Chemical Modification of Erythromycin: Synthesis and Preliminary Bioactivity of Selected Amides and Esters

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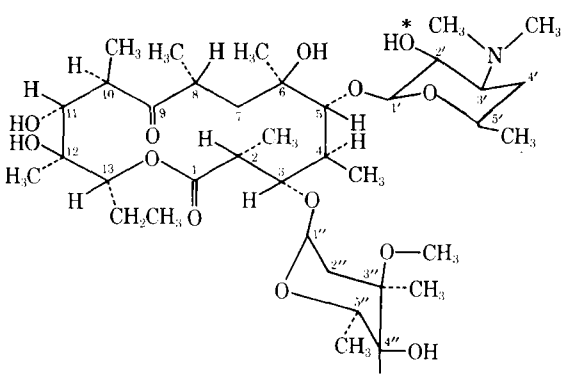
Abstract □ Erythromycin was chemically modified for the purpose of providing *in vivo* reversible tasteless derivatives of this antibiotic that might afford serum levels equivalent to erythromycin base. For improvement of taste properties and bioavailability, a series of erythromycin 2′-*N*-alkylsuccinamate and 2′-*N*-alkylglutaramate derivatives and several erythromycin 2′-alkylthiolsuccinate and 2′-alkylthiolglutarate derivatives were synthesized. The necessary chemical synthetic technology was developed to produce these derivatives and, in certain cases, alternative synthetic routes were utilized to improve yields. Most derivatives exhibited bioactivity comparable to erythromycin base in the mouse CD_{50} bioassay, and several derivatives produced serum levels of erythromycin equal to or greater than serum levels of animals dosed with erythromycin base. Several derivatives were tasteless and acceptable as candidates for pediatric formulation.

Keyphrases □ Erythromycin amides and esters—synthesis as tasteless derivatives, mouse CD_{50} bioassay and rat serum levels □ Tasteless erythromycin derivatives—synthesis, bioactivity □ Pediatric formulations, potential—synthesis and bioactivity of erythromycin amide and ester derivatives

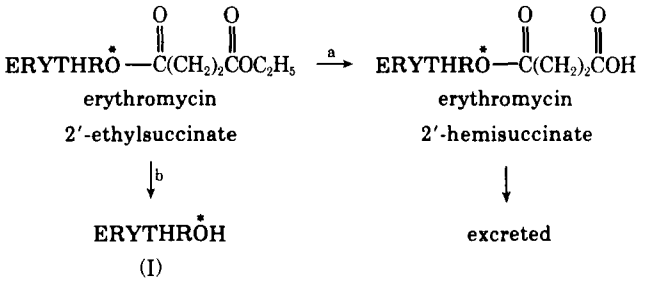
The purpose of this project was to obtain a tasteless pediatric form of erythromycin (I; ERYTHROH) that provided serum levels comparable to those of erythromycin base dosage forms. The results yielded several specially designed derivatives of erythromycin. These derivatives took the form of *N*-alkylsuccinamates and *N*-alkylglutaramates and, in four cases, erythromycin 2′-alkylthiolsuccinates and 2′-alkylthiolglutarates. These derivatives met the following requirements: *(a)* lack of bitterness (tastelessness), *(b)* bioactivity comparable to erythromycin base in the mouse CD_{50} bioassay, and *(c)* total whole blood levels (rat) equivalent to or greater than those of erythromycin base.

RESULTS AND DISCUSSION

The rationale for synthesizing a 2′-*N*-alkylglutaramate or 2′-*N*-alkylsuccinamate of erythromycin is that chemically (1–3) and, in many cases, enzymatically (4–8), amide linkages are more stable than ester linkages. Erythromycin 2′-ethylsuccinate (an ester) affords blood levels of erythromycin base consistently lower in human subjects than those of erythromycin base (9, 10). Similarly, erythromycin 2′-propionate lauryl sulfate salt affords low human blood levels of erythromycin base (11–14). This report, however, concerns itself only with comparisons to erythromycin base.



I: erythromycin (ERYTHROH)



Scheme I

Erythromycin 2′-ethylsuccinate has two ester linkages at which hydrolysis can occur (Scheme I): *(a)* hydrolysis at the terminal ester linkage of the ethylsuccinate chain yields erythromycin 2′-succinate hemiester, and *(b)* hydrolysis at the 2′-ester position yields erythromycin base.

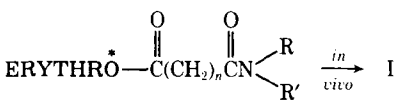
It appears that hydrolysis by route *(a)* should predominate since this ester is the least sterically hindered and would be most susceptible to enzymatic hydrolysis. If *(a)* is the predominant route of metabolism, then two factors become important:

1. The erythromycin succinate hemiester, being more polar than erythromycin ethylsuccinate or erythromycin base, would be more rapidly eliminated from the body and would tend to lower serum levels of this antibiotic. Monosubstituted succinic acid esters are known to be readily metabolized and excreted *in vivo* (15).

2. Erythromycin 2′-succinate hemiester possessed about 0.5 times the antibacterial activity as did erythromycin base in the mouse CD_{50} bioassay, implying that this derivative is less active than the free base (Table I).

In vitro results indicate about 50% base activity for erythromycin 2′-succinate hemiester (16). Thus, by all standards, it appears that erythromycin 2′-succinate hemiester is significantly less bioactive than erythromycin base itself.

