Potential Hypolipidemic Agents Derived from 3-Hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene

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Numerous aryloxy derivatives containing a lipophilic group have been found to possess hypolipidemic activity. This has prompted the preparation of various derivatives of 3-hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (**6a**). In **6a** the lipophilic biphenyl group is incorporated into the steroid nucleus. A three-step synthesis of **6a** from 17β-methylestradiol methyl ether was developed. The derivatives prepared were tested in rats made hypercholesterolemic with propylthiouracil. Several were found active in this test.

An elevated level of serum lipids has been identified as one of the risk factors associated with atherosclerosis. This has resulted in an intensive effort to develop agents which would reduce serum lipid levels.[1]

Currently, the most widely used hypolipidemic agent is clofibrate (**1**).[2] This substance is an aryloxy derivative, as are many other compounds which have been found to produce lipid-lowering effects, for example, boxidine (**2**),[3] nafenopin (**3**),[4] halophenate (**4**),[5] and treloxinate (**5**).[6] Each of these substances possesses a lipophilic group in the molecule. Since the biphenyl group has been reported to exhibit a high degree of hydrophobic bonding, even higher than that of the *p*-chlorophenyl or the 3,4-dichlorophenyl group,[7] we decided to examine the effects on lipids of various classes of compounds derived from 3-hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (**6a**). These derivatives could be considered as steroid analogues of substances known to have hypolipidemic activity.

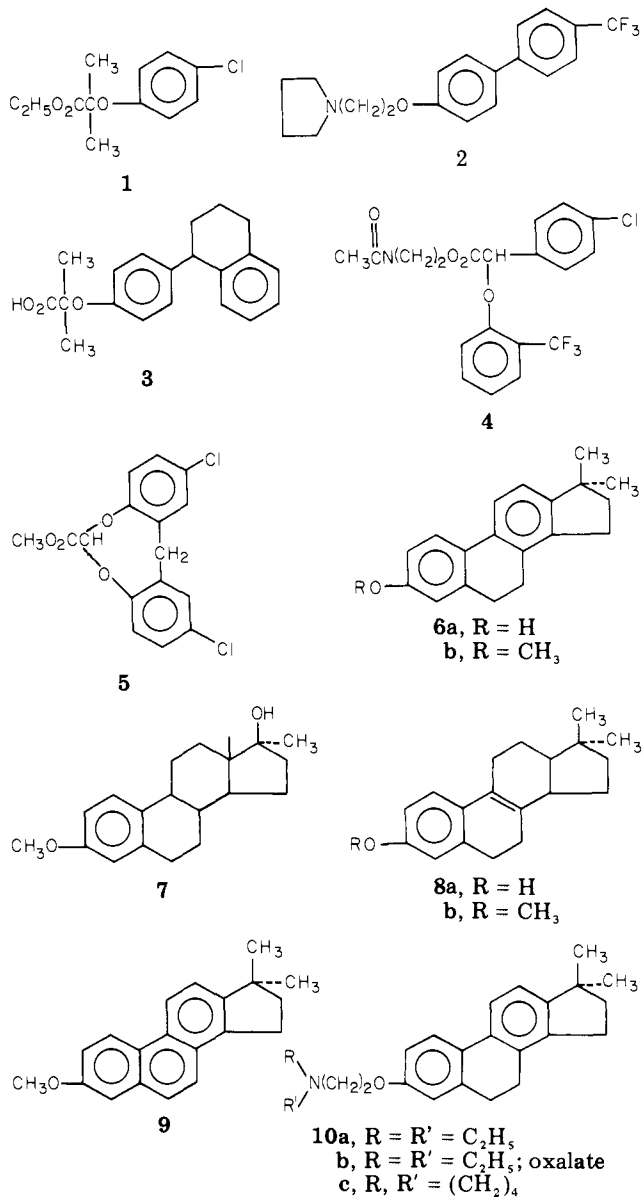
In **6a**, the biphenyl group is part of the steroid nucleus. The absence of chiral centers provided an added incentive for studying these derivatives as **6a** could, in principle, be generated from optically active as well as inactive compounds, irrespective of the stereochemistry of the starting material.

Initially, we sought to prepare **6a** from 17α-methylestradiol 3-methyl ether (**7**) by treatment with pyridine hydrochloride followed by dehydrogenation. A previous study on 17α-ethyl-19-nortestosterone[8] suggested that demethylation and dehydration could be achieved concurrently to furnish the Δ^8 compound, **8a**. Dehydrogenation of **8a** could conceivably then afford the desired product, **6a**. Experimentally, it was found that while treatment with pyridine hydrochloride at 220 °C resulted in demethylation and dehydration, partial dehydrogenation also occurred. In addition to the expected product **8a**, the reaction mixture also contained the desired biphenyl derivative **6a**.

