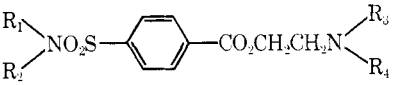
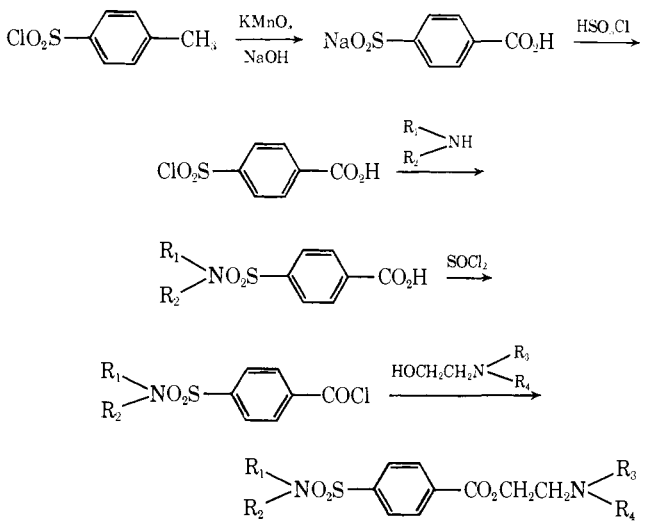
TABLE I



Compd	R_1	R_2	R_3	R_4	Yield, %	Mp, °C Base	Mp, °C HCl	$n^{19}D$ (base)	Formula[e]	Rel act.[f]
1	CH_3	CH_3	CH_3	CH_3	67	98	206[a]		$C_{13}H_{20}N_2O_4S$	–
2	CH_3	CH_3	C_2H_5	C_2H_5	72	57	177[a]		$C_{15}H_{24}N_2O_4S$	–
3	CH_3	CH_3	$-(CH_2)_5-$		81	103	200		$C_{16}H_{24}N_2O_4S$	0.3
4	CH_3	CH_3	$O(CH_2CH_2-)_2$		76	88	188		$C_{15}H_{22}N_2O_5S$	–
5	C_2H_5	C_2H_5	CH_3	CH_3	88	Oil	178[b]	1.5181	$C_{15}H_{24}N_2O_4S$	0.1
6	C_2H_5	C_2H_5	C_2H_5	C_2H_5	81	Oil	172[c]	1.5140	$C_{17}H_{28}N_2O_4S$	0.4
7	C_2H_5	C_2H_5	$-(CH_2)_5-$		69	Oil	178	1.5304	$C_{18}H_{28}N_2O_4S$	0.3
8	C_2H_5	C_2H_5	$O(CH_2CH_2-)_2$		77	Oil	188	1.5241	$C_{17}H_{26}N_2O_5S$	0.1
9	C_3H_7	C_3H_7	CH_3	CH_3	89	Oil	190	1.5165	$C_{17}H_{28}N_2O_4S$	0.5
10	C_3H_7	C_3H_7	C_2H_5	C_2H_5	86	Oil	171	1.5103	$C_{19}H_{32}N_2O_4S$	0.2
11	C_3H_7	C_3H_7	$-(CH_2)_5-$		81	Oil	207[d]	1.5239	$C_{20}H_{32}N_2O_4S$	0.5
12	C_3H_7	C_3H_7	$O(CH_2CH_2-)_2$		79	Oil	207	1.5165	$C_{19}H_{30}N_2O_5S$	–
13	$-(CH_2)_2-$		CH_3	CH_3	65	85	203		$C_{15}H_{22}N_2O_4S$	–
14	$-(CH_2)_4-$		C_2H_5	C_2H_5	68	50	175		$C_{17}H_{26}N_2O_4S$	0.4
15	$-(CH_2)_4-$		$-(CH_2)_5-$		73	133	160		$C_{18}H_{26}N_2O_4S$	–
16	$-(CH_2)_4-$		$O(CH_2CH_2-)_2$		69	140	195		$C_{17}H_{24}N_2O_5S$	–
17	$-(CH_2)_5-$		CH_3	CH_3	72	114	219		$C_{16}H_{24}N_2O_4S$	0.4
18	$-(CH_2)_5-$		C_2H_5	C_2H_5	65	62	123		$C_{18}H_{28}N_2O_4S$	0.2
19	$-(CH_2)_5-$		$-(CH_2)_5-$		61	101	130		$C_{19}H_{28}N_2O_4S$	0.2
20	$-(CH_2)_5-$		$O(CH_2CH_2-)_2$		95	102	207		$C_{18}H_{26}N_2O_5S$	0.1