It was previously stated that amide linkages in many cases are more resistant to hydrolysis than are ester linkages. Replacing the terminal ester linkage on an erythromycin 2′-alkyl hemiester with an amide linkage would enhance the stability of this portion of the molecule. If hydrolysis does occur, it should do so preferentially at the 2′-ester linkage rather than at the terminal amide linkage (Scheme II). This would ensure *in vivo* hydrolysis to erythromycin base rather than to the intermediate and less bioactive hemiester. Chemical modification would thus allow a one-step hydrolysis to the parent antibiotic erythromycin. Another important factor would be the slower rate of excretion of this amide derivative since it is not hydrolyzed to a polar intermediate. This would allow a longer residence time in the body and



$n = 2, 3$; R = H; R′ = C_2H_5 to $C_{12}H_{25}$

Scheme II

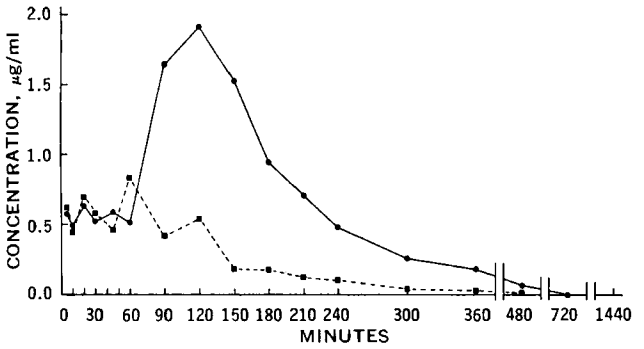


Figure 1—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-ethylsuccinate* (■).

permit the necessary esterases a longer time in which to hydrolyze this derivative to erythromycin base.

Hydrolysis of Erythromycin 2′-*N*-Alkylamides—The results of the antibacterial CD_{50} mouse bioassay illustrate some interesting points. All synthesized derivatives, with three exceptions, exhibited full antibacterial activity (compared to erythromycin base) in the mouse CD_{50} bioassay. This indicated that *in vivo* these ester-amides were being hydrolyzed to erythromycin base, affording subsequent antibacterial protection (Table I). The three exceptions to this generalization were erythromycin 2′-ethylthiolsuccinate, erythromycin 2′-ethylthiolglutarate, and erythromycin 2′-hexylthiolsuccinate. This trend is to be expected, however, since these compounds resemble erythromycin 2′-ethylsuccinate in all respects except that one is a "normal" ester (RCOOR′) and the three derivatives are thiol esters (RCOSR′). Based on what has been stated previously concerning erythromycin 2′-ethylsuccinate, this phenomenon is not unusual. The erythromycin 2′-ethylsuccinate oral CD_{50} value was on the lower end of the antibacterial bioactivity range (0.67)[1].

A more graphic illustration of the differences between erythromycin 2′-ethylsuccinate, erythromycin 2′-ethylthiolsuccinate, erythromycin 2′-ethylthiolglutarate, and the erythromycin 2′-amide derivatives is seen by a comparison of their respective blood level curves (Figs. 1–3)[2]. For one of the parameters measured (area under curve ± standard error), the ethylsuccinate, the ethylthiolsuccinate, and the ethylthiolglutarate derivatives exhibited decreased bioavailability when compared to erythromycin base (Table II). On the other hand, the ethyl- and hexylglutaramate and the dodecylsuccinamate derivatives showed bioavailability comparable to erythromycin base (Figs. 4–6). Erythromycin 2′-dodecylglutaramate and 2′-glutaryl-*N*-dicyclohexylurea exhibited bioavailability approximately 75% that of erythromycin base (Figs. 7 and 8). These blood level data thus support the contention that the amide terminal linkage in these derivatives stabilizes the molecule so that hydrolysis occurs preferentially at the 2′-ester linkage rather than at the terminal amide linkage (good hydrolysis was reflected in the oral CD_{50} values) and that these derivatives have a slower excretion rate, thus allowing a longer residence time which permits greater opportunity for hydrolysis. Extended T_{50} values reflected slow excretion of drug and bioactivity.

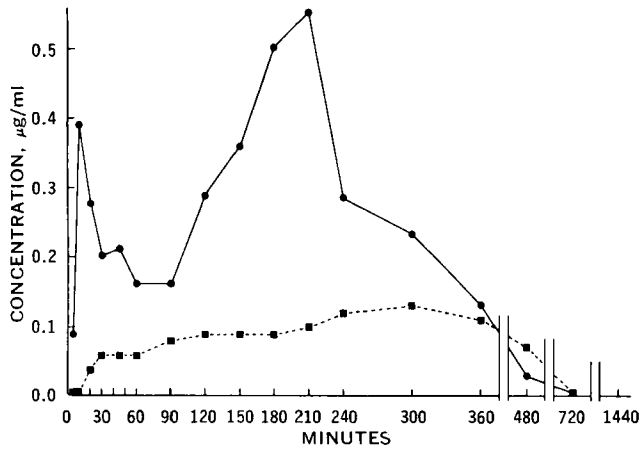


Figure 2—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-ethylthiolsuccinate* (■).

[1] Ratio of activity: CD_{50} erythromycin base/CD_{50} erythromycin 2′-ethylsuccinate.

[2] In certain figures, the concentration profile of erythromycin base is not consistently reproducible due to the variable absorption pattern of erythromycin in the rat. This phenomenon has also been noted in human subjects (15, 17). Comparison of the plasma level-time profiles between erythromycin base and ester is only valid for the series of rats dosed with a given derivative. The plasma concentrations of erythromycin are not necessarily therapeutic levels but rather reflect the amount of drug absorbed in a particular group of rats.