Although the desired product could be formed directly from **7**, the results were not always reproducible and the product could not be obtained pure. Attempts at altering the reaction conditions so that **6a** could be obtained as the sole product were unsuccessful. However, by modifying the conditions reported for the preparation of 3-methoxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (**6b**)[9] and for the preparation of 3-methoxy-17-methylgona-1,3,5(10),8,11,13-hexaene,[10] we succeeded in preparing **6a** from **7** in three steps in a fairly respectable yield.

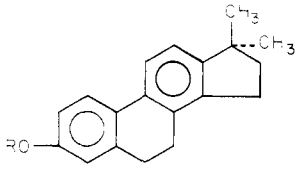
Kimura et al.[10] reported that treatment of 17α-methylestradiol 3-methyl ether (**7**) with concentrated sulfuric acid furnished the styrene derivative **8b** in 90% yield. We found that aromatization of ring C of the latter substance to afford **6b** could be accomplished in 50% yield with 2 equiv of chloranil. A by-product of the reaction was the corresponding phenanthrene derivative **9**, which, incidentally, could readily be prepared from **6b** by dehydrogenation with dichlorodicyanobenzoquinone. Demethylation of **6b** with pyridine hydrochloride at 220 °C gave 3-hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (**6a**) in quantitative yield.

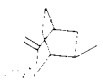
Alkylation of **6a** with diethylaminoethyl chloride gave **10a**, an oil which was characterized and tested as the oxalate salt **10b**. With pyrrolidinoethyl chloride hydrochloride, **10c** was obtained.



The clofibrate analogue **11a** and the lower homologues **11d** and **11e** were prepared by alkylation of **6a** with the appropriate α-bromo acids. As **11e** was very insoluble, the

Table I. Cholesterol-Lowering Effects of Derivatives of 3-Hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene in the Propylthiouracil-Induced Hypercholesterolemic Rats



R	Dose, mg ig[a]	Change in plasma cholesterol concn from control, %	t[b]
H (6a)	20	−10	2.2
	10	−16	2.5
	5	+4	0.2
CH_3 (6b)	10	+1	0.6
$(C_2H_5)_2N(CH_2)_2\cdot(CO_2H)_2$ (10b)	10	−19	2.5
	5	−14	2.0
c-$C_4H_8N(CH_2)_2$ (10c)	10	−17	2.4
	5	−5	0.9
$HO_2CC(CH_3)_2$ (11a)	10	−18	2.7
$C_2H_5O_2CC(CH_3)_2$ (11b)	10	−9	1.0
 (11c)	10	−4	0.6
$HO_2CCH(CH_3)$ (11d)	10	+3	0.5
$C_2H_5O_2CCH_2$ (11f)	10	+8	1.7
$HOCH_2C(CH_3)_2$ (12a)	10	−25	2.7
$CH_3O_2C(CH_2)_2CO_2CH_2C(CH_3)_2$ (12b)	10	−18	2.4
$HO_2C(CH_2)_2CO_2CH_2C(CH_3)_2$ (12c)	10	−7	1.7
Clofibrate	10	+1	0.2

[a] $n = 8$ for both the control and treated animals; ig = intragastric. [b] Student's t value. A t value greater than 1.65 indicates $p < 0.05$.

ethyl ester 11f was prepared for testing. The ethyl ester 11b and the azabicyclo ester 11c of **11a** were also synthesized.

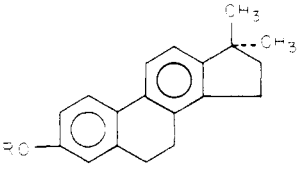
Reduction of **11a** with lithium aluminum hydride gave the alcohol **12a**, which was acylated to furnish the succinate ester 12b. Saponification of 12b regenerated the alcohol **12a**. Selective cleavage of the methyl ester portion of the molecule to afford the hydrogen succinate **12c** was accomplished with lithium iodide in refluxing pyridine.[11] When refluxing dimethylformamide was used instead, cleavage with lithium iodide afforded 3-hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (**6a**).

The preparation of **12b** and **12c** was prompted by reports that a number of arylalkyl hydrogen succinates exhibited hypocholesterolemic activity,[7,12] possibly as a result of inhibiting β-hydroxy-β-methylglutaryl CoA reductase.

