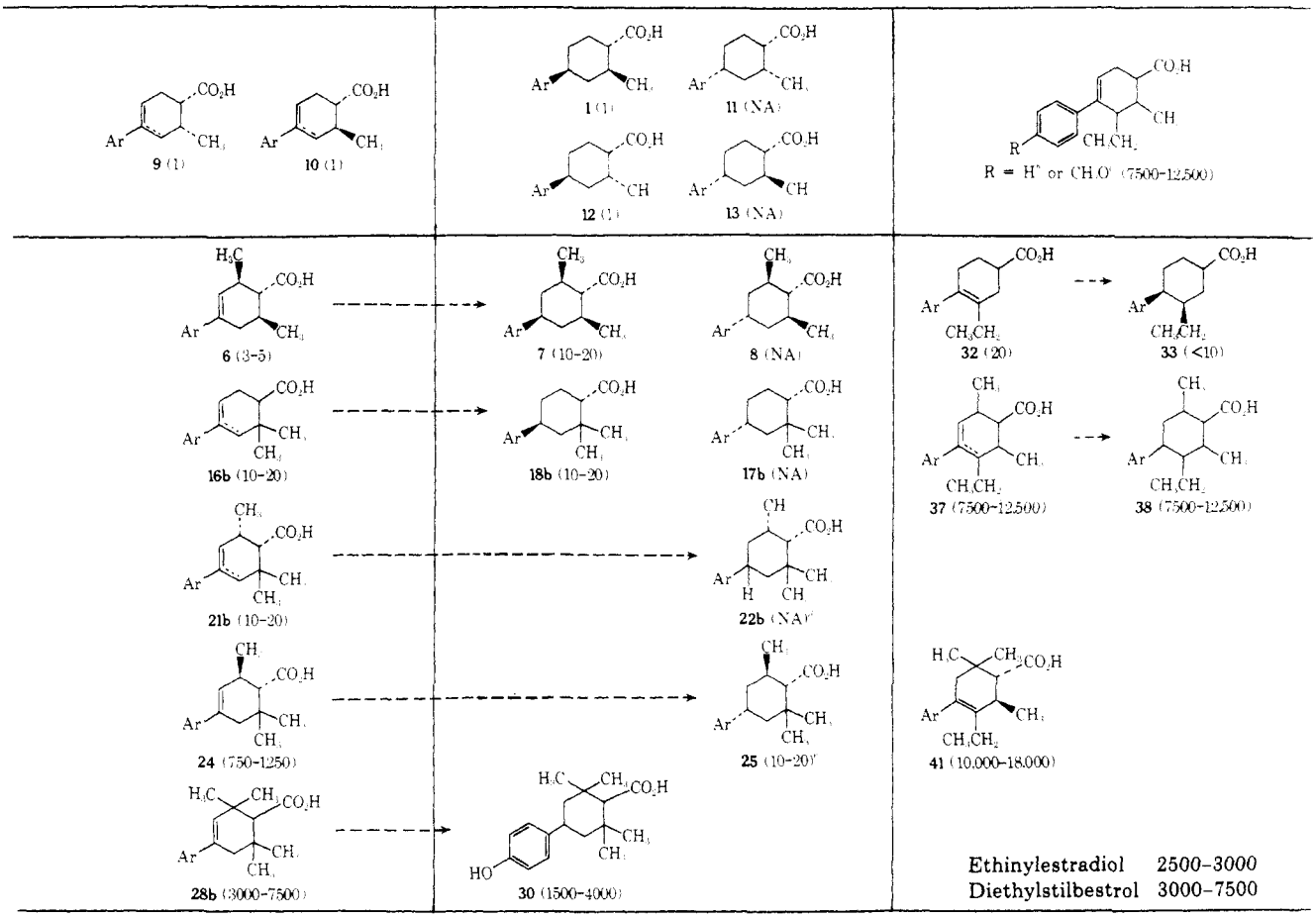


Ar = *p*-$CH_3OC_6H_4$; **a**, R = C_2H_5; **b**, R = H

one isomer which we have assigned as the cis configuration **20**. Treatment of the ester **20** with the Grignard reagent resulted in the *cis*-Δ^3,Δ^4-ene esters **21a**. Basic hydrolysis resulted in a low yield of the acid **21b**. Catalytic reduction of **21b** gave approximately a 2:1 mixture of C-4 epimers (**22b**). The sample of **22b** which was used for biological testing was an 80:20 mixture of C-4 epimers which was ultimately obtained from treatment of the saturated ester **22a** (from reduction of **21a**) with lithium 1-propanethiolate.[9]

Treatment of the ester **2** (Scheme IV) with lithium dimethylcuprate resulted in a 60/40 mixture of isomeric esters (**23a**), the minor component of which was identical

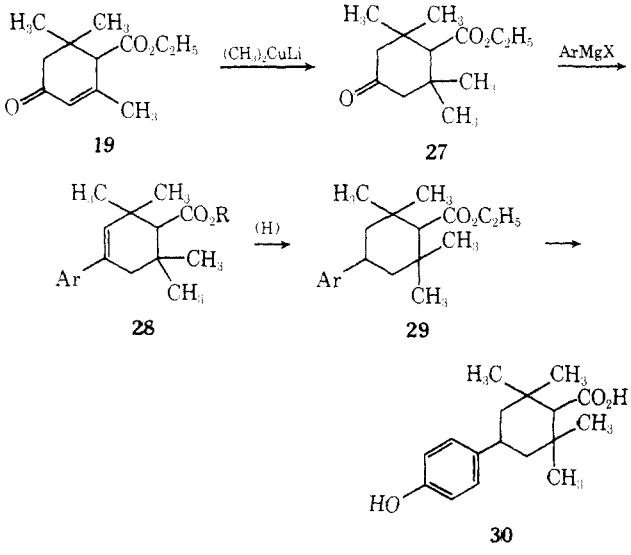
Table I. Relative Uterotropic Potencies of Various 2-, 3-, and 6-Alkyl-Substituted 4-(*p*-Methoxyphenyl)cyclohexene- and -cyclohexanecarboxylic Acids[a]