Table I—Antibacterial Oral Activity in *Staphylococcus aureus* (UC-76) Infected Mice

Compound	Oral CD_{50}, mg/kg[a]	Ratio of Activity[b]
Erythromycin 2′-*N*-ethylglutaramate	9.7 (7.0–12)	0.95
Erythromycin 2′-*N*-hexylglutaramate	11 (7.2–17)	0.82
Erythromycin 2′-*N*-dodecylglutaramate	14 (10–19)	2.14
Erythromycin 2′-*N*-dodecylsuccinamate	6.7 (4.6–9.7)	1.64
Erythromycin 2′-ethylthiolsuccinate	60 (45–77)	0.27
Erythromycin 2′-ethylthiolglutarate	36 (27–47)	0.44
Erythromycin 2′-hexylthiolsuccinate	40 (29–56)	0.42
Erythromycin 2′-hexylthiolglutarate	23 (17–31)	0.74
Erythromycin 2′-glutaryl-*N*-dicyclohexylurea	28 (21–38)	1.07
Erythromycin 2′-ethylsuccinate	45 (31–64)	0.67
Erythromycin 2′-succinate hemiester	19 (13–29)	0.48
Erythromycin 2′-glutarate hemiester	29 (21–42)	0.37

[a] Corrected to erythromycin base equivalent; numbers in parentheses indicate range. [b] CD_{50} erythromycin base/CD_{50} erythromycin 2′-ester.

Erythromycin 2′-hexylthiolsuccinate paralleled the time-concentration curve of erythromycin base and afforded serum levels equivalent to the base (Fig. 9). Erythromycin 2′-hexylthiolglutarate afforded levels of erythromycin base approximately twice that of erythromycin base (Fig. 10). No apparent explanation is available for the great differences in bioavailability between these hexylthiol esters and erythromycin base.

Chemistry—Erythromycin 2′-*N*-dodecylglutaramate (III) was synthesized by three different synthetic procedures, all active ester-mixed anhydride methods.

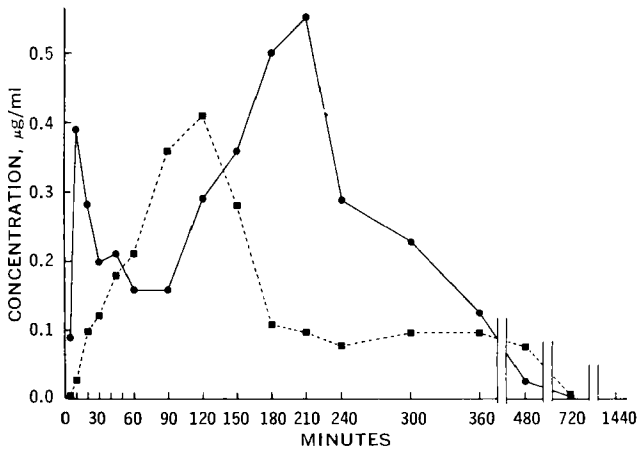


Figure 3—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-ethylthiolglutarate* (■).

Table II—Oral Blood Levels in Fasted Rats[a]

Compound	Area under Curve[b] ± *SE*	T_{50} of Area ± *SE*	Maximum Concentration, μg/ml	T_{max}, min	Ratio: Area–Control
Erythromycin 2′-*N*-ethylglutaramate	114 ± 1.4	171 ± 5.2	0.47	150	0.992
Erythromycin base	115 ± 33	193 ± 17	0.55	210	
Erythromycin 2′-*N*-hexylglutaramate	433 ± 32	278 ± 13	1.33	150	1.341
Erythromycin base	323 ± 144	180 ± 79	2.14	30	
Erythromycin 2′-*N*-dodecylglutaramate	228 ± 25	231 ± 28	0.80	60	0.77
Erythromycin base	304 ± 149	163 ± 29	1.90	120	
Erythromycin 2′-*N*-dodecylsuccinamate	103 ± 36	228 ± 18	0.35	120	0.925
Erythromycin base	112 ± 26	183 ± 22	0.42	150	
Erythromycin 2′-ethylthiolsuccinate	52.9 ± 7.6	298 ± 33	0.13	300	0.460
Erythromycin 2′-ethylthiolglutarate	80 ± 20	22 ± 60	0.41	120	0.698
Erythromycin base	115 ± 33	193 ± 17	0.55	210	
Erythromycin 2′-hexylthiolsuccinate	69 ± 17	102 ± 9	0.45	30	1.033
Erythromycin 2′-hexylthiolglutarate	144 ± 25	155 ± 3	0.61	45	2.155
Erythromycin base	67 ± 10	138 ± 9	0.40	90	
Erythromycin 2′-glutaryl-*N*-dicyclohexylurea	224 ± 124	270 ± 35	0.59	150	0.736
Erythromycin base	304 ± 149	163 ± 58	1.90	120	
Erythromycin 2′-ethylsuccinate	104 ± 23	90 ± 23	0.83	60	0.343
Erythromycin base	304 ± 149	163 ± 58	1.90	120	

[a] Single oral dose of 100 mg/kg of base equivalent. [b] Mean area from three rats.

Method I—The first method involved the use of erythromycin 2′-glutarate hemiester (II), dodecylamine, and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline[3] (IV) (18) (Scheme III). This reaction afforded crude material that was subjected to column chromatography for purification. The overall yield of pure derivative was low (43%).

Method II—The second method required the use of erythromycin 2′-glutarate hemiester, isobutyl chloroformate, triethylamine, and dodecylamine as starting materials (19–21) (Scheme IV). This method also required the use of column chromatography for purification. Again, overall yields of pure material were low (33%).

Method III—The third method involved the condensation of erythromycin base and *N*-dodecylglutaramic acid in the presence of isobutyl chloroformate (Scheme V). Column chromatography was again necessary for purification. Yields were slightly improved (~50%). Erythromycin 2′-*N*-dodecylsuccinamate was also synthesized by the third method (yield ~50%).