Biological Results. The cholesterol-lowering effects of the three classes of compounds (basic ethers, aryloxyalkanoic acids, and succinates) derived from **6a** were determined in male rats made hypercholesterolemic with propylthiouracil.[13] The compounds were tested orally and initially at 10 mg/kg (Table I).

Both **10b** and **10c**, the two basic ethers, showed significant cholesterol-lowering effects at 10 mg/kg. However, they also produced undesirable side effects. The former compound was observed to cause the accumulation of desmosterol.[1c,14] Although **10c** showed no untoward effects in immature mice at 25 mg/kg, five out of ten mice died when the compound was administered at 250 mg/kg. The effect of this compound on desmosterol was not determined. Neither compound showed uterotropic activity at 25 mg/kg (Table II).

Table II. Effects of Derivatives of 3-Hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene on Uterine Weight Gain in Immature Mice



R	Dose, mg/kg[a]	Uterine wt, mg: Control	Uterine wt, mg: Treated
H (6a)	625 sc	16.1 ± 4.6	34.9 ± 13.6
	250 sc	15.2 ± 1.7	22.6 ± 4.5
CH_3 (6b)	250 sc	15.3 ± 3.2	22.6 ± 3.0
$(C_2H_5)_2N(CH_2)_2\cdot(CO_2H)_2$ (10b)	25 sc	14.2 ± 4.7	16.4 ± 4.4
c-$C_4H_8N(CH_2)_2$ (10c)	25 sc	12.1 ± 2.3	11.2 ± 4.7
	250 ig	10.5 ± 2.5	8.1 ± 0.5[b]
$HO_2CC(CH_3)_2$ (11a)	25 sc	15.1 ± 2.8	17.2 ± 4.0
	250 ig	10.5 ± 2.5	8.4 ± 1.4
$HOCH_2C(CH_3)_2$ (12a)	250 sc	15.7 ± 5.0	13.4 ± 2.3
$CH_3O_2C(CH_2)_2CO_2CH_2C(CH_3)_2$ (12b)	250 sc	18.4 ± 5.3	15.7 ± 4.2
$HO_2C(CH_2)_2CO_2C(CH_3)_2$ (12c)	25 sc	15.3 ± 3.2	20.6 ± 3.3

[a] $n = 10$ for both control and treated animals. [b] Five out of ten animals died.

Table III. Hypolipidemic Effects in Normal Rats

Compd	Dose (in diet)[a]	Cholesterol, % of control	Triglyceride, % of control
11a	0.2%	75 ($t = 3.3$)[b]	69 ($t = 1.6$)[b]
Clofibrate	0.2%	72 ($t = 3.3$)[b]	43 ($t = 3.4$)[b]

[a] $n = 8$ for both control and treated animals. [b] Student's t value. A t value greater than 1.65 indicates $p < 0.05$.

Although clofibrate was inactive in the propylthiouracil-treated rat, the aryloxyacetic acid derivatives, **11a–d,f**, were tested, nevertheless, in this assay. Of these five compounds, only **11a** showed a reduction of cholesterol (Table I).

As **11a** is an analogue of clofibrate, the hypolipidemic effect of this compound was also studied in the normal rat. The test compound was administered in the diet at a level of 0.2% for a period of 5 days. In this test, 11a demonstrated a significant lowering of the cholesterol level. Although the triglyceride level was also reduced, the effect was not statistically significant. Clofibrate, on the other hand, was found to produce a significant decrease in both serum triglyceride and cholesterol levels in the normal rat (Table III).

Compound 11a was examined for its uterotropic effect.[15] Mice treated at both 250 and 25 mg/kg showed no significant uterine weight gain over control (Table II).

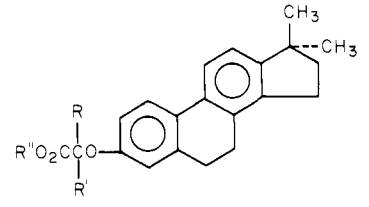
The alcohol **12a**, derived from the reduction of **11a**, produced a significant lowering of serum cholesterol in the hypercholesterolemic rat at 10 mg/kg, as did the succinate 12b. The hydrogen succinate **12c**, on the other hand, showed only marginal activity (Table I). In the uterotropic assay, **12a** and 12b were tested at 250 mg/kg, while **12c** was inadvertently tested at 25 mg/kg. Both **12a** and 12b were inactive while **12c** showed possibility of activity at the dose level tested (Table II).