[a] Numbers in parentheses refer to uterotropic potencies relative to compound 1 in immature 21-day-old rats (*cf.* ref 4 for methodology). All unsymmetrical structures represent racemates. Ar = *p*-$CH_3OC_6H_4$; NA means not active at 2500 μg unless otherwise noted. [b] A. Mebane, U. S. Patent 3,344,147 (1967). [c] Reference 13. [d] An 80/20 mixture of C-4 epimers; highest dose tested was 250 μg at which no indication of activity was seen; the chemical precursor **21b** showed a good response at the 250-μg dose level. [e] The response seen here may be due to a small contamination with the 4β epimer.

(glpc) with the cis ester **20**. Hydrolysis of **23a** resulted in the keto acid **23b** (which was enriched relative to **23a** in respect to the trans component). This acid was elaborated into a pure Δ^3-ene acid which proved different from **21b** and was thus assigned the trans structure **24**. Models suggested that catalytic reduction of **24** with hydrogen addition from the less hindered face should result in predominantly the α-aryl isomer **25**. Experimentally, it was found that reduction of **24** produced a 90/10 mixture of C-4 epimers; we therefore assigned the major and minor products as the 4α (**25**) and 4β (**26**) epimers, respectively. These isomers proved different (glpc analysis of methyl esters) from the two C-4 epimers **22** obtained *via* Scheme III.

Scheme V

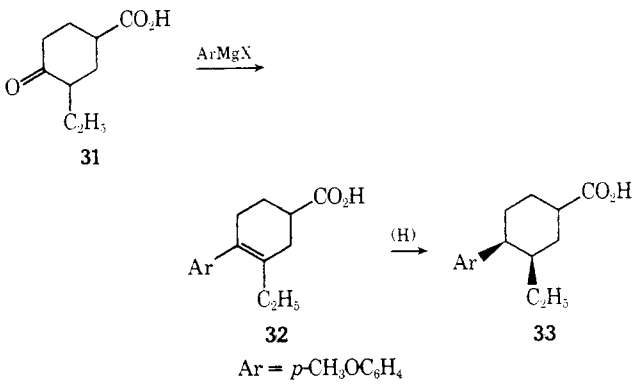


The tetramethyl derivative **28a** was obtained as indicated in Scheme V. Prolonged basic hydrolysis of **28a** gave a low yield of the corresponding acid **28b**. Hydrogenation of **28a** gave a mixture (2:1) of saturated esters **29**. Cleavage of the ester **29** with lithium 1-propanethiolate in HMPA resulted in concomitant cleavage of the methyl ether, a result which was not unexpected.[10,11] The product **30** which was screened biologically was a mixture of isomers in an approximate ratio of 60:40.

Scheme VI indicates the preparation of the acids **32** and **33** from the known keto acid **31**.[12] The saturated acid **33** is a mixture thought to be C-1 epimers.

Schemes VII and VIII depict synthetic routes used to obtain monomethyl and dimethyl analogs of the known potent estrogens 3-ethyl-4-(*p*-methoxyphenyl)-2-methyl-Δ^4- (and Δ^3-) cyclohexenecarboxylic acids.[13,14] Esters **2** and **19** were alkylated analogously to procedures reported

Scheme VI



Ar = *p*-$CH_3OC_6H_4$

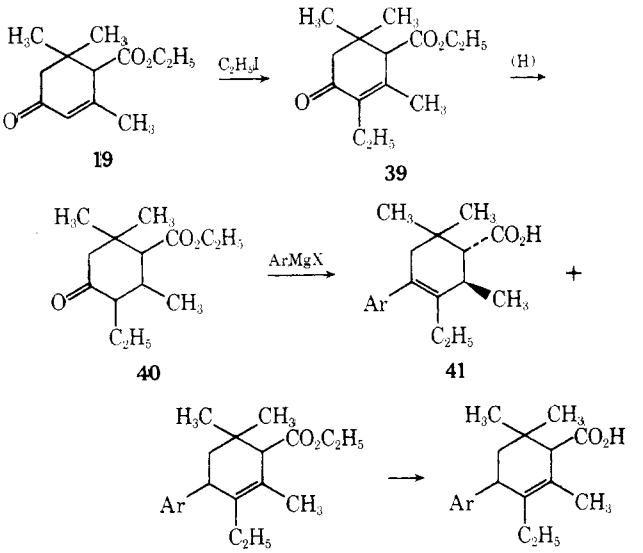
for alkylation of Hagemann's ester.[15,16] The acid **37** was a 50/50 mixture of Δ^3 and Δ^4 isomers but was unknown in regard to configurational composition. The saturated relative **38** was similarly unknown in regard to isomeric content. The acid **41** was obtained directly from reaction of the keto ester **40** with the Grignard reagent (after the usual treatment with acid), probably *via* intermediate lactone formation. The yield of **41** thus obtained was very low. The trans structure for **41** was assigned on the basis of nmr analysis which indicated trans-diaxial coupling for protons at C-1 and C-2. Additional work-up of the Grignard reaction mixture resulted in an ester which, after purification, was indicated by nmr and ir analysis to be the Δ^2 ester **42.** It is unknown whether isomerization of the double bond occurred during the purification or at an earlier stage. The Δ^2-ene acid **43** was obtained from **42.**

Biology. Methodology for the biological assays has been described previously.[4] All data are from oral dosing of aqueous solutions of sodium salts of the acids. Estrogenic data are summarized in a format (Table I) which provides for depiction of trends seen in uterotropic activity with the various methylated derivatives of **1.** For this purpose uterotropic activities are expressed as potencies relative to **1** which is assigned as unity. Four to six dose levels were examined for each active compound and the derived dose–response curve compared with that of **1** for an estimate of relative potency.