Erythromycin 2′-*N*-ethylglutaramate and erythromycin 2′-*N*-hexylglutaramate were prepared by Method II using ethylamine and hexylamine, respectively. Isobutyl chloroformate was again used as the condensing agent. Erythromycin 2′-*N*-ethylglutaramate was crystalline but difficult to purify because of its high water solubility. Erythromycin 2′-*N*-hexylglutaramate was also crystalline and purification posed no problem.

Erythromycin 2′-ethylthiolglutarate was synthesized by the reaction of erythromycin 2′-glutarate hemiester with ethanethiol in the presence of isobutyl chloroformate and triethylamine (Scheme VI). This material was completely crystalline and its R_f value[4] was comparable to that of erythromycin 2′-ethylsuccinate,

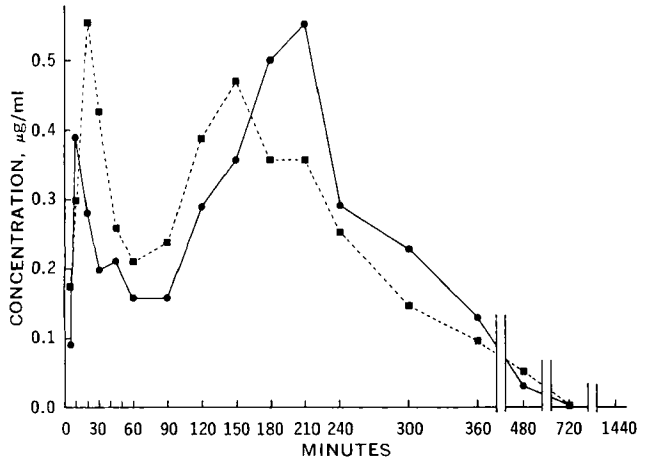


Figure 4—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-*N*-ethylglutaramate* (■).

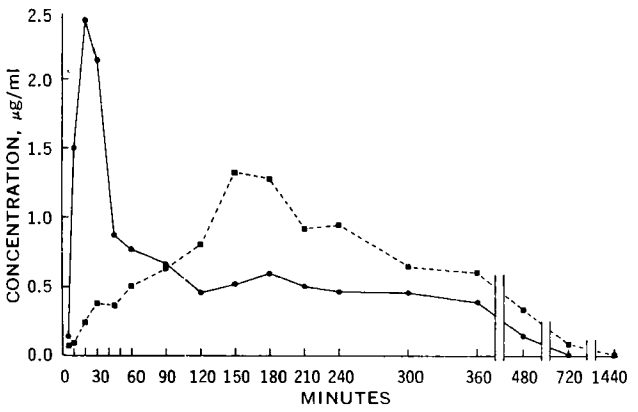


Figure 5—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-*N*-hexylglutaramate* (■).

[3] Aldrich Chemical Co., Milwaukee, Wis.

[4] Silica gel GF precoated TLC plates (Analtech, Inc., Newark, Del.), using acetone as developing solvent.

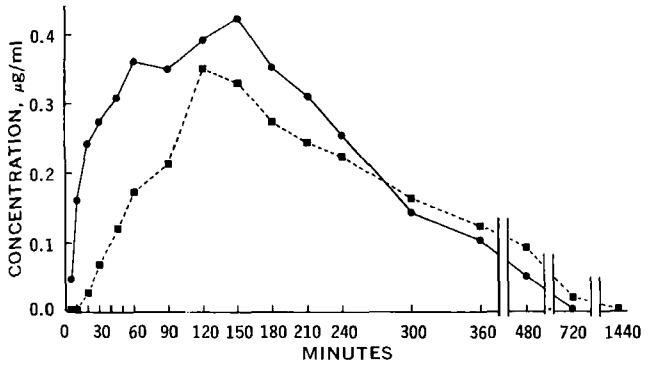


Figure 6—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-*N*-dodecylsuccinamate* (■).

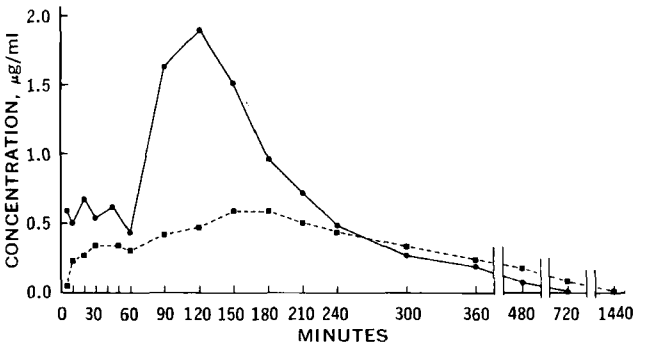


Figure 8—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-glutaryl-*N*-dicyclohexylurea* (■).

suggesting that the polarity of these substances may be similar[5].

Erythromycin 2′-ethylthiolsuccinate was synthesized in a similar manner using erythromycin 2′-succinate hemiester. Its R_f value was also similar to that of erythromycin 2′-ethylsuccinate, again suggesting a marked similarity in properties[4]. This material crystallized easily. The yield of these two thiol esters was approximately 20%.

The hexylthiolsuccinate and hexylthiolglutarate esters were synthesized similarly using hexanethiol. These esters were also highly crystalline and required minimal purification. The overall yield of the hexylthiol esters averaged 20%.

Erythromycin 2′-glutaryl-*N*-dicyclohexylurea was synthesized by condensation of erythromycin 2′-glutarate hemiester with dicyclohexylcarbodiimide. The reaction proceeded through the formation of the erythromycin 2′-glutaryl-*o*-dicyclohexylisourea intermediate, which rearranged to the urea (22–25) (Scheme VII). The overall yield of the pure derivative was about 40%.