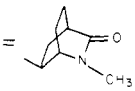
Because of the possible in vivo cleavage of **11a** and **12a–c** to afford 3-hydroxy-17,17-dimethylgona-1,3,5(10),8,13-

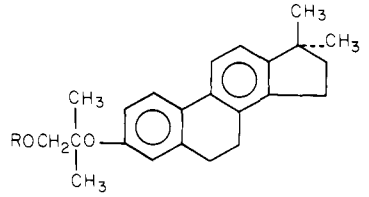
hexaene **6a**, the hypocholesterolemic and uterotropic effects of **6a**, as well as of the methyl ether **6b**, were examined.

Compound **6a** produced a cholesterol-lowering effect in the hypercholesterolemic animal at 10 and 20 mg/kg but not at 5 mg/kg. The ether **6b** was tested only at 10 mg/kg and was found to be inactive (Table I). When administered at 25 mg/kg, **6a** increased the uterine weight of immature mice. The uterotropic effect of **6a** was found to be considerably more pronounced at the higher dose level of 625 mg/kg. The methyl ether was tested only at 250 mg/kg, and it was found to promote uterine weight gain at that dose (Table II).

From these results, it would appear desirable to determine whether **11a** and **12a–c** dealkylate to furnish **6a** in vivo. If they do, it is conceivable that the hypocholesterolemic effects observed with these substances are associated in some ways with the estrogenicity of **6a**, in which case their potential use in humans would be severely limited.[1c]



11a, R = R′ = CH_3; R″ = H
b, R = R′ = CH_3; R″ = C_2H_5
c, R = R′ = CH_3; R″ = 
d, R = H; R′ = CH_3; R″ = H
e, R = R′ = R″ = H
f, R = R′ = H; R″ = C_2H_5



12a, R = H
b, R = $CH_3O_2C(CH_2)_2C{=}O$
c, R = $HO_2C(CH_2)_2C{=}O$

Experimental Section

Melting points were determined on a Fisher-Johns melting block and are uncorrected. NMR spectra were taken on a Varian A-60 instrument in deuteriochloroform with tetramethylsilane as an internal standard unless specified otherwise.

3-Hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (6a) and 3-Hydroxy-17,17-dimethylgona-1,3,5(10),8-tetraene (8a). a. A mixture of 3.0 g of 17α-methylestradiol 3-methyl ether (**7**) and ca. 15.0 g of freshly fused pyridine hydrochloride was maintained at 220 °C (bath temperature) for 1.5 h in an atmosphere of N_2. The cooled reaction mixture was diluted with a large volume of water, and the resultant solid was collected, washed well with water, and dried.

The solid was chromatographed on 300 g of silica gel. The column was eluted with varying proportions of benzene in hexane. Elution with a mixture of 80% benzene and 20% hexane afforded a crystalline product. Continued elution with 90% benzene and 10% hexane gave additional quantity of the crystalline product. The combined crystalline fractions were crystallized from ether–hexane to afford 1.18 g of a product, mp 149–152 °C. Another crystallization from ether–hexane raised the melting point to 151–154 °C: λ_{max}^{MeOH} 277.5–280 nm (ε 16 700).

GLC indicated that the product was a mixture of three substances present in a ratio of 71:22.5:6.5. The NMR spectrum displayed single methyl signals at 76, 60, and 56.5 Hz in decreasing intensity. The signal at 76 Hz was assigned to the *gem*-dimethyl group in **6a** (see below) while that at 60 Hz was attributed to the corresponding group in **8a** (see below). The signal at 56.5 Hz could be due to the *gem*-dimethyl group in an isomer of **8a**.

b. In another run in which the same quantity of reagents, the same conditions, and the same work-up were employed, 0.24 g of **8a** was obtained: mp 186–186.5 °C; λ_{max}^{MeOH} 271–271.5 nm (ε 15 050); NMR (Hz) 61 (s, 6 H, 17-CH_3). Anal. ($C_{19}H_{24}O$) C, H.

3-Methoxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (6b).[9] The procedure of Kimura et al.[10] was employed for the dehydration and rearrangement of 17α-methylestradiol 3-methyl ether (**7**). A mixture of 5.0 g of **7** and 75 ml of 18 M H_2SO_4 was stirred at room temperature for 5 min. The reaction mixture was diluted with H_2O, and the resultant mixture was then extracted with EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to afford 4.4 g of a crude product.