As indicated previously,[4] cyclohexene acids related to **1** (*cf.* **9** and **10**) are of approximately equal uterotropic potency with **1.** The various new cyclohexene derivatives prepared thus provide an additional end point for evaluating the effect of the placement of methyl groups on the parent structure.

The 2,6-dimethyl derivative **7**, which may be viewed as a "hybrid" of (+)- and (−)-**1**, had uterotropic activity

Scheme VIII



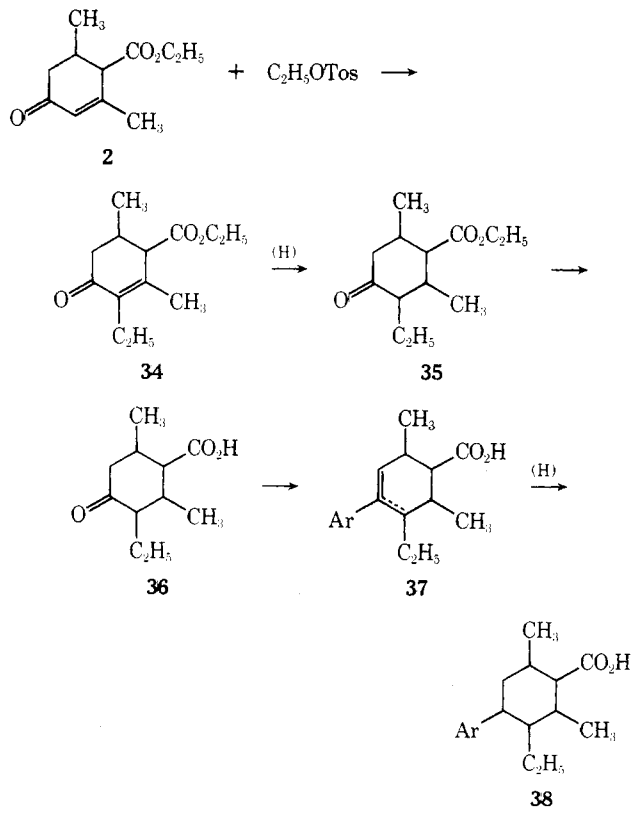
Ar = *p*-$CH_3OC_6H_4$

about 10–20 times that of **1.** The fact that activity was not only retained but was enhanced lends support to our earlier hypothesis[1] concerning the involvement of a spatial area C′ on an estrogenic receptor surface (*cf.* Figure 1) in the elicitation of a uterotropic response. An enhancement in activity was also seen in the related cyclohexene **6.** The lack of activity seen with **8** is expected by analogy with the inactive isomers **11** and **13** which possess a similar cis relationship between the aryl and carboxyl groups. The activity relationship between **7** and **8** complements arguments made above for their stereochemical assignments. A 10- to 20-fold enhancement of activity relative to **1** was seen in the 2,2-dimethyl derivatives **16b** and **18b.** In terms of our working receptor model (Figure 1), this indicates that areas C and D may not only be occupied simultaneously but that such an arrangement actually results in enhancement of response. Again, total inactivity (2500 μg) of the C-4 epimeric **17b** supports the stereochemical assignments made above.

Acid **24** may be viewed as a combination of structures **6** and **16b,** each of which showed enhancement of activity over the parent reference compound **10.** As noted in the table, a large synergistic effect is seen with a 750–1250 increase in potency relative to **10.** Interestingly, a similarly large increase in potency was not seen with **21b.** The activity level seen with acid **25** may be due to a small contamination with its C-4 epimer which we would expect, analogously with **24,** to have high activity.

In terms of the receptor model (Figure 1), **24** may be

Scheme VII



Ar = *p*-$CH_3OC_6H_4$

viewed as occupying simultaneously either areas C, D, and C′ or areas C, C′, and D′. The latter area D′ represents an area complementary to D, capable of accepting axial alkyl groups. Its spatial relationship with D is analogous to the enantiomeric relationship between C and C′.

The vast enhancements in activity seen with the tetramethyl derivatives **28b** and **30** lend credence to the existence of an area D′ and suggest that areas C, C′, D, and D′ may accommodate alkyl groups simultaneously with a resultant increase in biological response. The discrepancy between activities of **21b** and **24** suggests that these areas differ in their relative importance in terms of complexation for maximal response. Biological results with optical isomers of some of the methylated derivatives should shed light on this question.

Compounds **37**, **38**, and **41** represent methylated derivatives of the known potent estrogen 3-ethyl-4-(*p*-methoxyphenyl)-2-methyl-Δ^4-cyclohexenecarboxylic acid.[13] It is of considerable interest that **37** and **38**, although mixtures of isomers, maintain the high level of estrogenicity of the parent compound. An exceptionally high level of activity was seen with the derivative **41**. A total dose over 3 days of 0.3 μg of racemic **41** produced a uterine weight (114.8 mg) greater than that (95.6 mg) obtained with 1.0 μg of diethylstilbestrol (vehicle control uteri weight 20.8 mg).

Hypocholesterolemic activities of the acids in Table I generally paralleled their trends in estrogenicity. In the normal rat hypocholesterolemic assay,[4] representative results were **6** (5 mg/kg/day; −51% change in serum cholesterol; $p < 0.05$); **16b** (1; −43%; $p < 0.05$); **24** (0.05; −88%; $p < 0.001$); **28b** (0.05; −72%; $p < 0.05$); **30** (0.05; −75%; $p < 0.05$). Similarly, antifertility activities in various pre- and postcoital dosing regimens generally paralleled the estrogenic trends seen in Table I. Acid **24** was completely effective in preventing pregnancy in mated mice or rats dosed on days 1–5 postcoitum at 0.1 mg/kg/day. Acid **41** was completely effective in the same dosing regimen in rats at 0.1 mg/kg; an occasional pregnancy was seen at 0.05 mg/kg. Acid **41** was not tested for antifertility activity in mice.