EXPERIMENTAL

Biological

In Vitro—*In vitro* bioactivity was determined using the standard curve plate bioassay (26, 27). The sensitivity of the assay was 0.25 μg/ml using *Sarcina lutea* as the test organism. Erythromycin free base had an assigned potency of 1000 μg/mg, and the activities of the erythromycin derivatives were calculated and reported as the free base equivalents per milligram of antibiotic. All erythromycin derivatives tested showed less than 10% activity compared to erythromycin base (similar to other erythromycin 2′-esters) (28).

In Vivo—The *in vivo* protection studies were undertaken using CF-1 male albino mice randomly selected from an animal pool; they were experimentally infected using *Staphylococcus aureus* (UC-76). Experimental details were previously described (26, 27). Oral CD_{50} values obtained with the erythromycin derivatives are listed in Table I.

Blood Level Studies—The comparative absorption rates of erythromycin from the various erythromycin thiol esters and amides were determined using Sprague–Dawley (Upjohn strain) white, male rats weighing approximately 150 g. The rats were fasted for 24 hr and dosed orally by gavage with 0.5 ml of 0.25% methylcellulose[6] containing 100 mg/kg erythromycin base equivalent of derivative. The procedures for determining the quantity of erythromycin in the blood were described previously (29). In the blood level determinations, the parameters evaluated were: *(a)* total area under the curve ± standard error, *(b)* time in minutes at which 50% of the total area appeared under the curve (T_{50}) ± standard error, *(c)* maximum concentration (micrograms per milliliter) of erythromycin in the blood at time T (minutes), and *(d)* ratio of total areas of erythromycin base derived from derivative–erythromycin base control.

Synthetic

Typical examples of reaction conditions for erythromycin 2′-*N*-alkylamides and erythromycin 2′-alkylthiol esters are illustrated. Elemental analyses are listed in Table III.

Erythromycin 2′-*N*-Dodecylglutaramate (Method I)—*Erythromycin 2′-Glutarate Hemiester (16, 30)*—Erythromycin base (146.8 g, 0.2 mole) was dissolved in enough (1 liter) methylene chloride at 30° to effect complete solution. A filtered methylene chloride solution containing 24 g of glutaric anhydride was added to the solution of erythromycin base and stirred for 1 hr. The resulting crystals were collected, dried *in vacuo* at 60° for 48 hr, and used without further purification.

*Erythromycin 2′-*N*-Dodecylglutaramate*—Erythromycin 2′-glutarate hemiester (4.24 g, 0.005 mole) was dissolved in 100 ml of acetone (previously dried with 3A molecular sieve). A solution of 0.193 g (0.005 mole) of *n*-dodecylamine was dissolved in 15 ml of anhydrous acetone. A solution of 1.24 g (0.005 mole) of IV was

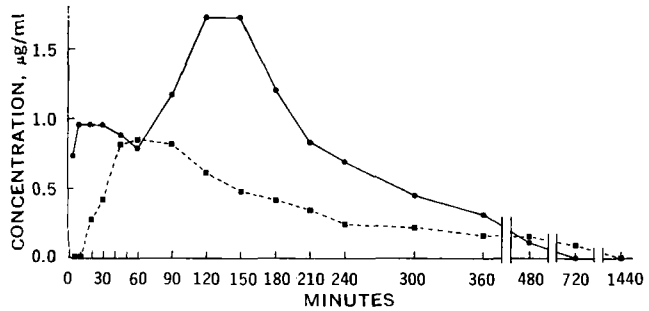


Figure 7—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-*N*-dodecylglutaramate* (■).

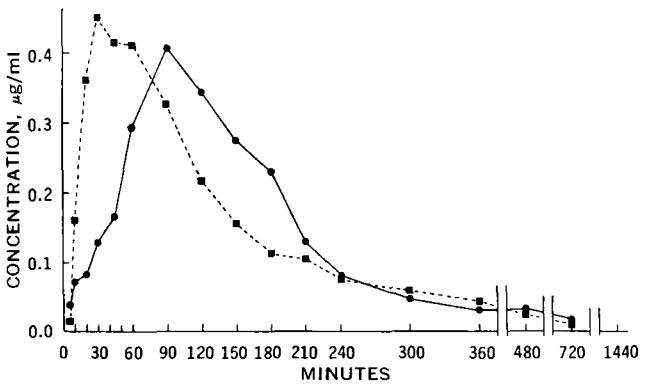


Figure 9—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-hexylthiolsuccinate* (■).

[5] Erythromycin 2′-ethylthiolglutarate, erythromycin 2′-ethylthiolsuccinate, and erythromycin 2′-ethylsuccinate were less polar in aqueous media (*via* comparison of R_f values) than any of the other derivatives synthesized in this study. The low rat blood level data suggested that the dissolution rates *in vivo* of these derivatives may be insufficient to provide adequate drug for absorption.

[6] Methocel HG, Dow Chemical Co., Midland, Mich.