This product was dissolved in 240 ml of dry benzene. After 3.8 g of chloranil was added, the reaction mixture was heated under reflux for 3 h in an atmosphere of N_2. Then another 3.8 g of chloranil was added, and the reaction mixture was heated under reflux for an additional 1 h. The cooled reaction mixture was diluted with H_2O and CH_2Cl_2. The organic phase was separated, washed successively with dilute Na_2SO_3 and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from CH_2Cl_2–MeOH to furnish 2.2 g of **6b**, mp 97–98 °C (lit.[9] 102–103 °C).

The mother liquor was evaporated to dryness. The residue was chromatographed on silica gel. The column was eluted with 0.25% of EtOAc in cyclohexane to give initially mixtures containing **6b**, the phenanthrene derivative **9** (see below), **8b**, and various isomers of **8b**, following which an additional quantity of pure **6b** was obtained.

3-Methoxy-17,17-dimethyl-1,3,5(10),6,8,11,13-heptaene (9). A solution of 5.0 g of dichlorodicyanobenzoquinone and 1.5 g of **6b** in 70 ml of C_6H_6 was stirred at room temperature for 18 h. The reaction mixture was diluted with C_6H_6 and washed successively with dilute Na_2SO_3 and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was crystallized from CH_2Cl_2–hexane to yield 0.86 g of **9**: mp 163–165 °C; λ_{max}^{MeOH} 224 nm (ε 19 900), 236 (23 000), 260 (68 000), 282 (15 600), 290 (13 000), 310 (9100); NMR (Hz) 236 (s, 3, OCH_3), 81 (s, 6, 17-CH_3). Anal. ($C_{20}H_{22}O$) C, H.

3-Hydroxy-17,17-dimethylgona-1,3,5(10),8,11,13-hexaene (6a). A mixture of 15 g of freshly fused pyridine hydrochloride and 2.6 g of **6b** was heated in an atmosphere of N_2 at 220 °C for 1.5 h. The warm reaction mixture was poured into ice water. The solid was collected, washed with water, and dried. It was crystallized from ether–hexane to afford 2.4 g of **6a**: mp 152–153 °C; λ_{max}^{MeOH} 280 nm (ε 22 400); ν($CHCl_3$) 3600 cm^{-1}; NMR (Hz) 76 (s, 6 H, 17-CH_3). Anal. ($C_{19}H_{20}O$) C, H.

3-(2-Diethylaminoethoxy)-17,17-dimethylgona-1,3,5(10),-8,11,13-hexaene Oxalate (10b). A solution of 0.40 g of **6a** in 30 ml of toluene was distilled at atmospheric pressure until 10 ml of distillate was collected. To the cooled residue was added 0.20 g of a 50% dispersion of NaH in mineral oil. The resultant mixture was stirred and heated under reflux in an atmosphere of N_2 for 45 min, after which it was cooled in an ice bath. A 0.30-g sample of diethylaminoethyl chloride was added. The reaction mixture was stirred and heated under reflux in an atmosphere of N_2 for 4 h and then stirred at room temperature for 18 h. Water was carefully added to the reaction mixture. The resultant mixture was extracted with C_6H_6. The C_6H_6 extract was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure to give **10a** as an oil in a quantitative yield.

A 0.54-g sample of **10a** was dissolved in 5 ml of absolute EtOH. To this solution was added a solution of 0.19 g of oxalic acid dihydrate in 5 ml of absolute EtOH. The crystalline product **10b** which resulted was collected by filtration, washed with absolute EtOH, and dried: yield 0.46 g; mp 173–175 °C; NMR [Hz, $(CD_3)_2SO$] 192.5 (q, 2 H, J = 7 Hz, CH_3CH_2), 75 (t, 3 H, J = 7 Hz, CH_3CH_2), 73 (s, 6 H, 17-CH_3). Anal. ($C_{27}H_{35}NO_5$) C, H.

