cy was observed in the case of the octyl compound 8. The binding ratio (1.0) indicated that it should be a nearly pure antagonist. However, no antagonist activity was detected in monkeys[10] (at 5.0 mg/kg) and only about 1% of the antagonist potency of nalorphine was observed in the guinea pig ileum.[5] As shown in Table I, 8 has weak but detectable analgesic potency. The reason for this discrepancy is not clear. The nonyl compound 9 and the decyl compound 10 were also predicted to be nearly pure antagonists based on this ratio (1.0 and 1.35, respectively). However, neither compound had sufficient potency in the hot-plate test, monkeys,[10] or the guinea pig ileum[5] for accurate characterization of its properties.

Some additional aspects of these results seem worthy of comment. The good correlation observed between binding and analgesic potency further suggests that this *in vitro* mixture contains a receptor involved in mediating the analgesic response *in vivo*.

Additionally, the observed correlation suggests minimal differences in metabolism and distribution at the time of peak analgesic effect in this series of compounds. Therefore, this series of compounds might be a useful tool in further investigation of the mechanism of opiate analgesia.

In summary, a statistical correlation was found between the *in vivo* analgesic potencies of a series of *N*-alkylnorketobemidones and both their relative binding abilities and their abilities to antagonize electrically induced contractions of the guinea pig ileum. The statistical correlation between *in vitro* binding in the presence of 100 m*M* sodium and *in vivo* determined analgesic potency was not as good as that which was found in the absence of sodium.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. The structures of compounds were in all cases supported by their nmr, ir, and mass spectra. Elemental analyses (indicated by C, H, Br, and N when within ±0.4% of theoretical values) were performed by the Section on Microanalytical Services and Instrumentation of this laboratory.

***N*-Octylnorketobemidone (8) Hydrobromide.** To 100 ml of DMF was added 5.0 g (0.021 mol) of norketobemidone, 4.5 g (0.024 mol) of 1-bromooctane, and 7.5 g of K_2CO_3, and the mixture was stirred overnight at 90–95°. The solvent was evaporated, the residue taken up in 250 ml of $CHCl_3$ and washed with H_2O, and the organic layer was dried (Na_2SO_4) and evaporated. This residue was dissolved in acetone, acidified to Congo Red with 33% HBr in AcOH, and crystallized by addition of ether. Recrystallization from ether–acetone gave white crystals, mp 137–139°. *Anal.* ($C_{22}H_{36}BrNO_2$) C, H, Br, N.

***N*-Nonylnorketobemidone (9) Hydrobromide.** Using the procedure described above for 8 white crystals were obtained, mp 146–148°. *Anal.* ($C_{23}H_{38}BrNO_2$) C, H, Br, N.

***N*-Decylnorketobemidone (10) Hydrobromide.** Using the procedure described above for 8 white crystals were obtained, mp 139–140°. *Anal.* ($C_{24}H_{40}BrNO_2$) C, H, Br, N.

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Hypocholesteremic Derivatives of Styrylacetic Acid. 1. *gem*-Dimethyl Analogs of Benzalbutyric Acid

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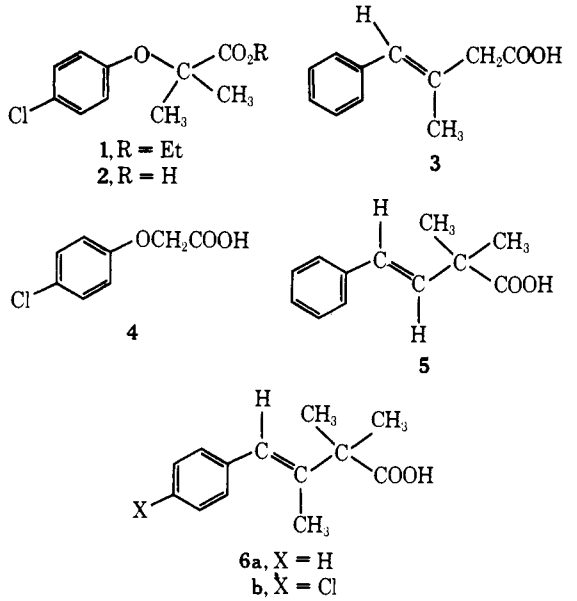
The preparation of α,α-dimethyl analogs of the hypocholesteremic and hypolipemic agent 3-methyl-4-phenyl-3-butenoic acid (β-benzalbutyric acid, BBA) is described. These compounds were prepared as part of a continuing program directed toward a study of the structure–activity interrelationships of styryl- and phenoxyacetic acid antilipemic agents and the preparation of metabolically resistent analogs of BBA. Preliminary results on the *in vitro* ability of the compounds to inhibit cholesterol biosynthesis indicate that α,α-dimethyl substitution reduces activity although the potency of the *p*-chloro analog **6b** was comparable to that of BBA.