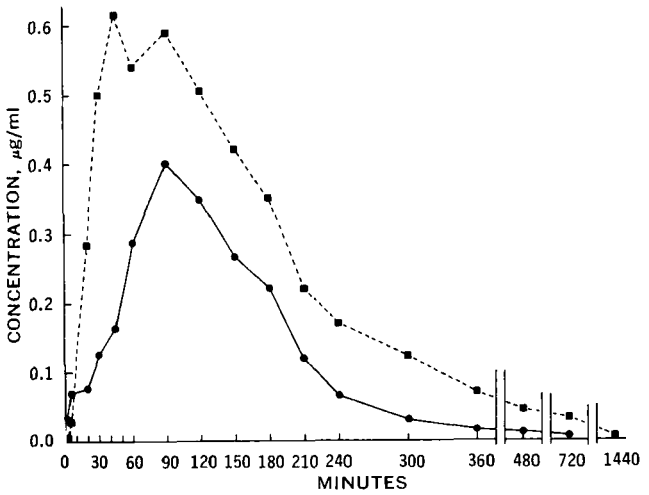
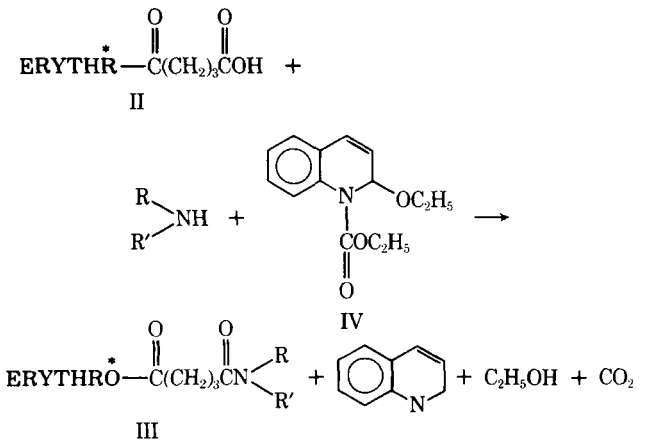


Figure 10—*Whole blood levels in rats dosed orally with erythromycin base* (●) *or erythromycin 2′-hexylthiolglutarate* (■).

dissolved in 10 ml of acetone, and the three solutions were combined at room temperature for 72 hr. The solvent was then removed *in vacuo*, and the yellow syrup was swirled with 50 ml of anhydrous ether. The resulting precipitate was discarded, and the ether was removed *in vacuo*.

One gram of the crude product was dissolved in 5 ml of anhydrous acetone, filtered, and placed on the top of a glass column containing 50 g of silica gel[7] (70–325 mesh) as an acetone slurry. The column was eluted with anhydrous acetone, and the fractions containing pure erythromycin 2′-*N*-dodecylglutaramate were collected. The solvent was removed at room temperature, and the resulting powder was dried *in vacuo* at 45°.

Erythromycin 2′-*N*-Dodecylglutaramate (Method II)—Erythromycin 2′-glutarate hemiester (33.92 g, 0.04 mole) was dissolved in acetone (200 ml) and cooled to −20°. Isobutyl chloroformate (5.44 g, 0.04 mole) and triethylamine (4.04 g, 0.04 mole) were added. *n*-Dodecylamine (7.4 g, 0.04 mole) was dissolved in 250 ml of acetone and added dropwise from a dropping funnel over 1 hr, keeping the reaction mixture at −20° until 15 min after the addition was completed. Then the reaction mixture was allowed to come to room temperature and was stirred for 24 hr. The solvent was removed *in vacuo*. Six grams of the dried powder was dissolved in 25 ml acetone, filtered, and placed on the top of a glass column containing 427 g silica gel (70–325 mesh) as an acetone slurry. The column was eluted with anhydrous acetone, the fractions containing pure erythromycin 2′-*N*-dodecylglutaramate were collected, the solvent was removed at room temperature, and the resulting powder was dried *in vacuo* at 45°.



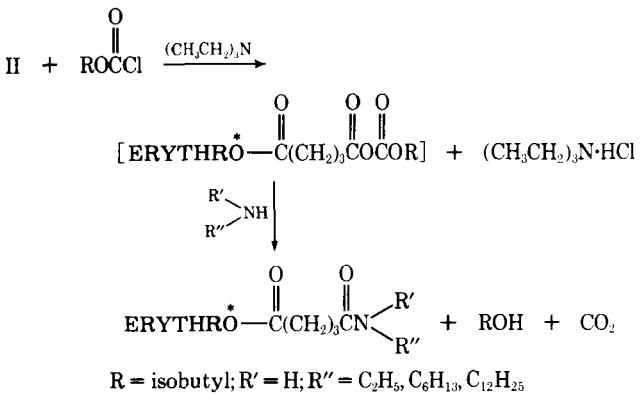
R = H; R′ = $C_{12}H_{25}$

Scheme III

Erythromycin 2′-*N*-Dodecylglutaramate (Method III)—*N-Dodecylglutaramic Acid*—Glutaric anhydride (11.41 g, 0.01 mole) was dissolved in 50 ml of acetone. *n*-Dodecylamine (16.68 g, 0.009 mole) was dissolved in 150 ml of acetone and warmed to 40°. The two solutions were thoroughly mixed and allowed to stand at room temperature for 1 hr. Then the resulting crystals were collected and dried.

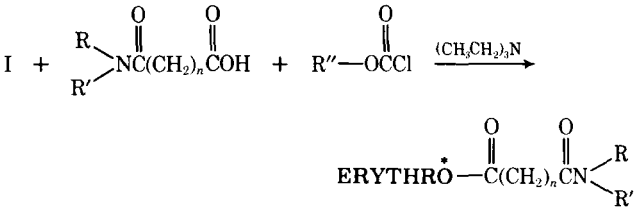
Erythromycin 2′-N-Dodecylglutaramate—Fifteen grams (0.05 mole) of *N*-dodecylglutaramic acid was dissolved in 750 ml of chloroform and cooled to −10°. To this solution were added 6.8 g (6.5 ml, 0.05 mole) of isobutyl chloroformate and 5.05 g (7 ml, 0.05 mole) of triethylamine, and the mixture was stirred well. To this mixture was added dropwise a solution of 36.65 g (0.05 mole) of erythromycin base in 250 ml. of chloroform. During addition, the temperature was maintained at −10°. The reaction mixture was warmed to room temperature and stirred for 24 hr. The solvent was then removed *in vacuo*, and the residue was dissolved in anhydrous acetone. The resulting precipitate of triethylamine hydrochloride was filtered, and the solvent was again removed *in vacuo*.

