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2-^{14}C-1-(2′-Deoxy-β-D-ribofuranosyl)-5-ethyluracil: Synthesis and Biotransformation in Rats

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Received December 15, 1975, from the *Research Laboratories, Pharmaceuticals Robugen GmbH, Alleenstrasse 22, D-73 Esslingen-Zell, West Germany.* Accepted for publication November 29, 1978.

Abstract □ With ^{14}C-urea as the starting material, 2-^{14}C-1-(2′-deoxy-β-D-ribofuranosyl)-5-ethyluracil [2-^{14}C-β-5-ethyl-2′-deoxyuridine] was synthesized for metabolic studies. After intravenous administration in rats, the radioactivity disappeared in the blood within 72 hr, being eliminated primarily in the urine (97%). The major elimination took place in the first 24 hr (92%). Little radioactivity was detected in the organs and tissues after 3 days. Approximately 50% of the urine radioactivity probably was due to an unidentified conjugate, 30% was due to Metabolite II, and 20% was unchanged drug. Metabolite II was identified as 5-ethyluracil by mass and ^{1}H-NMR spectra and by comparison with an authentic sample.

Keyphrases □ Ethyldeoxyuridine—synthesis, biotransformation in rats, metabolites □ Antiviral agents—ethyldeoxyuridine, synthesis, biotransformation in rats, metabolites

1-(2′-Deoxy-β-D-ribofuranosyl)-5-ethyluracil (β-5-ethyl-2′-deoxyuridine[1], VIII) has shown promising therapeutic activity against herpes simplex and vaccina viruses (1, 2) and against herpes keratitis in rabbits (3). Due to its compatibility with the ophthalmic mucous membrane, VIII is particularly suitable for the treatment of herpetic eye disease (4–7). Its excretion, distribution, and biotransformation were studied in rats using ^{14}C-VIII.

EXPERIMENTAL[2]

Synthesis—For metabolic studies, ^{14}C-β-5-ethyl-2′-deoxyuridine (VIII), specific activity 0.168 mCi/mmole, was synthesized (Scheme I). The starting material was ^{14}C-urea[3], specific activity 5.13 mCi/mmole.

2-^{14}C-5-Ethylbarbituric Acid (III)—Diethyl ethylmalonate (II) (9.4 g, 0.05 mole) was added dropwise to 10 ml of a 30% methanolic sodium methoxide solution. The reaction mixture was refluxed for 30 min with constant stirring. Then the ^{14}C-urea solution (3 g, 0.05 mole) in 10 ml of methanol was added quickly. The reaction mixture was refluxed with stirring for 6 hr under anhydrous conditions.

After cooling, the solvent was evaporated to dryness, and the residue was dissolved in 30 ml of hot water. The solution was acidified to pH 1–2 with 10 ml of concentrated hydrochloric acid. Crystals that precipitated overnight under refrigeration were filtered off, washed with ice water, and dried to yield 6.1 g of 2-^{14}C-III (78.3% based on ^{14}C-urea), mp 195–197° [lit. (8) mp 194–195°, (9) mp 193–194°].

2-^{14}C-5-Ethyl-6-chlorouracil (IV)—Phosphoryl chloride (14 ml) was added dropwise into a mixture of III (6.1 g, 0.035 mole) and water (0.5 ml). The reaction mixture temperature was slowly raised to 100° with constant stirring. After heating on an oil bath for 90 min at 90–100°, the reaction mixture was cooled. The remaining phosphoryl chloride was decomposed carefully with 25 ml of water.

The cooled mixture was stirred for 1 hr and allowed to stand in the refrigerator overnight. The white crystalline precipitate was filtered off, washed with ice water, and dried to yield 6.1 g of 2-^{14}C-IV (70% based on ^{14}C-urea), mp 215–217° [lit. (10) mp 215–217°].

2-^{14}C-5-Ethyluracil (V)—The solution of IV (6.1 g, 0.035 mole) in 2 *N* NaOH (58 ml) and 1% palladium-on-charcoal was hydrogenated at 60°. The hydrogen uptake virtually ceased after 3 hr. The catalyst was filtered off, and the filtrate was acidified with concentrated hydrochloric acid. After cooling, the precipitated crystals were filtered off, washed with cold water, and dried to yield 3.6 g of 2-^{14}C-V (51.4% based on ^{14}C-urea), mp 308–309° [lit. (8) mp 302–303°, (11, 12) mp 300–303°, (13) mp 300°, (14, 15) mp 308–309°].