3-(2-Pyrrolidinylethoxy)-17,17-dimethylgona-1,3,5(10),-8,11,13-hexaene (10c). A solution of 4.0 g of **6a** in 350 ml of

toluene was distilled at atmospheric pressure until 10 ml of distillate was collected. To the cooled residue was added 4.0 g of a 50% dispersion of NaH in mineral oil. The resultant mixture was stirred and heated under reflux in an atmosphere of N_2 for 1 h after which it was cooled in an ice bath. A 3.1-g sample of *N*-(2-chloroethyl)pyrrolidine hydrochloride was added. The reaction mixture was stirred and heated under reflux in an atmosphere of N_2 for 4 h and then stirred at room temperature for 18 h. Water was carefully added to the reaction mixture. The resultant mixture was extracted with C_6H_6. The C_6H_6 extract was washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residual solid was crystallized from ether–hexane to afford 2.8 g of **10c**: mp 85–87 °C; λ_{max}^{MeOH} 280 nm (ε 26 600); NMR (Hz) 76 (s, 6 H, 17-CH_3). Anal. ($C_{25}H_{31}NO$) C, H.

An additional 0.7 g of **10c** was obtained by chromatography of the mother liquor on silica gel in which 5% EtOAc in hexane was employed as the eluent.

2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-2-methylpropionic Acid (11a). The procedure described for the preparation of **10c** was utilized for the synthesis of **11a**. From 1.9 g of **6a**, 1.3 g of the 50% NaH dispersion, 100 ml of toluene, and 1.75 g of 2-bromoisobutyric acid, 1.5 g of **11a** was obtained after crystallization from ether–hexane: mp 167–170 °C; λ_{max}^{MeOH} 280 nm (ε 23 300); ν ($CHCl_3$) 1775, 1720 cm^{-1}; NMR (Hz) 97 (s, 6 H, α-CH_3), 76 (s, 6 H, 17-CH_3). Anal. ($CH_{23}H_{26}O_3$) C, H.

Ethyl 2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-2-methylpropionate (11b). A solution of 0.6 g of **11a** in 100 ml of absolute EtOH containing 1 ml of 18 M H_2SO_4 was heated under reflux for 16 h and then concentrated to 10 ml by distillation under reduced pressure. The residue was dissolved in C_6H_6. The C_6H_6 solution was washed with H_2O, dried (Na_2SO_4), and distilled to dryness. The residual oil was dissolved in ether. The ethereal solution was decolorized with Darco and then evaporated to dryness to furnish **11b** as a colorless oil: ν ($CHCl_3$) 1740 cm^{-1}; NMR (Hz) 254 (q, 2 H, J = 7 Hz, CH_3CH_2), 96.5 (s, 6 H, α-CH_3), 76 (s, 6 H, 17-CH_3), 74 (t, 3 H, J = 7 Hz, CH_3CH_2). Anal. ($C_{25}H_{30}O_3 \cdot 0.25H_2O$) C, H.

2-Methyl-3-oxo-2-azabicyclo[2.2.2]octan-6-*exo*-yl 2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-2-methylpropionate (11c). A solution of 1.5 g of **11a** in 15 ml of thionyl chloride was allowed to stand at room temperature for 5 h. Then it was distilled to dryness under reduced pressure with gentle heating. The residue was dissolved in 10 ml of dry C_6H_6, and the resultant solution was again distilled to dryness under reduced pressure. This process was repeated twice with fresh portions of dry C_6H_6. The residual yellow oil was dissolved in 10 ml of C_6H_6. After the addition of 0.75 g of *exo*-6-hydroxy-2-methyl-3-oxo-2-azabicyclo[2.2.2]octane[16] and 0.55 ml of pyridine, the reaction mixture was stirred at room temperature for 2 days. Then it was extracted with ether. The ether extract was washed successively with 1.2 M HCl and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residual solid (1.1 g) was crystallized from MeOH to afford 0.8 g of **11c**: mp 157–158 °C; λ_{max} (MeOH) 280 nm (ε 23 500); ν ($CHCl_3$) 1735, 1670 cm^{-1}; NMR (Hz) 176 (s, 3 H, NCH_3), 98 (s, 6 H, α-CH_3), 76 (s, 6 H, 17-CH_3). Anal. ($C_{31}H_{37}NO_4$) C, H.

2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-propionic Acid (11d). The procedure described for the preparation of **10c** was utilized. From 0.50 g of **6a**, 0.34 g of the 50% NaH dispersion, 50 ml of toluene, and 0.28 ml of 2-bromopropionic acid, 0.48 g of **11d** was obtained after crystallization from CH_2Cl_2–hexane: mp 168–170 °C; λ_{max} (MeOH) 280 nm (ε 24 200); ν ($CHCl_3$) 1735 cm^{-1}; NMR (Hz) 99.5 (d, 3 H, J = 7 Hz, α-CH_3), 77 (s, 6 H, 17-CH_3). Anal. ($C_{22}H_{24}O_3$) C, H.