Sixty-two grams of crude erythromycin 2′-*N*-dodecylglutaramate, dissolved in 150 ml of anhydrous acetone, was introduced to a 183 × 10-cm glass column containing 6 kg of previously treated silica gel[7] (70–325 mesh) for column chromatography. The material was eluted with anhydrous acetone, and the column was run at a flow rate of 17 ml/min. One thousand-milliliter cuts were



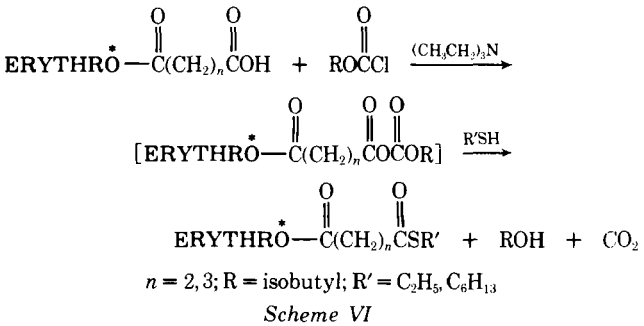
R = isobutyl; R′ = H; R″ = C_2H_5, C_6H_{13}, $C_{12}H_{25}$

Scheme IV



n = 2, 3; R = H; R′ = $C_{12}H_{25}$; R″ = isobutyl

Scheme V



n = 2, 3; R = isobutyl; R′ = C_2H_5, C_6H_{13}

Scheme VI

[7] This silica gel (Merck), prior to placement in the column, was suspended in anhydrous acetone and the "fines" were decanted. This procedure was repeated several times. The silica gel was then isolated by vacuum filtration and washed five times with 200-ml portions of chloroform (AR). The silica gel was then dried on a vacuum filter prior to suspension in anhydrous acetone.

Table III—Analytical Data for Erythromycin 2′-Esters[a]

Erythromycin Ester	Empirical Formula	Molecular Weight	Analysis, % Calc.	Analysis, % Found	Melting Point	Yield, %
2′-*N*-Ethylglutaramate	$C_{44}H_{78}N_2O_{15}$	875.11	C 60.39 H 8.98 N 3.20	C 60.03 H 9.05 N 3.15	138–141°	53
2′-*N*-Hexylglutaramate	$C_{48}H_{86}N_2O_{15}$	931.22	C 61.91 H 9.31 N 3.01	C 62.19 H 9.17 N 3.34	110–113°	54
2′-*N*-Dodecylglutaramate	$C_{54}H_{98}N_2O_{15}$	1015.34	C 63.89 H 9.73 N 2.76	C 63.74 H 9.88 N 2.70	83–85°	50
2′-*N*-Dodecylsuccinamate	$C_{53}H_{96}N_2O_{15}$	1001.35	C 63.57 H 9.66 N 2.80	C 63.10 H 9.69 N 2.72	90–93°	50
2′-Ethylthiolsuccinate	$C_{43}H_{75}NO_{15}S$	878.13	C 58.81 H 8.61 N 1.59 S 3.65	C 59.29 H 8.45 N 1.58 S 3.31	138–141°	21
2′-Ethylthiolglutarate	$C_{44}H_{77}NO_{15}S$	892.16	C 59.24 H 8.70 N 1.57 S 3.59	C 59.39 H 8.79 N 1.60 S 3.49	128–132°	20
2′-Hexylthiolsuccinate	$C_{47}H_{83}NO_{15}S$	934.24	C 60.43 H 8.96 N 1.50 S 3.43	C 60.10 H 8.99 N 1.49 S 3.13	95–96°	20
2′-Hexylthiolglutarate	$C_{48}H_{85}NO_{15}S$	948.27	C 60.80 H 9.04 N 1.48 S 3.38	C 61.17 H 9.04 N 1.36 S 3.45	91–93°	20
2′-Glutaryl-*N*-dicyclohexylurea	$C_{55}H_{95}N_3O_{16}$	1054.38	C 62.25 H 9.08 N 3.99	C 62.54 H 8.55 N 3.71	143–146°	40
N-Dodecylsuccinamic acid	$C_{16}H_{31}NO_3$	285.42	C 67.33 H 10.95 N 4.91	C 67.55 H 11.00 N 4.86	117–119°	94
N-Dodecylglutaramic acid	$C_{17}H_{33}NO_3$	299.45	C 68.17 H 11.11 N 4.70	C 68.13 H 11.03 N 4.46	95–96°	93

[a] Melting points are of the hydrated samples. They were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. The elemental analysis values are corrected for water content.

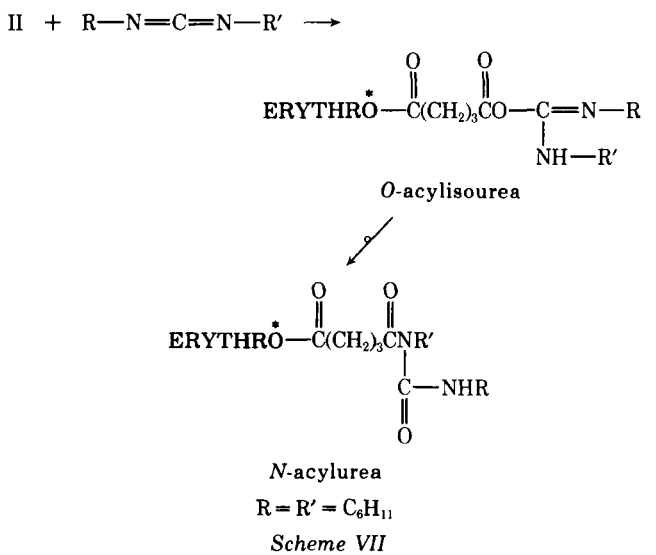
collected, fractions 7–12 were collected and pooled, and the solvent was removed *in vacuo*.