2-^{14}C-2,4-Bis-O-(trimethylsilyl)-5-ethylpyrimidine (VI)—Compound V (3.6 g, 25.5 mmoles), hexamethyldisilazane (21 ml), and dimethylformamide (1.8 ml) were heated on an oil bath for 12 hr at 160–170° with constant stirring under anhydrous conditions. After cooling, the solvent was removed under reduced pressure. The residue, an oily yellow liquid, was used without further purification in the next step.

2-^{14}C-1-(3′,5′-Di-O-p-chlorobenzoyl-2′-deoxy-α,β-D-ribofuranosyl)-5-ethyluracil (VIIa and VIIb)—The reaction of VI with 3,5-di-*O*-*p*-chlorobenzoyl-2′-deoxy-α,β-D-ribofuranosyl chloride (16) to produce 1-(3′,5′-di-*O*-*p*-chlorobenzoyl-2′-deoxy-α,β-D-ribofuranosyl)-5-substi-

[1] Edurid, Robugen GmbH, 7300 Esslingen/Neckar, West Germany.

[2] Melting points were taken on a Tottoli (Büchi, Switzerland) apparatus and are uncorrected. Mass spectra were measured at an ionizing potential of 70 ev with a CH-7 Varian MAT spectrometer using a direct evaporator inlet system. ^{1}H-NMR spectra were recorded on a Varian T-60 spectrometer using dimethyl sulfoxide-d_6 as the solvent and trimethylsilane as the internal standard.

[3] New England Nuclear Corp., Boston, Mass.

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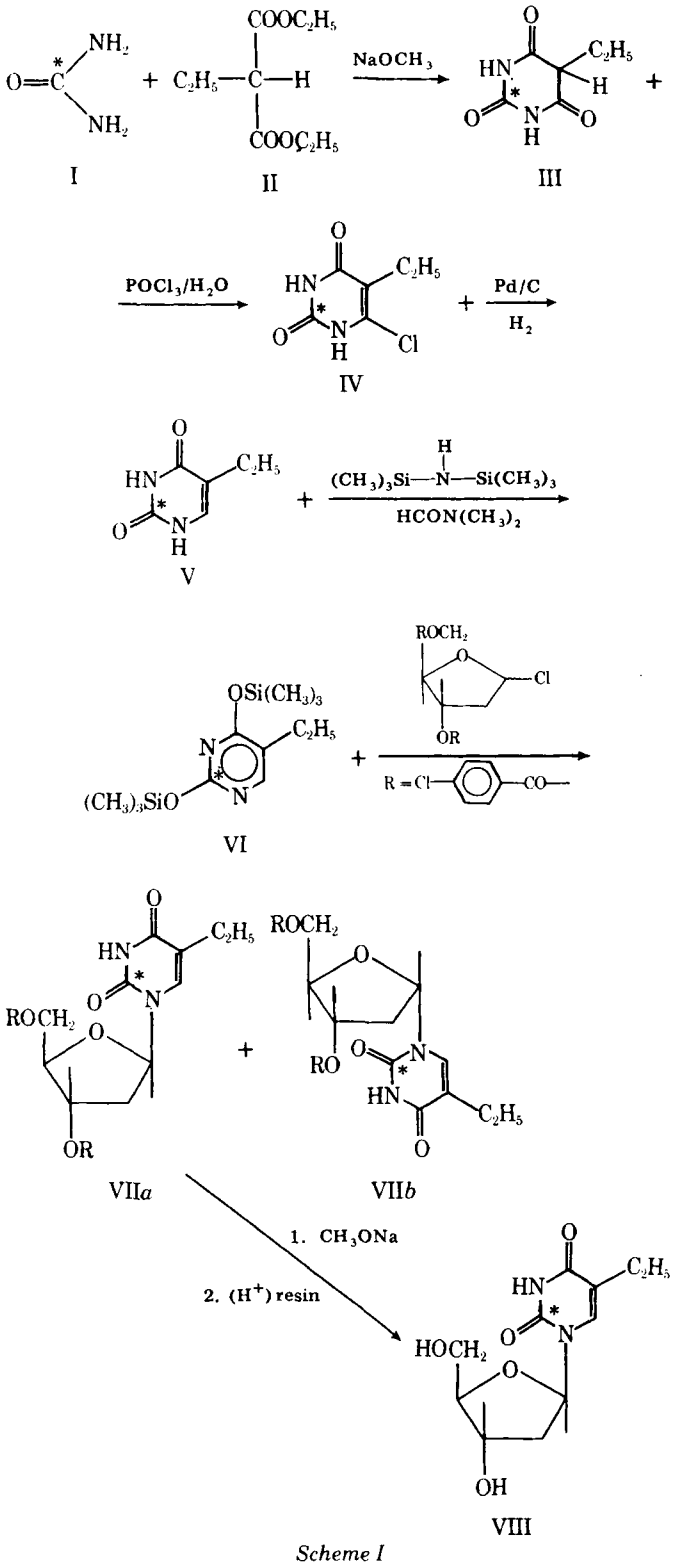