In summary, the data in Table I indicate a remarkable increase in uterotropic activity with the systematic placement in proper stereochemical orientations of alkyl groups on the parent structure **1** and thereby lend support to our proposed estrogenic receptor model.

After this work was completed, Akhrem, *et al.*, reported the synthesis of 4-(*p*-methoxyphenyl)-2,3,6-trimethyl-Δ^4-cyclohexenecarboxylic acid, but no biological activity was given.[17]

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, while nmr data reported for compounds **41** and **43** were obtained on a Varian HA-100 spectrometer. Mass spectral data were obtained on a LKB 9000 mass spectrometer. Where elemental analyses are indicated by symbols of the elements, analytical results were within ±0.4% of the theoretical values.

2β,6β-Dimethyl-4-ketocyclohexane-α-carboxylic Acid (4). A solution of ethyl 2,6-dimethyl-4-keto-Δ^2-cyclohexenecarboxylate[2] (22.7 g, 0.116 mol) in EtOH (300 ml) containing 5% Pd/C (0.8 g) was shaken under an initial hydrogen pressure of 50 psi for 2 hr. Removal of the catalyst and evaporation of the solvent gave a quantitative yield of ethyl 2,6-dimethyl-4-ketocyclohexanecarboxylate (**3**). A solution of **3** (22.2 g, 0.112 mol) in MeOH (60 ml) and H_2O (60 ml) containing KOH (9.35 g, 0.167 mol) was stirred at 25° for 2 hr. Most of the MeOH was removed and the solution was extracted with CH_2Cl_2. The aqueous layer contained 1.96 g (10%) of a mixture of three isomeric acids; this product was discarded. The CH_2Cl_2 was evaporated and the residue was dissolved in EtOH (325 ml) and H_2O (65 ml) containing KOH (8.75 g, 0.156 mol). The solution was heated at reflux for 16 hr and then was worked up as above. The aqueous layer was acidified (3 *N* HCl) and then was extracted with Et_2O. Evaporation of the Et_2O left 13.07 g (69%); recrystallization (MeCN) gave 7.59 g (40%) of **4**, mp 162–164°. *Anal.* ($C_9H_{14}O_3$) C, H.

4-(*p*-Methoxyphenyl)-2β,6β-dimethyl-Δ^3-cyclohexene-α-carboxylic Acid (6). A Grignard solution, prepared from *p*-bromoanisole (16.6 g, 0.09 mol) and Mg (2.16 g, 0.09 g-atom) in THF (75 ml), was added to a solution of the keto acid **4** (7.39 g, 0.04 mol) in THF (100 ml) with ice cooling. The mixture was stirred at reflux for 18 hr. Aqueous 1 *N* HCl (100 ml) was added with cooling and then the THF was removed (15 mm). The aqueous residue was extracted with Et_2O. Washing the Et_2O with H_2O caused precipitation of the intermediate hydroxy acid **5** (5.2 g) which was separated by filtration; a 1.5-g aliquot was recrystallized (MeCN) to give **5**, mp 207–208°. *Anal.* ($C_{16}H_{22}O_4$) C, H. The remainder of **5** plus 9.7 g of semisolid isolated by evaporation of the Et_2O were dissolved in toluene (120 ml) containing *p*-TosOH·H_2O (0.8 g). The solution was refluxed for 2 hr with provision for azeotropic removal of H_2O. Standard work-up[4] yielded as the crude product an acid (7.37 g, 75%), mp 138–140.5°, which appeared to be homogeneous (glpc analysis after CH_2N_2); recrystallization (MeCN) gave **6**, mp 142–143°. *Anal.* ($C_{16}H_{20}O_3$) C, H.

4β-(*p*-Methoxyphenyl)-2β,6β-dimethylcyclohexane-α-carboxylic Acid (7). A solution of **6** (6.00 g, 0.023 mol) in absolute EtOH (300 ml) containing 5% Pd/C (0.6 g) was shaken under an initial H_2 pressure of 50 psi for 16 hr. Removal of the catalyst and evaporation gave a quantitative yield of a mixture of **7** and **8** in a ratio of 3:1 (determined by glpc,‡ after CH_2N_2 treatment; retention times 6.45 and 7.4 min for **8** and **7**, respectively). A 2.0-g aliquot was recrystallized (MeCN) to give pure **7** (0.5 g), mp 131.5–132.5°. *Anal.* ($C_{16}H_{22}O_3$) C, H.

4α-(*p*-Methoxyphenyl)-2β,6β-dimethylcyclohexane-α-carboxylic Acid (8). The crude acids (3.71 g) from the above reduction of **6** were converted to methyl esters by treatment with CH_2N_2. The mixture was refluxed for 19 hr in *t*-BuOH containing KO-*t*-Bu. Usual work-up[4] gave a product essentially unchanged in isomeric content. A 2.77-g (0.01 mol) quantity of this product in EtOH (40 ml) and H_2O (8 ml) containing KOH (1.30 g, 0.02 mol) was heated at reflux for 20 hr. Usual work-up[4] gave 0.61 g (23%) of acids **7** and **8** in a ratio of 1:4, respectively. (The unhydrolyzed ester was shown to be pure **7** methyl ester.) Recrystallization (MeCN) gave **8**, mp 161.5–164°, of >95% purity. *Anal.* ($C_{16}H_{22}O_3$) C, H.