[a] Reference 4; melting point was not given. [b] Reference 2; melting point was not given. [c] Lit.[5] mp 175–177°. [d] Reference 3; melting point was not given. [e] All compounds prepared were subjected to ir and nmr spectroscopy and showed the expected absorptions. Analytical data were within ±0.30% of theoretical values. [f] Procaine hydrochloride as a 1% solution by subcutaneous injection = 1; – = inactive.

SCHEME I



chloride. The corresponding piperidinoethyl ester (**11**) was as active as cocaine hydrochloride. Subcutaneous injections of 0.5 ml of 1% solutions of **9** or **11** showed an activity comparable with that of 0.5 ml of 0.5% of procaine hydrochloride. The local anesthetic activity of the compounds prepared are included in Table I.

Experimental Section[7]

***p*-Pyrrolidinosulfonylbenzoic Acid.**—To a solution of 7.1 g (0.1 mole) of pyrrolidine in 40 ml of 10% aqueous NaOH was added with stirring 11 g (0.05 mole) of *p*-chlorosulfonylbenzoic acid.[8] After 3 hr the clear solution was acidified with HCl and the precipitate was filtered and recrystallized from MeOH–Me_2CO to give 6 g (56%) of product, mp 239°, ir and nmr (CF_3COOH) as expected. *Anal.* ($C_{11}H_{13}NO_4S$) N.

***p*-Pyrrolidinosulfonylbenzoyl Chloride.**—*p*-Pyrrolidinosulfonylbenzoic acid (2.55 g 0.01 mole), and 5 ml of $SOCl_2$ were allowed to react 1 hr at room temperature, then excess $SOCl_2$ was distilled off and the residue was washed with ice–water and filtered until dry. The yield was almost quantitative, mp 154°. *Anal.* ($C_{11}H_{12}ClNO_3S$) Cl.

***p*-Pyrrolidinosulfonylbenzoic Acid Dimethylaminoethyl Ester (13).**—To *p*-pyrrolidinosulfonylbenzoyl chloride (2.7 g, 0.01 mole) in 20 ml of dry C_6H_6 was added 0.9 g (0.01 mole) of dimethylaminoethyl alcohol in 5 ml of dry C_6H_6. The mixture was allowed to stand overnight at room temperature and then the solvent was evaporated *in vacuo*. The crystalline mass was recrystallized (EtOAc–EtOH) to give 2.3 g (65%) of product, mp 203°. The free base was recrystallized from EtOH–H_2O; mp 85°, ir and nmr ($CDCl_3$) as expected. *Anal.* See Table I.

(7) Melting points were taken on a Kofler hot stage microscope. The ir spectra were determined with a Leitz Model III spectrograph (KBr). Nmr spectra were obtained on a Varian A60A instrument using Me_4Si as internal standard.

(8) S. Smiles and O. C. Harrison, *J. Chem. Soc.*, **121**, 2022 (1922).