Ethyl 2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]acetate (11f). The procedure described for the preparation of **10c** was employed. From 1.5 g of **6a**, 250 ml of toluene, 1.0 g of the 50% NaH dispersion, and 1.2 g of bromoacetic acid, 0.97 g of the acetic acid derivative **11e**, which precipitated from the reaction mixture, was obtained by filtration.

A mixture of 0.77 g of **11e**, 200 ml of absolute EtOH, and 2 ml of 18 M H_2SO_4 was heated under reflux for 16 h after which it was concentrated to 10 ml by distillation under reduced pressure. The cooled residue was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residue was crystallized from ether–hexane to give 0.37 g of **11f**: mp 96–97 °C; ν ($CHCl_3$) 1760 cm^{-1}; NMR (Hz) 257 (q, 2 H, J = 7.5 Hz, CH_2CH_3), 77 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 76 (s, 6 H, 17-CH_3). Anal. ($C_{23}H_{26}O_3$) C, H.

2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-2-methylpropanol (12a). A solution of 4.7 g of **11a** in 80 ml of THF was added dropwise to a solution of 1.7 g of $LiAlH_4$ in 40 ml of THF. The reaction mixture was stirred at room temperature for 16 h. Then it was carefully treated with EtOAc and H_2O. The resultant mixture was extracted with EtOAc. The EtOAc extract was washed successively with 10% KOH and H_2O, dried (Na_2SO_4), and distilled nearly to dryness under reduced pressure. The residue was crystallized from CH_2Cl_2–hexane to give 2.54 g of **12a**: mp 122–125 °C; ν ($CHCl_3$) 3600 cm^{-1}; NMR (Hz) 217.5 (s, 2 H, CH_2OH), 78 (s, 6 H, α-CH_3), 76 (s, 6 H, 17-CH_3). Anal. ($C_{23}H_{28}O$) C, H.

2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-2-methylpropyl Methyl Succinate (12b). To a solution of 2.2 g of **12a** in 90 ml of pyridine, cooled in an ice bath, was added slowly and in an atmosphere of N_2 13.0 g of β-carbomethoxypropionyl chloride.[17] The reaction mixture was vigorously stirred at room temperature for 3 h and then poured into H_2O. The resultant mixture was extracted with EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residue was chromatographed on 800 g of silica gel. Elution of the column with 10% EtOAc in C_6H_6 afforded a crystalline product which was crystallized from MeOH to yield 0.99 g of **12b**: mp 58–60 °C; ν ($CHCl_3$) 1745 cm^{-1}; NMR (Hz) 250 (s, 2 H, CH_2O_2C), 222 (s, 3 H, OCH_3), 80 (s, 6 H, α-CH_3), 76 (s, 6 H, 17-CH_3). Anal. ($C_{28}H_{34}O_5$) C, H.

2-[17,17-Dimethylgona-1,3,5(10),8,11,13-hexaen-3-yloxy]-2-methylpropyl Hydrogen Succinate (12c). a. A mixture of 0.70 g of **12b** and 2.4 g of anhydrous lithium iodide in 70 ml of dry pyridine was heated under reflux in an atmosphere of N_2 for 3 days. The cooled reaction mixture was poured into 1.2 M HCl, and the resultant mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed successively with 1.2 M HCl and H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residue was crystallized from ether–hexane to afford 0.39 g of **12c**: mp 118–120 °C; ν ($CHCl_3$) 1740, 1720 cm^{-1}; NMR (Hz) 250 (s, 2 H, CH_2O_2C), 80 (s, 6 H, α-CH_3), 76 (s, 6 H, 17-CH_3). Anal. ($C_{27}H_{32}O_5$) C, H.

b. A mixture of 25 mg of **12b** and 100 mg of anhydrous lithium iodide in 5 ml of dry DMF was heated under reflux in an atmosphere of N_2 for 2 days. The cooled reaction mixture was poured into 1.2 M HCl, and the resultant mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed successively with 1.2 M HCl and H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. TLC and NMR spectroscopy indicated that the residual product was the phenol **6a** and not **12c**.

c. To a solution of 50 mg of **12b** in 5 ml of MeOH was added 0.22 ml of 1 N NaOH. The reaction mixture was stirred at room temperature in an atmosphere of N_2 for 18 h. Then it was poured into 1.2 M HCl, and the resultant mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to afford 44 mg of a solid. TLC, as well as IR and NMR spectroscopy, indicated that the product was the alcohol **12a**.