2,2-Dimethyl-4-ketocyclohexanecarboxylic Acid (15b). To a solution of 0° of lithium dimethylcuprate[5] (0.88 mol) in Et_2O (1 l.) there was added, dropwise with stirring, a solution of Hagemann's ester **14** (73.0 g, 0.40 mol) in Et_2O (500 ml). The mixture was stirred at 22° for 40 min and then was poured into an aqueous solution (pH 8–10) of NH_3 and NH_4Cl. The resultant mixture was extracted several times with Et_2O, and the combined extracts were washed with water and dried (Na_2SO_4). Removal of the solvent and distillation of the residue gave 68.1 g (81%), bp 72–85° (0.1–0.2 mm), of ethyl 2,2-dimethyl-4-ketocyclohexanecarboxylate (**15a**) containing about 10% of unchanged Hagemann's ester. The latter was removed in the next step since it is unstable to basic hydrolysis.[15]

A solution of the product (68.0 g, 0.34 mol) in EtOH (400 ml) containing KOH (23.0 g, 0.4 mol) and water (80 ml) was heated at reflux for 5 hr. The alcohol was removed at 10 mm and the solution was extracted with Et_2O. The aqueous phase was cooled and acidified (3 *N* HCl). The title acid was extracted into Et_2O and the solution was washed, dried, and evaporated to leave 46.6 g; distillation gave **15b** (33.2 g, 57%) of bp 113–119° (0.2 mm) as a waxy, hygroscopic solid [lit.[7] bp 160–170° "bath temperature" (0.4 mm)].

4-(*p*-Methoxyphenyl)-2,2-dimethyl-Δ^4(Δ^3)-cyclohexenecarboxylic acid (16b) was prepared from the acid **15b** and 2 equiv of *p*-methoxyphenylmagnesium bromide as described above for **6**. The yield of **16b** after recrystallization (MeCN) was 53%; mp 137.5–139° (previous softening from 135°); shown by nmr analysis of the vinylic proton pattern to be a mixture of Δ^4:Δ^3 in approximate ratio of 3:1, respectively. *Anal.* ($C_{16}H_{20}O_3$) C, H.

***cis*-4-(*p*-Methoxyphenyl)-2,2-dimethylcyclohexanecarboxylic Acid (17b).** Catalytic reduction of **16b** (4.00 g) in EtOH (100 ml) containing 5% Pd/C (0.5 g) gave quantitatively an 80:20 mixture of **17b** and the trans isomer **18b**, respectively (glpc‡ retention

‡Glass column, 6 ft × 6 mm i.d., packed with 3% butane-1,4-diol succinate polyester on 100–120 Gas-Chrom Q (Applied Science Laboratories, Inc.) at 190° isothermally, flow rate of 60 ml of He/min.

times, Me esters, 7.0 and 9.25 min, respectively). Recrystallization (MeCN) gave 2.83 g containing about 12% **18b**. This product was dissolved in hot EtOH (25 ml); as the solution cooled, **18b** crystallized as beads. The solution was observed further until formation of white needles was seen; at this point the supernatant was decanted and allowed to stand at 25° for 16 hr. Filtration gave pure **17b** (1.23 g, 31%), mp 153–154.5°. *Anal.* ($C_{16}H_{22}O_3$) C, H.

trans-**4-(*p*-Methoxyphenyl)-2,2-dimethylcyclohexanecarboxylic Acid (18b).** The ethyl ester **16a** (prepared by Fischer esterification of **16b**) was hydrogenated as above to an 80:20 mixture of cis–trans isomers. A solution of the product (7.50 g, 0.026 mol) in *t*-BuOH (75 ml) containing KO-*t*-Bu (from 0.34 g, 0.009 g-atom) was refluxed for 17 hr. Usual work-up gave a product (5.93 g, 81%) indicated by glpc to be a 20:80 cis–trans mixture. Hydrolysis in refluxing aqueous ethanolic KOH for 16 hr as described above gave as the crude product 3.21 g (59%) of an acid containing 95% of the trans isomer **18b**. Recrystallization (EtOH) gave pure **18b**, mp 171–173°. *Anal.* ($C_{16}H_{22}O_3$) C, H.

Ethyl *cis*-4-keto-2,2,6-trimethylcyclohexanecarboxylate (20) was prepared from reduction of ethyl 2,6,6-trimethyl-4-keto-2-cyclohexene-1-carboxylate[8] (**19**) as described above for **4**. The crude product (100%) was used directly in the next step; glpc§ (retention time, 10.35 min) indicated 99% purity.

cis-**4-(*p*-Methoxyphenyl)-2,6,6-trimethyl-$\Delta^3(\Delta^4)$-cyclohexenecarboxylic Acid (21b).** The ethyl ester **21a** was prepared from the keto ester **20** and 1 equiv of *p*-methoxyphenylmagnesium bromide as described above for **6**. The yield of **21a**, bp 141–143° (0.05 mm), was 43%. *Anal.* ($C_{19}H_{26}O_3$) C, H. A solution of **21a** (9.69 g, 0.032 mol) in EtOH (100 ml) and H_2O (20 ml) containing KOH (9.00 g, 0.16 mol) was refluxed for 5 days. Standard work-up gave 2.40 g of crude acid which was recrystallized twice from MeCN to give **21b** (0.86 g, 10%), mp 134–136° (softening at 132°) shown by glpc (after CH_2N_2 treatment) to be a 95:5 mixture of ene isomers; the nmr spectrum showed a multiplet pattern for the vinylic proton which appeared consistent for the Δ^3 isomer as the major component. *Anal.* ($C_{17}H_{22}O_3$) C, H.