The etiology of atherosclerosis has continued to remain obscure despite considerable research in this area. Therapeutic approaches to this disease have therefore centered on the minimization of epidemiologically defined risk factors and primarily on the reduction of serum lipoprotein levels with particular emphasis on cholesterol and its es-

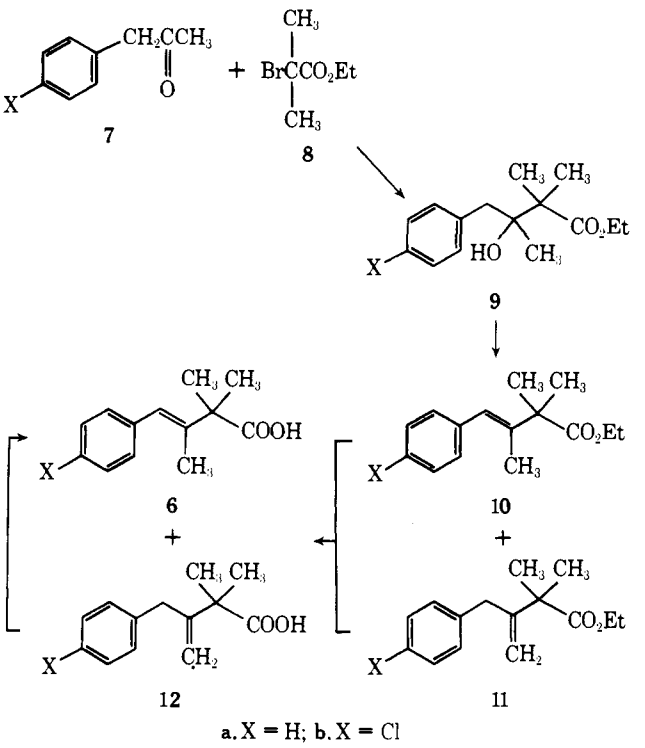
ters. Although undesirable clinical findings of compounds introduced during the 1950's led to a reduction in research efforts directed toward the discovery of hypocholesteremic agents, the report of hypocholesteremic activity of ethyl 2-(4′-chlorophenoxy)-2-methylpropanoate (**1**, clofibrate) by Thorp in 1962[1] and the realization that its hydrolysis product **2** was the effective agent prompted a renewed emphasis toward the development of cholesterol biosynthesis inhibitors. The mechanism of action of **2** and its analogs is multifaceted;[2] however, at clinical dosages their ability to inhibit cholesterol biosynthesis is apparently rather low. It would therefore be desirable to find compounds which would retain the pharmacological profile of **1** while enhancing its cholesteropenic activity.

In 1959 Canonica and associates reported the synthesis[3] and hypocholesteremic activity[4] of 3-methyl-4-phenyl-3-butenoic acid (**3**, BBA). Subsequent investigations with this compound and its amide derivative have shown that its pharmacological profile in man and laboratory animals is quite similar to that of **1**[5] and that its ability to penetrate tissue barriers is somewhat greater.[6] While there appears to be some disagreement as to the exact site of cholesterol biosynthesis inhibition by **3**,[7] both **2** and **3** exert their principal effect *in vitro*, at some stage prior to the formation of mevalonic acid.[8] An important disadvantage of BBA, however, is its ready susceptibility to metabolic hydroxylation,[9] resulting in a much shorter duration of action. Since *p*-chlorophenoxyacetic acid (**4**) is also an effective inhibitor of cholesterol biosynthesis *in vitro*,[10] it seems possible that both types of compounds might be affecting the same receptor site(s). We therefore set out to prepare analogs of **3** in order to investigate structure–activity interrelationships between compounds of these two series and to provide compounds which might retain the biological activity of **3** and be less prone to metabolism. In this report we describe our initial efforts toward this goal with the investigation of compounds **5** and **6**.