Scheme I

tuted pyrimidines is reported in the literature (17–21). With this method, ^{14}C-labeled VII*a* and VII*b* were synthesized in 43.4% yield based on ^{14}C-urea, mp 169–174°. The mixture of the blocked anomers was crystallized fractionally from toluene, yielding the pure α- and β-anomers: VII*a* (β-anomer), mp 196–197° [lit. (22) mp 195–196°]; and VII*b* (α-anomer), mp 186–188° [lit. (22) mp 186.5–187.5°].

2-^{14}C-1-(2′-Deoxy-β-D-ribofuranosyl)-5-ethyluracil (VIII)—To a suspension of the protected nucleoside VII*a* (3.6 g, 6.75 mmoles) in anhydrous methanol (35 ml), 30% methanolic sodium methoxide (0.65 ml) was added. The solution was stirred at room temperature for 4 hr, and methanol was evaporated under reduced pressure. Water (15 ml) was added to the oily residue. The formed ester was removed by extracting

Table I—Elimination of Radioactivity in Urine and Feces of Rats after 20 mg of 2-^{14}C-β-5-Ethyl-2′-deoxyuridine/kg iv

Sample	Hours	Radioactivity, % of applied dose
Urine	24	92
	48	5.1
	72	0.1
	96	—
Feces	24	0.5
	48	0.3
	72	0.1
	96	—

several times with methylene chloride. To the resulting solution an ion-exchange resin[4] (H^+), previously washed with methanol, was added. Stirring was continued for 5 min.

The solution was filtered from the resin, the filtrate was evaporated *in vacuo* to dryness, and acetone (25 ml) was added to the residue. After overnight refrigeration, the crystalline product was collected by filtration to yield 1.59 g of VIII (92% based on the blocked nucleoside VII*a*), mp 152–153° [lit. (22) mp 152–153°], specific activity 0.168 mCi/mmole. Radiochemical purity was checked by radioscanning of the TLC plates.

Radioactivity Measurements—The radioactive zones on TLC plates were located with a thin-layer scanner[5]. The radioassay in the solution was carried out in a two-canal liquid scintillation counter[6], using a scintillation solution of 1000 ml of dioxane, 180 g of naphthalene, 8 g of 2,5-diphenyloxazole, and 0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene. The external standardization technique was employed.

Application—A 1% solution of ^{14}C-VIII was made in normal saline. One-half milliliter was injected into the tail vein of 250-g male Wistar rats. The animals were kept in metabolic cages, which enabled collection of feces and urine separately. They were given free access to food[7] and water and were kept at 22° temperature and 55% humidity.

Sample Preparation—*Feces, Organs, and Tissues*—For qualitative and quantitative determination of the radioactivity, the fecal samples were dried at 60°, powdered, homogenized, and extracted with methanol in a soxhlet apparatus for 48 hr. The organs and tissues were cut into fine pieces and extracted with methanol for 72 hr. An aliquot (0.1 ml) of these samples was taken for required measurements.

Urine—An aliquot (0.1–0.2 ml) was mixed directly into the scintillation solution, and radioactivity was measured.

Chromatography—Analytical TLC was performed on 20 × 5-cm silica gel plates[8]. Solvent systems were (volume per volume): (*a*) chloroform–ether (8:2) for the blocked nucleoside, (*b*) chloroform–isopropanol (7:3) for the deblocked nucleoside, and (*c*) chloroform–isopropanol (5:5) for the metabolites.

Column chromatography was carried out on 0.05–0.2-mm silica gel with increasing proportions of isopropanol in chloroform.

Metabolite Isolation—The urine radioactivity could not be extracted with ether. The urine was evaporated to dryness, and the residue was extracted three times with isopropanol, yielding about 50% of the radioactivity (Extract A). The rest of the radioactivity could not be removed with organic solvents and possibly was due to conjugates (Extract B). The hydrolysis of the conjugate with β-glucuronidase and arylsulfatase[9] and subsequent isolation and identification of the fractions presented some difficulties and is in progress.