4-(*p*-Methoxyphenyl)-2,2,6α-trimethylcyclohexane-α-carboxylic Acid (22b). Catalytic hydrogenation of the ester **21a** (12.40 g, 0.041 mol) in EtOH (200 ml) containing 5% Pd/C (1.20 g) gave quantitatively a 90/10 mixture of the C-4 epimeric esters **22a**. A solution of **22a** (6.67 g, 0.022 mol) and 150 ml of 0.45 *M* lithium 1-propanethiolate in HMPA[9] was stirred under nitrogen at 25° for 40 hr. The mixture was poured onto 0.5 *N* NaOH and was extracted several times with Et_2O. The aqueous phase was acidified and then extracted with fresh Et_2O. Work-up gave 2.00 g of acid; two recrystallizations (MeCN) gave 0.64 g (11%) of **22b**, mp 139.5–141.5° (softening from 135°), indicated by glpc (after CH_2N_2) to be an 80:20 mixture. *Anal.* ($C_{17}H_{24}O_3$) C, H.

4-Keto-2,2,6-trimethylcyclohexanecarboxylic Acid (23b). The ester **23a** was prepared from the ester **2**[2] and lithium dimethylcuprate[5] by the procedure described above for **15a**: yield, 67%; bp 83–90° (0.3–0.5 mm); indicated by glpc§ to be a 38:62 mixture of cis–trans isomers (retention times, 10.35 and 11.10 min, respectively). A solution of **23a** (24.00 g, 0.113 mol) in ethylene glycol (57 ml) and H_2O (7 ml) containing KOH (6.93 g, 0.136 mol) was refluxed for 18 hr. Usual work-up gave a mixture (15.68 g, 76%) of isomeric acids (**23b**), approximate 1:3 cis–trans content; an analytical sample was prepared by short-path distillation, bp 142–148° (0.2 mm). *Anal.* ($C_{10}H_{16}O_3$) C, H.

trans-**4-(*p*-Methoxyphenyl)-2,6,6-trimethyl-Δ^3-cyclohexenecarboxylic acid (24)** was prepared from the keto acid **23b** and 2 equiv of *p*-methoxyphenylmagnesium bromide as described above for **6**. Two recrystallizations (MeCN) of the crude product gave **24** (yield, 21%): mp 165–166.5°; shown to be different (glpc after CH_2N_2) from either of the isomers of **21b**; as with **21b**, the nmr spectrum suggested the Δ^3 assignment. *Anal.* ($C_{17}H_{22}O_3$) C, H.

4α-(*p*-Methoxyphenyl)-2,2,6β-trimethylcyclohexane-α-carboxylic Acid (25). The acid **24** (4.00 g, 0.0146 mol) was hydrogenated in EtOH containing 5% Pd/C as previously described to give quantitatively a 90:10 mixture of C-4 epimers assigned as the 4α and 4β epimers, respectively. Recrystallization (MeCN–EtOH) gave **25** (98% pure), mp 163.5–167°. *Anal.* ($C_{17}H_{24}O_3$) C, H.

A sample of the acid **21b** was similarly hydrogenated to yield a 2:1 mixture of C-4 epimers; the mixture was shown by glpc (after CH_2N_2) to be different from the mixture of C-4 epimers obtained from hydrogenation of **24**.

Ethyl 4-keto-2,2,6,6-tetramethylcyclohexanecarboxylate (27) was prepared from the ester **19**[8] and lithium dimethylcuprate[5] by the procedure described above for **15a**: yield, 82%; bp 86–87° (0.2 mm). *Anal.* ($C_{13}H_{22}O_3$) C, H.

4-(*p*-Methoxyphenyl)-2,2,6,6-tetramethyl-Δ^3-cyclohexenecarboxylic Acid (28b). The ethyl ester **28a** was prepared from the keto ester **27** and 1 equiv of *p*-methoxyphenylmagnesium bromide as described above for **6**: yield of **28a** was 65%; bp 151–154° (0.3 mm). *Anal.* ($C_{20}H_{28}O_3$) H; C: calcd, 75.91; found, 75.41. A solution of **28a** (12.01 g, 0.038 mol) in EtOH (100 ml) and H_2O (20 ml) containing KOH (11.20 g, 0.20 mol) was heated at reflux for 64 hr. Usual work-up yielded 0.45 g (4%) of acid **28b**, mp 142–144° after recrystallization (MeCN). *Anal.* ($C_{18}H_{23}O_3$) C, H.

Ethyl 4-(*p*-Methoxyphenyl)-2,2,6,6-tetramethylcyclohexanecarboxylate (29). Catalytic reduction of the ester **28a** in EtOH containing 5% Pd/C gave quantitatively **29** as a mixture (*ca.* 2:1) of cis–trans isomers: bp 152–155° (0.3 mm). *Anal.* ($C_{20}H_{30}O_3$) C, H.

4-(*p*-Hydroxyphenyl)-2,2,6,6-tetramethylcyclohexanecarboxylic Acid (30). Treatment of the ester **29** with lithium 1-propanethiolate at 25° as described above for **22a** resulted in insignificant amounts of cleavage. The quality of the reagent was shown to be satisfactory when we repeated the reported[9] quantitative cleavage of methyl mesitoate. The reagent then was condensed with **29** in HMPA at 65° for 21 hr. Work-up as described above gave **30**: yield, 38%, after trituration under CH_2Cl_2; mp 174–178° (*ca.* 60:40 mixture of cis–trans isomers). *Anal.* ($C_{17}H_{24}O_3$) C, H.

3-Ethyl-4-(*p*-methoxyphenyl)-Δ^3-cyclohexanecarboxylic acid (32) was prepared from 3-ethyl-4-ketocyclohexanecarboxylic acid[12] by the procedure described above for **6**: yield of **32**, 43%; mp 127–129° (after CH_2N_2, glpc indicated 98% pure). *Anal.* ($C_{16}H_{20}O_3$) C, H.