Chemistry. The synthesis of **5** has been previously described.[11] Compounds **6a** and **6b** were prepared by a similar sequence of reactions as shown in Scheme I. Standard procedures[12] for the Reformatsky reaction were employed to obtain the β-hydroxy esters, **9**, which were dehydrated with $POCl_3$ to a mixture of olefins **10** and **11**. Huffman and Bethea[13] have previously reported that dehydration of **9a** with $POCl_3$ leads to a mixture of olefins which they assumed to be **10a** and its corresponding cis isomer. Analysis of the nmr spectrum of this mixture and that of its saponification product, however, revealed that the mixture consisted of position isomers **10a** and **11a** in approximately a 3:2 ratio, respectively. The relative proportion of isomers was determined from integration of the vinyl proton region of the spectrum with the benzal proton of **10a** appearing at 6.45 ppm and the methylene protons of **11a** of 4.65 and 5.15 ppm.

Scheme I



The desired acids **6** could be separated by repeated chromatography on silica gel; however, this procedure was unsatisfactory for preparation of the compounds on a scale required for biological studies. Several methods of isomerization were attempted and it was found that the mixture could be nearly quantitatively converted to the desired isomers by treatment with KO-*t*-Bu in *t*-BuOH–DMSO (2:1). As previously observed for isomerizations of this type, migration of the double bond into the position of conjugation fails to occur when *t*-BuOH alone is used as the solvent[14] and in fact affords **12** as the predominant isomer.

Biological Results. The compounds prepared were tested for their ability to inhibit *in vitro* incorporation of acetate-2-^{14}C into nonsaponifiable sterols at concentrations varying from 0.1 to 10 m*M*. The data obtained are shown in Table I along with the data obtained for the parent compound, BBA (**3**), using the same test procedure.

These results show that *gem*-dimethyl groups can be introduced α to the carboxyl function without destruction of activity. On the basis of the activities of compounds **5** and **6a**, however, it would appear that this structural alteration causes a significant reduction in potency with regard to *in vitro* activity. In this regard, the greater activity of **6b** with respect to **6a** is surprising since introduction of the *p*-chloro substituent into BBA has been previously reported to result in a less potent compound.[15] The reason for this discrepancy is not clear although one possible explanation is that the material used for biological examination of the *p*-chloro analog of BBA might have been contaminated with a substantial amount of the α,β-unsaturated isomer. We found the α,β-unsaturated isomer of BBA to be essentially

Table I. Effect of BBA and Analogs on Incorporation of Acetate-2-^{14}C into Neutral Sterols by Liver Homogenate Preparations

Compd no.	Concn, m*M*	% incorporation[a]
3	10	6.7 ± 0.51[b]
	1	40.9 ± 0.95
	0.5	54.4 ± 2.0
	0.1	72.7 ± 9.9
5	10	3.4 ± 0.67
	1	89.8 ± 2.0
	0.5	99.3 ± 5.0
	0.1	94.0 ± 0.78
6a	10	7.4 ± 0.43
	5	41.5 ± 3.2
	1	91.8 ± 3.4
	0.5	95.6 ± 4.8
6b	10	0.8 ± 0.08
	5	1.9 ± 0.25
	1	43.5 ± 2.9
	0.5	60.1 ± 3.2
	0.1	79.8 ± 3.9

[a] Relative to incorporation in controls defined as 100%. [b] Standard error of mean of three experiments.

inactive as an inhibitor of cholesterol biosynthesis in our *in vitro* procedure (unpublished data).