Erythromycin 2′-Thiol Esters—*Erythromycin 2′-Ethylthiolsuccinate*—Erythromycin base (146.8 g, 0.2 mole) was dissolved in 700 ml of anhydrous acetone. Succinic anhydride (22.01 g, 0.22 mole) was dissolved in 300 ml of anhydrous acetone. The two solutions were combined and stirred for 2 hr. Precipitation began after 30 min and the yield was 155 g (92%). This compound was used without further purification (16, 30).

Erythromycin 2′-succinate hemiester (50.04 g, 0.06 mole) was dissolved in 500 ml of anhydrous acetone, and 7.8 g (10.7 ml, 0.067 mole) of triethylamine was added to this solution. The mixture was cooled to −10°, 9.15 g (8.7 ml, 0.067 mole) of isobutyl chloroformate was added dropwise, and the reaction mixture was allowed to warm to room temperature. The mixture was stirred at room temperature for 12 hr, and the solvent was removed *in vacuo*. Ten grams of the resulting white powder was dissolved in 250 ml of acetonitrile, 75 g of silica gel GF (TLC grade) was added, and the mixture was swirled and warmed to 50°. The filtrate was added to 1 liter of distilled water, resulting in the precipitation of fine, white crystals. The material was pure by TLC (acetone, silica gel).

Erythromycin 2′-Hexylthiolglutarate—Erythromycin 2′-glutarate hemiester (50.88 g, 0.06 mole) was dissolved in 500 ml of anhydrous acetone. Seven and eight-tenths grams (0.067 mole) of triethylamine was added to this solution. The mixture was cooled to −10°, 9.15 g (8.7 ml, 0.067 mole) of isobutyl chloroformate was added dropwise, and the reaction mixture warmed to room temperature. The mixture was recooled to −10°, and 7.1 g (0.067 mole) of hexanethiol was added. The mixture was stirred at room temperature for 48 hr, recooled to −10°, an additional 4 g of hexanethiol was added, and the mixture was rewarmed to room temperature. Then the mixture was stirred for 12 hr and the solvent was evaporated. Ten grams of the resulting impure mixture was treated with 3 g of TLC grade silica gel in 100 ml of acetonitrile. The silica gel was removed by filtration, and the solvent was removed *in vacuo*, resulting in 2 g of pure erythromycin 2′-hexylthiolglutarate.

Erythromycin 2′-Glutaryl-N-dicyclohexylurea—Twelve and seven-tenths grams (0.015 mole) of erythromycin 2′-glutarate hemiester was dissolved in 100 ml of anhydrous acetone. Three and eight-tenths grams (0.018 mole) of dicyclohexylcarbodiimide was dissolved in 75 ml of anhydrous acetone. The two solutions were combined and stirred for 3 hr. The resulting mixture was fil-



Scheme VII

tered, and the solvent was removed *in vacuo*. The powder was pulverized and dried *in vacuo* at 40° for 2 hr. The yield was essentially quantitative by TLC.

Six grams of impure erythromycin 2′-glutaryl-*N*-dicyclohexylurea was dissolved in 25 ml of anhydrous acetone, and the solution was filtered and placed on a column packed by acetone slurry with 427 g silica gel[7] (70–325 mesh). The column was eluted with anhydrous acetone at a rate of 12 ml/min, and tubes numbering 30–79 were collected and pooled. The solvent was removed at room temperature, and 2.4 g of pure erythromycin 2′-glutaryl-*N*-dicyclohexylurea was collected.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 26, 1973, from the *Pharmacy Research Department, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication January 2, 1974.

Presented in part to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, San Diego, Calif., November 1973.

The author gratefully acknowledges the technical assistance of M. P. Jurgensen, J. F. Ochs, and J. F. Glasscock for synthesis of several of the compounds utilized in this work. Thanks are also extended to C. Lewis and associates for biological data.

Preparation and Pharmacological Screening of Indanethylamines Related to Tryptamine

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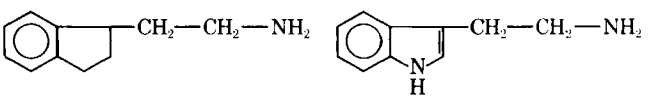
Abstract □ A series of mono- and di-*N*-substituted 2-(1-indanyl)-ethylamines was prepared and screened for monoamine oxidase inhibition and serotonin antagonism. Synthetic methods for their preparation are described, and the procedures used for their screening along with the activity found are discussed.

Keyphrases □ Indanethylamines, related to tryptamine—synthesis and pharmacological screening for monoamine oxidase inhibition and serotonin antagonism □ Tryptamine-related compounds—synthesis and screening of indanethylamines as monoamine oxidase inhibitors and serotonin antagonists □ Monoamine oxidase inhibition—synthesis and screening of indanethylamines □ Serotonin antagonism—synthesis and screening of indanethylamines

During an investigation in these laboratories directed toward the synthesis of compounds related to reserpine, the occasion arose to prepare 2-(1-indanyl)-ethylamine (I). The close isosteric relationship of I to tryptamine (II) aroused interest with reference to its pharmacological activity.

A review of the literature indicated that a biological investigation of I had not been published and only recently was its preparation reported (1). Further search revealed that pharmacological studies with 1-, 2-, and 3-indanylmethyl-, indanylethyl-, and indanylpropylamines have been confined chiefly to phenyl-substituted indanamines (2–8). Very few studies concerning these amines with substituents on the cyclopentano moiety of indan used substituents other than a phenyl group (9, 10). Since most studies



I II