3-Ethyl-4-(*p*-methoxyphenyl)cyclohexanecarboxylic Acid (33). The acid **32** was reduced catalytically in EtOH by the usual procedure described above: yield of **33**, 38% after two recrystallizations from methylcyclohexane; mp 74–80°; shown by glpc (after CH_2N_2) to be a 92:8 mixture of isomers. *Anal.* ($C_{16}H_{22}O_3$) C, H.

2,6-Dimethyl-3-ethyl-4-ketocyclohexanecarboxylic Acid (36). The keto ester **2**[2] (21.45 g, 0.109 mol) was added over 15 min to a stirred solution of NaOEt (from 2.64 g of Na, 0.115 g-atom) in absolute EtOH (90 ml). The solution was stirred at 25° for 40 min, and then ethyl *p*-toluenesulfonate (23.00 g, 0.115 mol) was added. The mixture was heated under reflux for 3.5 hr. Work-up gave a product (22.36 g) indicated (glpc) to be a mixture of the alkylated ester **34** (56%), unchanged **2** (35%), and unknown impurities (possibly C-1 alkylated products).[16] Pure **34** could not be obtained from distillation (2-ft spinning-band column). The best fractions [bp 77° (0.07 mm)] contained 82% **34** (two diastereomers) and 14% **2** (two diastereomers). Hydrogenation (5% Pd/C in EtOH as above) gave a product which was distilled (2-ft spinning-band column) to give the ester **35** [bp 64–65.5° (0.06 mm), 10.60 g] as a mixture of isomers. Refluxing for 16 hr of **35** in aqueous ethanolic KOH as above yielded **36** (7.59 g, 82%) as an oil (mixture of isomers) which was used in the following preparation.

2,6-Dimethyl-3-ethyl-4-(*p*-methoxyphenyl)-$\Delta^3(\Delta^4)$-cyclohexenecarboxylic acid (37) was prepared from the keto acid **36** as described above for **6**: yield, 2.23 g (20%); mp 144–158°; indicated by nmr to be *ca.* 50/50 mixture of Δ^3 and Δ^4 isomers. *Anal.* ($C_{18}H_{24}O_3$) C, H.

2,6-Dimethyl-3-ethyl-4-(*p*-methoxyphenyl)cyclohexanecarboxylic acid (38) was prepared from catalytic hydrogenation (5% Pd/C in EtOH) of **37**: yield of **38**, 52%, after two recrystallizations (MeCN) to mp 135–155°; glpc (after CH_2N_2) indicated a minimum of four isomers, the major of which comprised about 70% of the total. *Anal.* ($C_{18}H_{26}O_3$) C, H.

Ethyl 3-Ethyl-4-keto-2,6,6-trimethyl-Δ^2-cyclohexenecarboxylate (39). The ester **19**[8] (25.0 g, 0.119 mol) was added to a solution of KO-*t*-Bu (from 4.64 g of K, 0.119 g-atom) in *t*-BuOH (95 ml) while cooling to maintain the temperature below 38°. A solution of ethyl iodide (18.6 g, 0.119 mol) in *t*-BuOH (20 ml) was added and the mixture was stirred under reflux for 3.5 hr. Usual work-up gave **39** (26.24 g, 93%), bp 86–89° (0.1 mm). *Anal.* ($C_{14}H_{22}O_3$) C, H.

Ethyl 3-Ethyl-4-keto-2,6,6-trimethylcyclohexanecarboxylate (40). A solution of **39** (20.1 g, 0.085 mol) in EtOH (90 ml) containing 3 *N* aqueous HCl (12 ml) and 5% Pd/C (1.1 g) was shaken under H_2 (50 psi initially) until theoretical uptake was observed. Usual work-up gave **40**, bp 64–65° (0.1 mm). *Anal.* ($C_{14}H_{24}O_3$) C, H.

trans-**3-Ethyl-4-(*p*-methoxyphenyl)-2,6,6-trimethyl-Δ^3-cyclohexenecarboxylic acid (41)** was prepared from the keto ester **40** and 1 equiv of *p*-methoxyphenylmagnesium bromide as described

§Glass column, 6 ft × 6 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 60 ml of He/min.

above for 6. After treatment with p-TosOH·H_2O and usual work-up the crude yield of 41 was 5% which recrystallized (MeCN) to pure 41: mp 164.5–167.5°; ir (KBr) 1700 cm^{-1} (C=O); nmr ($CDCl_3$) δ 7.0–7.7 (q, 4, J = 8.5 Hz, aromatic), 3.78 (s, 3, CH_3O), 2.7 (m, broad, 1, CH_3CH), 2.4–1.8 (overlapping m, 5, $CHCO_2H$ and $CH_2C{=}CCH_2$), 1.2–1.05 [doublet overlapping two singlets, J = 6 Hz, 9, $CHCH_3$ and $C(CH_3)_2$], and 0.87 (t, 3, J = 7.5 Hz, CH_2CH_3); scale expansions and spin decouplings confirmed the assignments as well as establishing the stereochemistry; coupling constant of the C-1 proton (10 Hz) was indicative of trans-diaxial coupling; mass spectrum (70 eV) m/e 302. *Anal.* ($C_{19}H_{26}O_3$) C, H.

3-Ethyl-4-(*p*-methoxyphenyl)-2,6,6-trimethyl-Δ^2-cyclohexenecarboxylic Acid (43). The Et_2O extracts which remained after the base extractions in the above experiment were worked up to leave an oil. Distillation [bp 173–182° (0.05 mm)] gave the Δ^2-ene ester 42 (yield, 42%): nmr ($CDCl_3$) δ 1.70 (d, 3, J = 2 Hz, $CH_3C{=}C$). *Anal.* ($C_{21}H_{30}O_3$) C, H. A solution of the ester 42 (1.10 g, 0.003 mol) in ethylene glycol (18 ml) and H_2O (3.6 ml) containing KOH (2.00 g, 0.036 mol) was heated at reflux for 88 hr. Usual work-up gave the acid 43 (0.67 g, 67%): mp 131–132° (MeCN); ir (KBr) 1697 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.76 (d, 3, J = 2 Hz, $CH_3C{=}C$); mass spectrum (70 eV) m/e 302. *Anal.* ($C_{19}H_{26}O_3$) C, H.