Based on a comparison of compounds **5** and **6a**, it would appear that the methyl group in the 3 position of the side chain is of relatively little importance with regard to *in vitro* potency of the compounds. The importance of this observation will only become clear upon *in vivo* examination of the effects of these compounds since the principal goal of these structural alterations was to produce compounds which would be incapable of undergoing the same metabolic transformations known to occur with the parent compound.

The results presented in this report do not appear to support the suggestion that the two series of hypocholesteremic agents derived from **2** and **3** are affecting the same receptor sites since the presence or absence of the *gem*-dimethyl groups in the clofibrate series did not significantly affect *in vitro* activity.[10] We are preparing and testing asymmetric analogs of **3** which should provide further insight into this question.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, the analytical results for these elements were within ±0.4% of the theoretical value. Ir spectra were recorded on a Beckmann Acculab 3 spectrophotometer. Nmr spectra were obtained with a Hitachi Perkin-Elmer Model R-24 spectrometer using TMS as the internal standard. Unless otherwise noted, the spectra were obtained using $CDCl_3$ as the solvent. For the biological evaluations, ^{14}C counting was performed with a Packard Tri-Carb liquid scintillation spectrometer, Model 3375.

3-Methyl-4-phenyl-3-butenoic acid (3) was prepared by the method of Valcavi and Gaudenzi[15] affording white crystals from hexane: mp 108–109° (lit.[7a] 112–113°). *Anal.* ($C_{11}H_{12}O_2$) C, H.

2,2-Dimethyl-4-phenyl-3-butenoic Acid (5). The ethyl ester of **5**[11] (8.7 g, 0.04 mol) was dissolved in 50 ml of MeOH and 6.6 g (0.1 mol) of 85% KOH was added. The mixture was heated at reflux for 2 hr, cooled, and diluted with 200 ml of H_2O. The resulting mixture was extracted with Et_2O and the aqueous solution acidified and extracted with 3 × 75 ml of Et_2O. Combined Et_2O extracts were washed with saturated NaCl solution and dried ($MgSO_4$) and the solvent was removed under reduced pressure affording 7.3 g (96%) of crude **5**. Recrystallization from hexane after treatment with charcoal afforded pure **5** as short white needles: mp 111–112° (lit.[11] 111.5–112.5°); nmr δ 1.57 (s, 6, *gem*-Me_2), 6.60 (s, 2, vinyl protons), 7.48 (s, 5, phenyl), 10.40 (br s, 1, COOH). *Anal.* ($C_{12}H_{14}O_2$) C, H.

2,2,3-Trimethyl-4-phenyl-3-butenoic Acid (6a). A mixture of phenylacetone (26.8 g, 0.20 mol) and activated Zn[16] (14.3 g, 0.22 g-atom) in 150 ml of dry benzene under a N_2 atmosphere was heated to 80° and ethyl 2-bromoisobutyrate (42.9 g, 0.22 mol) added dropwise over 20 min. The resulting mixture was then heated at reflux an additional 2 hr. After cooling the mixture in an ice bath to 15°, 200 ml of 2 *N* H_2SO_4 was added with the temperature maintained below 25°. The resulting layers were separated and the aqueous phase was extracted with 3 × 100 ml of Et_2O. The combined organic layers were washed with 2 × 100 ml of saturated NaCl solution and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was distilled affording 32.4 g (65%) of hydroxy ester **9a**, bp 115–120° (0.5 mm) [lit.[14] bp 145–150° (6 mm)].

A mixture of the hydroxy ester (26.9 g, 0.108 mol) and $POCl_3$ (35 ml) in 120 ml of pyridine was heated at reflux. After 4 hr, the solution was cooled in an ice bath and the excess $POCl_3$ cautiously was decomposed by the addition of ice chips (100 g) and 200 ml of 2 *N* H_2SO_4 was added. The resulting mixture was then extracted with Et_2O (3 × 100 ml) and the combined organic layers were washed with saturated NaCl solution and dried (Na_2SO_4), and the solvent was removed *in vacuo*. Distillation of the residue afforded 23.5 g (94%) of the unsaturated esters: bp 95–98° (0.06 mm) [lit.[13] bp 134–138° (5 mm)].