Inhibition of Cholesterolgenesis *in Vitro* by Chlorophenoxyacetic Acids. Effect of α-Methyl Groups[1a]

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Received March 10, 1969

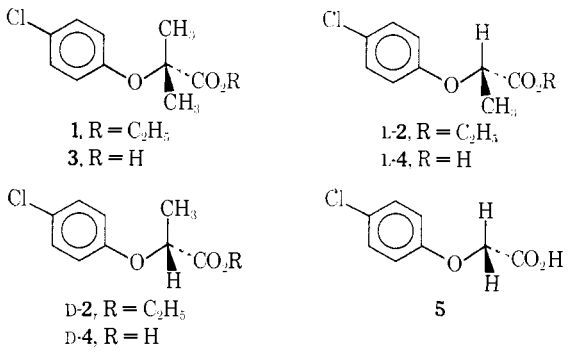
We recently reported that the two isomeric desmethyl analogs of ethyl α-(4-chlorophenoxy)-α-methylpropionate (**1**)[2] differ in their hypocholesterolemic activity in rats; only the L isomer **2** is equally as active as **1** *in vivo*.[3] The D isomer **2** is not significantly active. Experiments in this laboratory have shown that like **1**, which readily undergoes *in vivo* and *in vitro* hydrolysis to **3**, esters L-**2** and D-**2** are readily hydrolyzed to their respective acids L-**4** and D-**4** by rat liver, serum, and

(1) (a) Presented to the Division of Medicinal Chemistry, 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 13–18, 1969. (b) American Foundation for Pharmaceutical Education Fellow. (c) National Science Foundation Senior Visiting Foreign Scientist 1967–1968.

(2) Clofibrate: chlorpenisate, ICI-28257, CPIB, Atromid-S, Regelan.

(3) D. T. Witiak, T. C.-L. Ho, R. E. Hackney, and W. E. Connor, *J. Med. Chem.*, **11**, 1086 (1968).

intestinal preparations.[4] The acid **3** is known to block sterol biosynthesis after mevalonate[5] and before squalene[6] with a minimal effect before mevalonate.[6] For these reasons we have now examined the effects of **3**, L-**4**, D-**4**, and **5**, added *in vitro*, on the incorporation of acetate-1-^{14}C and mevalonate-2-^{14}C into nonsaponifiable material, squalene and cholesterol.



The inhibitory effect of these compounds on the incorporation of acetate-1-^{14}C into nonsaponifiable material is shown in Table I. At a concentration of 1.5 m*M* acids, **3**, L-**4**, and **5** inhibited acetate incorporation about 30%. The inhibition at the 1.5-m*M* dose was slightly greater when acetate rather than mevalonate (Figure 1) was used, but with either precursor the difference in activity between **3**, L-**4**, and **5** was not significant. The D-**4** isomer exhibited less than one-fourth the activity ($P < 0.02$) of either **3**, L-**4**, or **5** when acetate was the precursor. Dose–response curves (Figure 1) further substantiate the greater *in vitro* activity of acids **3**, L-**4**, and **5**. Within experimental error these three compounds exhibited essentially the same curve with approximately 90% inhibition of incorporation of mevalonate-2-^{14}C into nonsaponifiable material at a concentration of 9 m*M*. At this concentration D-**4** inhibited mevalonate incorporation not more than 20%.

TABLE I

EFFECT OF COMPOUNDS **3**–**5** ADDED *In Vitro* ON THE INCORPORATION OF ACETATE-1-^{14}C INTO NONSAPONIFIABLE MATERIAL OF RAT LIVER HOMOGENATE

Compd (1.5 m*M*)	% inhib of ^{14}C incorporn[a]
3	30 ± 5[b]
L-**4**	33 ± 7
D-**4**	7 ± 5
5	27 ± 6

[a] Average value from five determinations. [b] Standard error of the average value.

Combinations of either 1.5 m*M* **3** plus 1.5 m*M* L-**4** or 1.5 m*M* **3** plus 1.5 m*M* **5** gave the same per cent inhibition of incorporation of mevalonate-2-^{14}C into nonsaponifiable material as did 3 m*M* **3** alone; *i.e.*, no synergistic effects are observed. Had a synergistic effect been found, this might have suggested two different sites in the over-all pathway of sterolgenesis for the action of L-**4** and **3** or **5**.