Extract A TLC showed radioactive peaks I and II in a 4:6 ratio, I being more polar than II. Extract A was concentrated to dryness and taken up in chloroform. It was loaded on a glass column (80 × 3 cm) filled with silica gel and eluted with increasing proportions of isopropanol in chloroform using a automatic fraction collector. Three hundred 20-ml fractions were collected. A radioassay was carried out with each fraction. The loaded radioactivity was recovered fully. Eluates 107–172 (7.5% isopropanol in chloroform) were pooled and concentrated to dryness, and the residue was recrystallized twice from methanol, mp 302–303°. It was designated Metabolite II.

Eluates 228–307 (20% isopropanol in chloroform) were concentrated, the solvent was removed, and the residue was recrystallized from toluene,

[4] Dowex 50 WX4, Dow Chemical Co., Midland, Mich.
[5] Prof. Dr. Rudolf Berthold Co., Wildbad, West Germany.
[6] Tri-Carb model 3950, Packard Instrument Co., La Grange, Ill.
[7] Altromin, Altromin, Lage/Lippe, West Germany.
[8] Silica gel HF_{254}, 0.25 mm, E. Merck AG, Darmstadt, West Germany.
[9] Glusulase, Boehringer, Mannheim, West Germany.

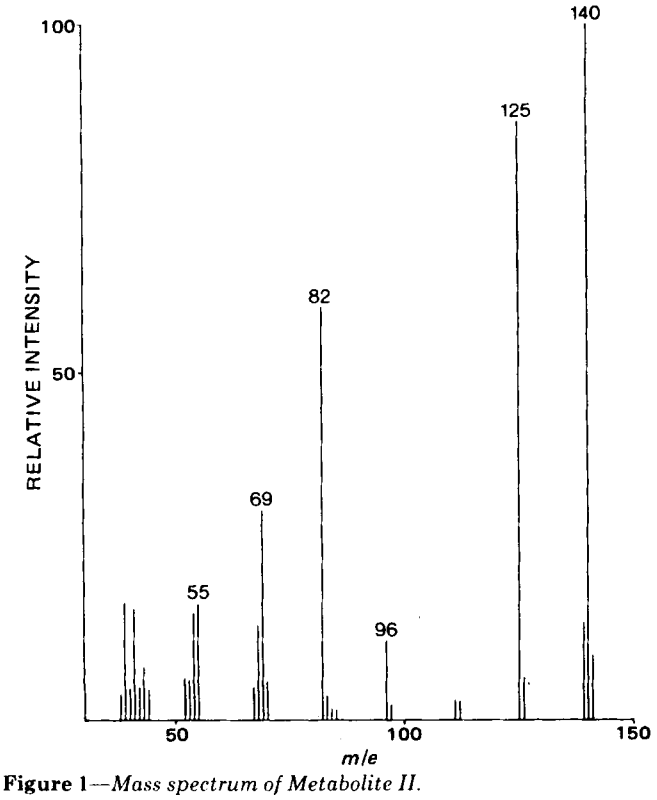


Figure 1—*Mass spectrum of Metabolite II.*

mp 152–153°. It was designated Metabolite I. By mixed melting-point determination and by comparison of the R_f values and mass spectra with an authentic VIII sample, Metabolite I could be identified as unchanged β-5-ethyl-2′-deoxyuridine (VIII).

RESULTS AND DISCUSSION

Excretion—Table I shows the elimination of urine and feces radioactivity up to 96 hr. Almost all of the radioactivity (98%) was extracted within 3 days. Moreover, the major part of the activity (92%) was excreted in the first 24 hr. Therefore, the kidneys are the main elimination organs in the excretion of β-5-ethyl-2′-deoxyuridine and its metabolites. Only a small amount of radioactivity could be found in the feces.

Distribution—In most organs and tissues as well as in the blood, <0.1% of the radioactivity could be found after 72 hr. After intravenous administration, VIII spontaneously disappeared from the blood and was completely eliminated.