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p-Aminobenzoic Acid Derivatives as Inhibitors of the Cell-Free H_2-Pteroate Synthesizing System of *Escherichia coli*

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A heterogeneous series of compounds, derived from *p*-aminobenzoic acid (PABA), has been investigated for their PABA-antagonistic potency in a cell-free H_2-pteroate synthesizing system of *E. coli*. A prerequisite of compounds, other than sulfones or sulfonamides, to compete with PABA for the enzyme H_2-pteroate synthetase appeared to be the presence of a *p*-aminobenzoyl moiety. Substitution of the carboxyl group of PABA by an ester, an amide, or a ketone function, however, strongly reduces the ability to interact with the PABA binding site on the enzyme. This decrease in affinity probably has to be ascribed to the inability to create a sufficient negative charge in the carbonyl part of these *p*-aminobenzoyl derivatives. The relatively high affinities of L-PABG (**16**), PABP (**22**), and the α-phenyl derivative of **22**, as compared with the other substituted *p*-aminobenzamides and *p*-aminobenzene-1-alkanones, are explained by assuming that these compounds, besides interfering with the PABA receptor site, also interact with an accessory area on the enzyme.

The discovery in 1940 that *p*-aminobenzoic acid (PABA) antagonizes the bacteriostatic action of the sulfonamides[1] initiated the investigation of numerous derivatives of PABA for their antibacterial activity. With the exception of the tuberculostatic *p*-aminosalicylic acid, however, none of these derivatives showed useful antimicrobial activity (for reviews see ref 2 and 3).

The elucidation of the folate-synthesizing system in bacteria,[4-6] plants,[7,8] and protozoa[9-11] definitely showed the sulfonamides to exert their bacteriostatic action by inhibiting competitively the enzyme H_2-pteroate synthetase, the enzyme which catalyzes the condensation of PABA with the pteridine moiety to give 7,8-dihydropteroic acid (H_2-pteroate), the precursor of the biologically important folates.

In today's antiinfectious therapy the sulfonamides had to make way for the antibiotics, not lastly because of some serious side effects that may occur with the former. Yet, inhibitors of the pteroate-synthesizing system, at least in theory, would be ideal chemotherapeutic agents because higher organisms, e.g., mammals, do not possess this biosystem. Therefore, it seemed worthwhile to pay some more attention to derivatives of PABA as potential PABA antagonists.

Earlier investigations already revealed that, because of steric effects, the introduction of substituents into the benzene nucleus of PABA reduces the affinity for the enzyme H_2-pteroate synthetase.[12,13] The present paper deals with the interaction of several PABA analogues, modified in the amino and/or carboxyl group, with the cell-free system of *Escherichia coli*. The results, in general, confirm earlier views about the structural requirements necessary to interfere with the PABA-converting enzyme system.[2,3] In addition, indications are obtained for the existence of an accessory binding area on the enzyme. Such a binding area might be used as a target for further exploration, possibly leading to useful antibacterial drugs of a new structural type.

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Experimental Section

Determination of the Enzyme Activity. Cell-free extracts of *E. coli* B were prepared as described previously.[14] The formation of H_2-pteroate in a reaction mixture was determined by a radioassay method. The mixtures were prepared to contain in a volume of 0.45 ml: 0.5 μmol of ATP, 2 μmol of $MgCl_2$, 0.5 mg of sodium ascorbate, 0.1 mmol of Tris–HCl buffer (pH 8.0), 20 nmol of 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine,[14] and enzyme preparation containing 400–600 μg of protein. After incubation at 37 °C for 10 min, 50 μl of a [7-^{14}C]-PABA solution (0.1–0.3 M) was added and the incubation was continued for another 40 min. The reaction was stopped by adding 1.5 ml of a concentrated citric acid phosphate buffer, pH 3.8, whereafter [^{14}C]-PABA, which was not incorporated into H_2-pteroate, was extracted by ether. The radioactivity remaining in the water layer is a quantitative measure of the H_2-pteroate formed.[12]

Determination of the Inhibition Index. The inhibition of the cell-free H_2-pteroate synthesis was followed at six inhibitor concentrations in duplicate experiments. The inhibition index, I.I. ($=I_{50}/S$), was determined by plotting the percent inhibition vs. the logarithm of the inhibitor concentration. Interpolation for 50% inhibition and dividing the inhibitor concentration (I_{50}) by the substrate concentration (S) will give the relative affinity of the compound, expressed as I.I. These determinations were performed at least at two different PABA concentrations in order