Analysis by gas–liquid partition chromatography (see Experimental Section) indicated that cholesterol accounted for approximately 85% of the nonsaponifiable material extracted from rat liver homogenate preparations incubated without added drugs (controls) while squalene accounted for less than 2% of the total fraction. When mevalonate-2-^{14}C was added to the control rat liver homogenate preparations and the squalene and cholesterol were separated on acid-washed alumina, the ratio of radiolabeled cholesterol to squalene was approximately 150:1. The purity of the cholesterol and squalene fractions eluted from the alumina column was substantiated by glpc.

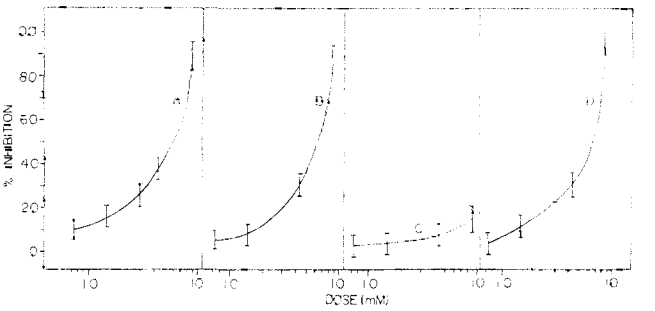


Figure 1.—Dose–response curves for the per cent inhibition of incorporation of mevalonate-2-^{14}C into nonsaponifiable material in fortified rat liver homogenates: (A) α-(4-chlorophenoxy)-α-methylpropionic acid (**3**), (B) L-(−)-α-(4-chlorophenoxy)propionic acid (**4**), (C) D-(+)-α-(4-chlorophenoxy)propionic acid (**4**), (D) α-(4-chlorophenoxy)acetic acid (**5**).

Addition of 3.0 m*M* **3**, L-**4**, or **5** to rat liver homogenate preparations, followed by incubation and isolation of the radiolabeled squalene and cholesterol, showed, within experimental error, the same per cent inhibition of incorporation of mevalonate-2-^{14}C into these compounds as into nonsaponifiable material. These results with **3** agree with those obtained by others,[5,7] who found that various concentrations (0.25–2.5 m*M*) of **3** inhibited cholesterol biosynthesis to the same extent as nonsaponifiable material both in liver and aortic preparations. Our results show that like **3**, L-**4** and **5** interfere with cholesterol biosynthesis between mevalonate and squalene. The considerably lower drug activity of D-**4** *in vitro* indicates that insertion of CH_3 in the wrong configuration might inhibit the binding of the parent structure **5**, yet apparently does not inhibit the association of **3** with the (presumed) common binding site(s) on the drug-sensitive enzymes.

Experimental Section

α-(4-Chlorophenoxy)-α-methylpropionic acid (3), L-(−)-α-(4-chlorophenoxy)propionic acid (4), and D-(+)-α-(4-chlorophenoxy)propionic acid (4) were synthesized according to methods previously published.[3] **4-Chlorophenoxyacetic acid (5)** was purchased from Eastman Kodak Co., Rochester, N. Y. Solutions (60 m*M*) of **3**, L-**4**, and D-**4** were prepared by dissolving the drugs directly in 0.1 *M* phosphate buffer, pH 7.4.