Identification of Metabolite II—Metabolite II showed R_f values identical with those of 5-ethyluracil. In the mass spectrum (Fig. 1), the metastable peaks established the following fragmentation:

$$m/e\ 140 \xrightarrow{m^*\ 111.6} m/e\ 125 \xrightarrow{m^*\ 53.9} m/e\ 82$$

This pattern is characteristic of 5-ethyluracil, in which a methyl group is cleaved first. The hydrogen isocyanate (HCNO) loss in the final sequence is characteristic of uracil and barbituric acid derivatives in a retro-Diels-Alder fragmentation (23–25). For comparison, the mass spectrum of VIII was recorded (Fig. 2). The fragmentation of VIII can be formulated according to Scheme II.

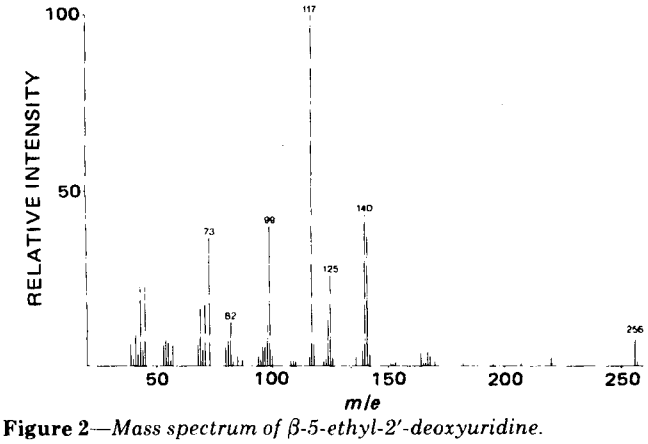
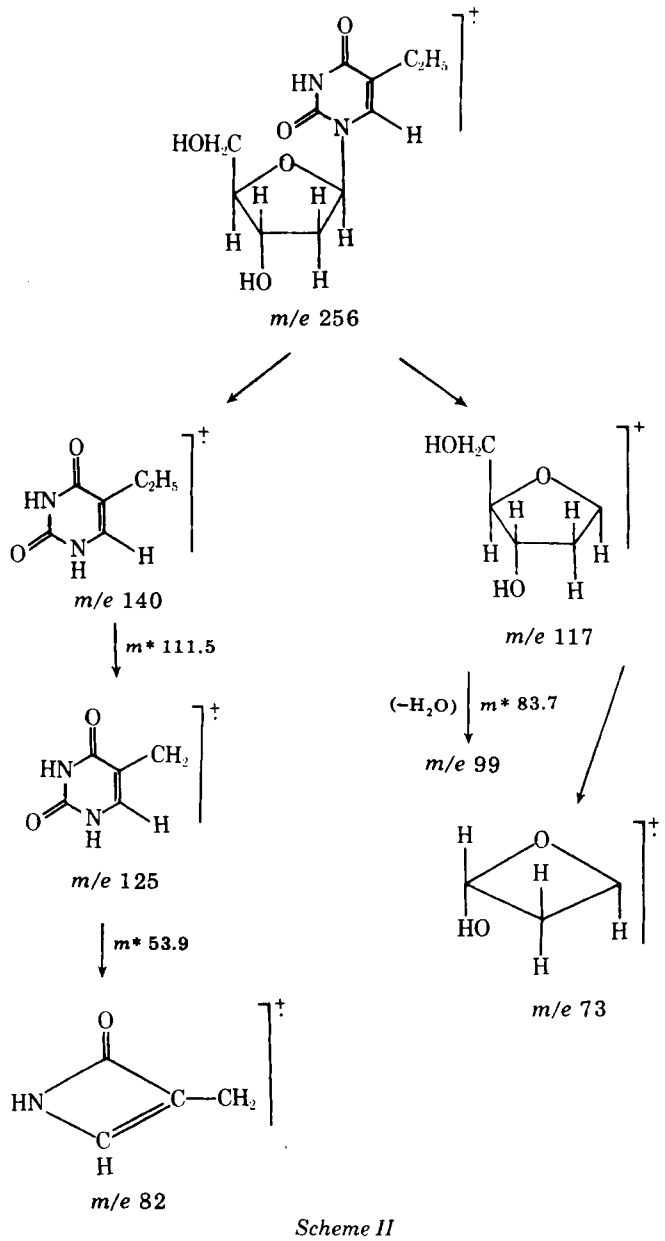


Figure 2—*Mass spectrum of β-5-ethyl-2′-deoxyuridine.*



Scheme II

The ^{1}H-NMR spectrum of II in dimethyl sulfoxide-d_6 showed the presence of eight protons. Three protons appeared as a triplet at δ 1.02 ppm, and two protons appeared as a quadruplet at δ 2.2 ppm. The broad doublet (one proton) appeared at δ 7.17 ppm ($J_{6\text{-H/N}^1\text{-H}}$ = 6 Hz). The broad lower field signals at δ 10.26 and 10.66 ppm were in close agreement with the N^1- and N^3-protons of thymine (N^1-H 10.55 ppm, N^3-H 10.95 ppm, dimethyl sulfoxide-d_6), respectively.