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References

(1) R. R. Crenshaw, T. A. Jenks, G. M. Luke, and G. Bialy, *J. Med. Chem.*, **17**, 1258 (1974) (paper 6).
(2) E. C. Horning, M. O. Denekas, and R. E. Field, "Organic Syntheses," Collect. Vol. III, Wiley, New York, N.Y., 1955, p 317.
(3) T. L. Jacobs, R. Reed, and E. Pacovska, *J. Amer. Chem. Soc.*, **73**, 4505 (1951).
(4) R. R. Crenshaw, G. M. Luke, T. A. Jenks, R. A. Partyka, G. Bialy, and M. E. Bierwagen, *J. Med. Chem.*, **16**, 813 (1973).
(5) H. Gilman, R. G. Jones, and L. A. Woods, *J. Org. Chem.*, **17**, 1630 (1952).
(6) H. O. House and W. F. Fischer, Jr., *J. Org. Chem.*, **33**, 949 (1968).
(7) P. B. Talukdar and P. Bagchi, *J. Org. Chem.*, **20**, 25 (1955).
(8) J. D. Surmatis, A. Walser, J. Gibas, and R. Thommen, *J. Org. Chem.*, **35**, 1053 (1970).
(9) P. A. Bartlett and W. S. Johnson, *Tetrahedron Lett.*, 4459 (1970).
(10) G. I. Feutrill and R. N. Mirrington, *Tetrahedron Lett.*, 1327 (1970).
(11) J. K. MacLeod, B. R. Worth, and R. J. Wells, *Tetrahedron Lett.*, 241 (1972).
(12) M. Artico, R. Giuliano, and V. Nacci, *Ann. Chim. (Rome)*, **53**, 1797 (1963).
(13) A. H. Nathan and J. A. Hogg, *J. Amer. Chem. Soc.*, **78**, 6163 (1956).
(14) A. A. Akhrem, Y. A. Titov, and I. S. Levina, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 624 (1967); *Chem. Abstr.*, **67**, 100306f (1967).
(15) L. I. Smith and G. F. Rouault, *J. Amer. Chem. Soc.*, **65**, 631 (1943).
(16) G. Sarkar and D. Nasipuri, *J. Indian Chem. Soc.*, **45**, 200 (1968).
(17) A. A. Akhrem, L. E. Kulikova, I. S. Levina, and Y. A. Titov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 588 (1971); *Chem. Abstr.*, **75**, 88259y (1971).

Synthesis and Antifolate Activity of Isoaminopterin†

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The synthesis of isoaminopterin has been carried out by two methods. The first parallels the method employed for constructing the isofolate framework recently developed in this laboratory, which involves a novel ring closure of a substituted pyrimidine. The second involves the displacement of the halogen of 2,4-diamino-6-chloropteridine by α-amino-p-toluic acid. Isoaminopterin has been tested for its ability to inhibit the growth of two folate-requiring organisms. Inhibition studies of this compound were carried out on the enzyme dihydrofolate reductase. While isoaminopterin is equally potent in its bacteriostatic activity as aminopterin, it is somewhat less inhibitory for dihydrofolate reductase.

As part of a continuing program aimed at the design, synthesis, and biological evaluation of folate analogs which would be less toxic and more specific in their action than currently available drugs, we have recently reported 9-oxofolic acid[1] and isofolic acid.[2] Although the altered isomeric framework of isofolic acid could contribute specific interference with the metabolic pathways in which 5,10-methylenetetrahydrofolate takes part as a cofactor, its 4-amino analog might also elicit antimetabolic activity by inhibiting the enzyme dihydrofolate reductase.[3-5] Since no 4-amino analogs of isofolic acid have been prepared, the preceding assumption of inhibition of DHFR by 4-aminoisofolate is only speculative and warranted examination. It appeared that although 4-aminoisofolate possesses the 2,4-diamino functions at the pteridine moiety as with the classical aminofols, the reversal of substituents at the C_9–N_{10} positions could alter its binding characteristics for DHFR, which should be reflected in altered inhibitory characteristics. Some evidence to support this type of reasoning is available. For example, it has been noted that dihydroisofolic acid, which differs from dihydrofolic acid only in the exchange of the amino and methylene groups at positions 9 and 10, does not behave as a substrate for DHFR.‡

Another reason for the synthesis of 4-aminoisofolic acid comes from the work of Kisliuk and coworkers who investigated the ability of the classical aminofols aminopterin,[6] methotrexate,[7] and their dihydro and tetrahydro derivatives to inhibit the enzyme thymidylate synthetase and concluded that the tetrahydro forms are more powerful inhibitors than the parent compounds. Since formaldehyde

†Trivial names in general usage will be used for these compounds: isoaminopterin = *N*-[*p*-[[(2,4-diamino-4-deoxy-6-pteridinyl)amino]methyl]benzoyl]glutamic acid; methotrexate = *N*-[*p*-[[(2,4-diamino-6-pteridinyl]-methyl]methylamino]benzoyl]glutamic acid; aminopterin = *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]glutamic acid; folic acid = *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]glutamic acid; homofolic acid = *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)ethyl]amino]benzoyl]glutamic acid. Other abbreviations include DHFR, dihydrofolate reductase; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; DEAE, diethylaminoethyl; DMF, dimethylformamide; Boc, *tert*-butyloxycarbonyl.

‡C. M. Baugh, R. L. Kisliuk, and M. G. Nair, unpublished work, University of South Alabama and Tufts University, 1974.