Biological.—Livers were obtained from healthy male Wister rats (200–250 g). Liver homogenates were prepared by Bucher's method[8] and debris was removed by slow centrifugation (300*g*, 2.5 min). One liver (10 g) yielded approximately 20 ml of supernatant. Each incubation flask contained 2 ml of supernatant, 2.5 μCi of acetate-1-^{14}C, 0.1 ml of stock drug solutions, and cofactors[9] with added sodium citrate (final concentration 2 m*M*) made up to 4.0 ml with 0.1 *M* phosphate buffer, pH 7.4. In order to accommodate a greater volume of 50 m*M* drug solution for dose–response studies when mevalonate-2-^{14}C (0.25 μCi) was em-

(4) D. T. Witiak and M. W. Whitehouse, *Biochem. Pharmacol.*, in press.
(5) D. L. Azarnoff, D. R. Tucker, and G. A. Barr, *Metabolism*, **14**, 959 (1965).
(6) D. H. Huffman and D. L. Azarnoff, *Steroids*, **9**, 41 (1967).
(7) D. R. Avoy, E. A. Swyryd, and R. G. Gould, *J. Lipid Res.*, **6**, 369 (1965).
(8) N. L. R. Bucher, *J. Am. Chem. Soc.*, **75**, 498 (1953).
(9) W. L. Holmes and J. D. Bentz, *J. Biol. Chem.*, **235**, 3118 (1960).

ployed, 1.5 ml of supernatant was utilized. Concentrations of all cofactors remained the same and the final volume was similarly made up to 4.0 ml with 0.1 *M* phosphate buffer. After 1 hr of incubation in air with slow shaking at 37° the reaction was stopped by adding 3.0 ml of 15% KOH in 50% EtOH followed by heating at 65–70° in a water bath for 10 min. The partially saponified reaction mixture was transferred to a 20-ml glass-stoppered centrifuge tube and the flask was rinsed with 3.0 ml of the alcoholic KOH solution which was added to the contents of the centrifuge tube. Saponification was completed by heating the tightly stoppered tube in a water bath (75–80°) for 1 hr.

The saponified mixtures were extracted three times with 5.0-ml portions of petroleum ether (bp 60–80°). The combined extracts were diluted to 25.0 ml and dried (Na_2SO_4). Five milliliters of the dried petroleum ether extract was diluted with 10.0 ml of PPO (2,5-diphenyloxazole) solution (0.4% PPO in PhMe–95% EtOH, 70:30 v/v) in standard counting vials. The radioactivity was determined with a Packard Tri-Carb liquid scintillation counter. A sufficient number of counts was taken to reduce the statistical error of counting to less than 5%. In each run, as a further check on the extraction procedure (and as a measure of possible contamination), the contents of one incubation flask was treated with KOH solution prior to incubation; the radioactivity in the petroleum ether extract derived from these alkali pretreated incubations was always found to be negligible.

Gas–liquid partition chromatography[10] of the nonsaponifiable material was accomplished as follows. The petroleum ether extract of nonsaponifiable material (15 ml) was concentrated to dryness (water bath, 50°) under N_2. The residue was dissolved in 0.5 ml of petroleum ether and gas chromatographed on 10% silicone gum rubber (SE-30) on Chromosorb W (80–100 mesh), 4 ft × 0.25 in. glass column with the column temperature 240°, detector temperature 285°, injection port temperature 355°, inlet pressure of 2.8 kg/cm², and carrier gas (He) flow rate 50 ml/min; this gave a retention time for squalene of 13.0 min and for cholesterol, 24.4 min.

Separation of the nonsaponifiable extract into squalene and cholesterol was accomplished by the method of Langdon and Bloch.[11] The petroleum ether extract (15 ml) was concentrated to dryness in a water bath (50°) under N_2. The residue was chromatographed on acid-washed alumina[12] using petroleum ether (60–70°). The squalene fraction was checked for purity by glpc. Sterols were then eluted with Me_2CO–Et_2O (1:1 v/v). The cholesterol fraction was similarly checked for purity using glpc. Squalene- and cholesterol-containing eluates were concentrated to dryness in a water bath (50°) under N_2 in standard counting vials. PPO solution (10 ml) was added and the radioactivity was determined with a Packard Tri-Carb liquid scintillation counter.

Acknowledgment.—We are grateful to the National Institutes of Health for support of this work through Grant HE 12740-01.

(10) The F and M Model 402 biomedical gas chromatograph equipped with flame ionization detector was employed in these studies.