These data establish Metabolite II as 5-ethyluracil. The metabolite structure was further confirmed by an independent synthesis; the synthetic 5-ethyluracil mass spectrum and ^{1}H-NMR data were identical with those of II.

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Antimuscarinic Agents: Furan Analogs of Benzilate Esters

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Abstract □ The methiodide and ethiodide salts of 5-(dimethylaminomethyl)- and 5-(diethylaminomethyl)-α,α-diphenylfurfuryl alcohol were prepared. These compounds may be considered as furan analogs of dialkylaminoethyl benzilate alkiodides. The pA_2 values of these compounds as antagonists of acetylcholine were determined on rat jejunum preparation. All four compounds were significantly less potent than the analogous ester antimuscarinic lachesine. The furan ring cannot be substituted for the ester moiety of typical antimuscarinics. Possible modes of binding by antagonists to the receptor proposed previously are considered that might account for this less-than-expected antimuscarinic activity.

Keyphrases □ Antimuscarinic agents—furan analogs of benzilate esters, synthesis and activity □ Furan analogs—of benzilate esters, synthesis and antimuscarinic activity □ Benzilate esters, furan analogs—synthesis and antimuscarinic activity □ Structure-activity relationships—effect of furan analogs of benzilate esters on receptor binding activity, synthesis of antimuscarinic agents

A fundamental problem in medicinal chemistry is the determination of the topography of drug receptors, *i.e.*, the nature and relative position of groups (subsites) on the receptor that interact with groups on the drug molecule. Knowledge of receptor topography usually comes from the study of the relative activities of structurally different drugs that are used as probes of the receptor surface. In general, present knowledge of topography for the autonomic drug receptors is unsatisfactory.

BACKGROUND

An example is the muscarinic cholinergic receptor. For typical potent agonists such as acetylcholine, Bovet's acetal (I), and methylfurtrethonium (II), it usually is assumed that each compound combines with the same receptor site by interaction of the positive nitrogen with an anionic subsite and with the ester group, acetal group, or a portion of the furan ring interacting with an ester binding subsite.

Several investigators noted that extension of the carbon chain in potent muscarinic agonists caused a progressive decline in agonist potency and the appearance of competitive antagonist properties (1–3). It is uncertain just how the competitive antagonists combine with the muscarinic receptor relative to the acetylcholine receptor site. In the classical view, the competitive antagonists can bind to the anionic and ester binding subsites in the same manner as acetylcholine. The hydrocarbon rings then are believed to bind to a hydrophobic area located just beyond the ester binding subsite (Fig. 1).

Several discrepancies in the known structure–activity relationships are difficult to explain according to this classical view. Many potent antagonists do not contain an ester or comparable group between the nitrogen and the hydrophobic rings. The distance between the nitrogen and the rings is too short in many cases to bind as shown in Fig. 1. Even in ester-type antagonists, many changes in structure or stereochemistry cause changes in activity that do not parallel the effect of that change on agonist activity (4).

As a possible means of explaining the discrepancies between muscarinic agonist and antagonist structure–activity relationships, an alternative mode of binding for the antagonists was proposed (5, 6). In this view, the antagonist is believed to bind so that the cationic head overlaps the anionic subsite used by acetylcholine but the remainder of the molecule does

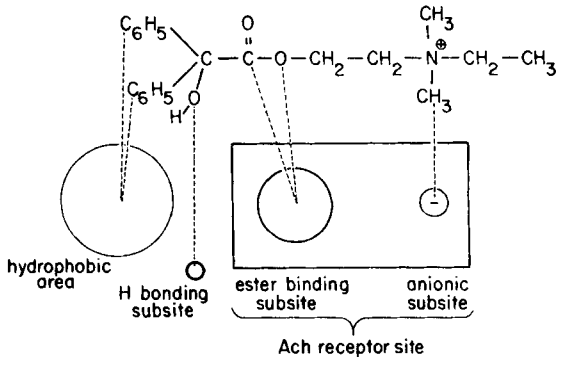

Figure 1—*Classical view of binding of lachesine to the muscarinic receptor (Ach = acetylcholine).*

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