A 10.6-g (0.046 mol) sample of the mixture of olefinic esters was subjected to hydrolysis by refluxing with 5 g of NaOH in 50 ml of MeOH for 20 hr (shorter reaction times resulted in incomplete hydrolysis and recovery of a mixture of esters). The reaction mixture was diluted with 200 ml of H_2O and extracted with 3 × 50 ml of Et_2O. The remaining aqueous solution was acidified with 10% H_2SO_4 and extracted with 4 × 50 ml of Et_2O. The combined Et_2O extracts were washed with saturated NaCl solution and dried (Mg_2SO_4), and the solvent was removed *in vacuo* affording 8.0 g (86%) of a slightly yellow solid, mp 72–78°.

A 9.7-g (0.048 mol) sample of the mixture of isomeric acids was dissolved in 150 ml of DMSO and added to a solution prepared from 9.3 g (5 equiv) of K and 300 ml of *t*-BuOH. The solution was allowed to stir at 70–75° for 45 min, poured into ice (300 g), acidified with 20% H_2SO_4 (300 ml), and extracted with Et_2O (5 × 150 ml). The combined Et_2O extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The DMSO was removed under high vacuum affording a yellow solid which was dissolved in hot heptane, treated with charcoal, and allowed to cool affording 8.4 g (87%) of a slightly yellow solid, mp 93.5–96°. Concentration of the filtrate and cooling in refrigerator afforded an additional 1.2 g (12%) of a white solid, mp 79–86°. The latter material could be recycled such that the isomerization was essentially quantitative. An additional recrystalization of the higher melting solid from heptane afforded colorless plates: mp 96–98°; nmr δ 1.43 (s, 6, *gem*-Me_2), 1.85 (s, 3, vinyl Me), 6.51 (s, 1, vinyl H), 7.30 (s, 5, aromatic protons), and 11.34 (s, 1, CO_2H). *Anal.* ($C_{13}H_{16}O_2$) C, H.

2,2,3-Trimethyl-4-(4′-chlorophenyl)-3-butenoic acid (6b) was prepared by a similar sequence of reactions employed for the preparation of **6a**. Thus, from 8.43 g (0.05 mol) of *p*-chlorophenylacetone[17] was obtained 11.25 g (79%) of **9b**: bp 115–120° (0.03 mm); ir 3480 (OH), 1720 (CO_2Et), and 1250 cm^{-1} (*gem*-Me_2). This hydroxy ester (10.0 g, 0.035 mol) was dehydrated to give 6.8 g (73%) of a mixture of olefins **10b** and **11b**, bp 112–118° (0.2 mm), which was hydrolyzed affording 4.1 g (67%) of an off-white solid, mp 80–100° (heptane), consisting of a mixture of acids **6b** and **12b**. Isomerization of 1.40 g (5.9 mmol) of the mixture of acids gave 1.3 g (97%) of crude **6b** as a pale yellow solid, mp 92–97°. Recrystallization from hexane afforded pure **6b**: mp 99–100°; nmr δ 1.45 (s, 6, *gem*-Me_2), 1.85 (br s, 3, vinyl Me), 6.48 (br s, 1, vinyl H), 7.28 (m, 4, aromatic), and 11.05 (s, 1, CO_2H). *Anal.* ($C_{13}H_{15}O_2Cl$) C, H, Cl.

2,2-Dimethyl-3-(4′-chlorobenzyl)-3-butenoic Acid (12b). Treatment of 4.5 g of the above mixture of **6b** and **12b** with a solution prepared from 4.48 g (6 equiv) of K and 200 ml of *t*-BuOH at 60° for 1 hr followed by reisolation of the acidic material in the usual manner afforded an off-white solid which was dissolved in hot hexane. The hexane solution was allowed to cool to room temperature affording 2.8 g of impure **12b**, mp 120–123°. Recrystallization afforded a pure sample: mp 125–126°; nmr δ 1.43 (s, 6, *gem*-Me_2), 3.40 (s, 2, benzyl CH_2), 4.70 and 5.20 (2 s, 2, vinyl CH_2), 7.21 (m, 4, aromatic), and 10.6 (br s, 1, CO_2H). *Anal.* ($C_{13}H_{15}O_2Cl$) C, H, Cl.