(11) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 129 (1953).

(12) Alupharm Chemicals, New Orleans, La.

2-Benzyl-5-aminolevulinic Acid, an Analog of Glycyl-DL-phenylalanine[1a]

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Numerous studies have indicated that peptides have biological activities which differ from the activities displayed by the free constituent amino acids.[2] Interpretation of the results of such studies is complicated by the hydrolysis of the peptides to form free amino acids as often occurs in biological systems. In an attempt to produce a peptide analog whose study would not be complicated by hydrolysis, 2-benzyl-5-aminolevulinic acid was synthesized. 2-Benzyl-5-aminolevulinic acid (I) can be viewed as an analog of glycylphenylalanine wherein the labile CONH group of the peptide has been replaced by the nonlabile $COCH_2$ group.

All compounds used in biological testing were of the racemic form. 2-Benzyl-5-aminolevulinic acid was tested for its effect upon the growth of *Escherichia coli* 9723 and *Leuconostoc mesenteroides* P-60. Growth of *E. coli* was not inhibited by I when tested in the basal salts–glucose medium. However, as shown in Table I,

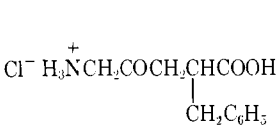
TABLE I

EFFECT UPON GROWTH OF *E. coli* 9723[a] BY 2-BENZYL-5-AMINOLEVULINIC ACID (BALA) IN MEDIA CONTAINING β-2-THIENYL-DL-ALANINE (TA), GLYCYL-β-2-THIENYL-DL-ALANINE (GTA), GLYCYLGLYCYL-β-2-THIENYL-DL-ALANINE (G_2TA), AND GYLCYL-DL-PHENYLALANINE (GPA)

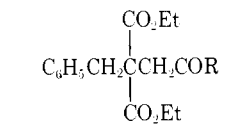
	mg of dry wt of cells/ml of culture — Supplements			
BALA, mμmoles/ml	TA[b]	GTA[c]	G_2TA[d]	TA, GTA, GPA[e]
0	0	0	0	0.13
20				0.12
60		0.01		0.10
200	0	0.08	0	0.06
600	0	0.20	0	0.01

[a] Incubated 15 hr at 37°. [b] Medium contained 2 mμmoles of TA/ml. [c] Medium contained 6 mμmoles of GTA/ml. [d] Medium contained 6 mμmoles of G_2TA/ml. [e] Medium contained 200 mμmoles of TA, 200 mμmoles of GTA, and 20 mμmoles of GPA/ml.

I may either stimulate or inhibit the growth of *E. coli* depending upon the way the medium is supplemented with peptides of β-2-thienylalanine or phenylalanine.

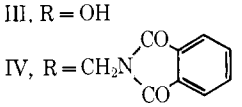


I



II, R = $OCH_2C_6H_5$

III, R = OH



IV, R = CH_2N

If growth was inhibited by glycyl-β-2-thienylalanine, reversal of inhibition occurred when I was added to the medium. When growth was inhibited by the tripeptide glycylglycyl-β-2-thienylalanine, I was ineffective as a growth stimulant. When the *E. coli* medium was sup-

(1) (a) This investigation was supported by U. S. Public Health Service Grant No. AI03710 from the National Institutes of Health and Biochemistry Training Grant GM-203. (b) Taken in part from the M. S. Thesis of Sherrel C. Smith, University of Tennessee, June 1965. (c) To whom requests for reprints should be directed: Department of Chemistry, Abilene Christian College, Abilene, Texas 79601.

(2) (a) F. W. Dunn, *J. Biol. Chem.*, **234**, 802 (1959); (b) S. Shankman, V. Gold, S. High, and R. Squires, *Biochem. Biophys. Res. Commun.*, **9**, 25 (1962); (c) W. Shive and C. G. Skinner in "Metabolic Inhibitors," Vol. I, R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 1, pp 37–39.