Inhibition of Acetate-2-^{14}C Incorporation into Neutral

Sterols. Livers were obtained from male Sprague–Dawley rats and homogenates prepared according to the method of Bucher[18] using 0.1 *M* phosphate buffer, pH 7.3, as the suspending medium except that debris was removed by centrifugation at 500g at room temperature for 5 min. Each incubation flask was made to contain 1.0 ml of supernatant, 0.1 ml of stock drug solution, cofactors as described by Holmes and Bentz,[19] and 1.0 μCi of acetate-2-^{14}C and brought to a final volume of 2.5 ml with the phosphate buffer. Stock drug solutions were prepared in 0.5 *N* NaOH solution such that 0.1 ml diluted to 2.5 ml would give the desired final concentration. An identical quantity of 0.5 *N* NaOH solution was added to each of the control flasks. Results for each experiment were obtained by averaging the data from triplicate flasks. Flasks were incubated for 1 hr in air at 37° with slow shaking (60 cpm) after which they were placed on ice and 3.0 ml of 15% KOH in 50% EtOH was added to stop the reaction. The contents of each flask were then transferred to a 15-ml glass-stoppered centrifuge tube and the flask was rinsed with 3.0 ml of the KOH solution. The reaction mixture was then saponified by heating the stoppered centrifuge tubes in a water bath at 75–80° for 1 hr.

The cooled saponification mixtures were extracted three times with 3.0 ml of hexane and the combined extracts diluted with hexane to 10.0 ml and dried (Na_2SO_4) overnight. A 5.0-ml aliquot of the dried hexane solution was added to 10.0 ml of scintillation solution (0.4% PPO in PhMe–95% EtOH, 70:30 v/v) in a standard counting vial. A sufficient number of counts were obtained to reduce the standard deviation to 1.0% or less. The data obtained were used directly to determine the % cpm from flasks which contained the test compounds relative to controls (defined as 100% incorporation).

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Antiviral Quinolinehydrazones. A Modified Free-Wilson Analysis

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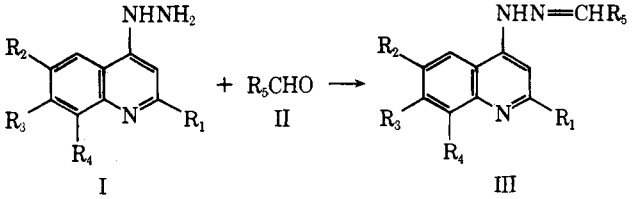
Eighty-four 4-quinolinehydrazones were synthesized and tested for antiviral activity. Thirty-nine derivatives were active against Influenza A_2 and/or Coxsackie B1 in mice at a dose of 25 mg/kg sc. Structure–activity relationships of 44 derivatives (21 inactive) were analyzed qualitatively using a modified Free–Wilson approach.

4-Quinolinehydrazones have antimalarial,[1] antimycoplasmal,[2] anticestode,[3] and tuberculostatic[4] activity. In light of their broad antiinfective profile, we elected to evaluate their effects on the replication of viruses of clinical concern.

Eighty-four 4-quinolinehydrazones (Tables I and II) were prepared by condensation of the appropriate 4-hydrazinoquinoline and aldehyde. They were tested in a unique *in vivo* antiviral screen *vs.* three viruses: Influenza A_2, Coxsackie B1, and Herpes simplex as described in the Experimental Section. Thirty-nine of the compounds were active against Influenza A_2; thirteen were active against Coxsackie B1; none was active against Herpes simplex.

Experimental Section

Chemistry. The compounds were prepared by refluxing equimolar amounts of 4-hydrazinoquinoline[3,5] or its HCl salt (I) and the appropriate carboxaldehyde (II) in EtOH for 1–2 hr. On cool-



I III

where R_1–R_5 are listed in Tables I and II