(6) E. Riehm and K. K. Gauri, *Dtsch. Ophthalmol. Ges., Heidelberg*, **1968**, 69.
(7) W. Lerche and B. Schmolke, *Albrecht Graefes Arch. Klin. Exp. Ophthalmol.*, **184**, 193 (1972).
(8) J. Shapira, *J. Org. Chem.*, **27**, 1918 (1962).
(9) A. von Merkats, *Ber. Dtsch. Chem. Ges.*, **52**, 869 (1919).
(10) K. K. Gauri, Patent Robugen, DP 16 20 185 (1966).
(11) J. H. Burckhalter and H. C. Scarborough, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 545 (1955).
(12) J. Tafeland and H. B. Thompson, *Ber. Dsch. Chem. Ges.*, **10**, 4498 (1907).
(13) T. B. Johnson and G. A. Menge, *J. Biol. Chem.*, **2**, 105 (1906).
(14) M. Shakun-Todorovic and V. Tezdic, *J. Labelled Compd.*, **9**, 475 (1973).
(15) A. Guyot, J. Chopin, and C. Mentzer, *Bull. Soc. Chim. Fr.*, **1960**, 1596.
(16) M. P. Kotick, C. Szantai, and T. J. Bardos, *J. Org. Chem.*, **34**, 3806 (1969).
(17) E. Wittenburg, *Z. Chem.*, **4**, 303 (1964).
(18) E. Wittenburg, *Chem. Ber.*, **101**, 1095 (1968).
(19) T. Nishimura and J. Iwai, *Chem. Pharm. Bull.*, **12**, 357 (1964); through *Chem. Abstr.*, **60**, 15968 (1964).
(20) T. Nishimura, B. Shimizu, and J. Iwai, *Chem. Pharm. Bull.*, **12**, 1471 (1964); through *Chem. Abstr.*, **62**, 12236 (1965).
(21) T. Nishimura and B. Shimizu, *Chem. Pharm. Bull.*, **13**, 803 (1965); through *Chem. Abstr.*, **63**, 13388 (1965).
(22) M. Swierkowski and D. Shugar, *J. Med. Chem.*, **12**, 533 (1969).
(23) T. Nishiwaki, *Tetrahedron*, **22**, 3117 (1966).
(24) J. M. Rice, G. O. Dudek, and M. Barber, *J. Am. Chem. Soc.*, **87**, 4569 (1965).
(25) R. F. Skinner, E. G. Gallaher, and D. B. Predmore, *Anal. Chem.*, **45**, 574 (1973).

ACKNOWLEDGMENTS

The authors thank Miss Silvia Müller for technical assistance.

Antimuscarinic Agents: Furan Analogs of Benzilate Esters

JAMES F. STUBBINS ˣ, PATRICIA M. HUDGINS *, and DAGMAR C. MURPHY

Received July 23, 1979, from the *Departments of Pharmaceutical Chemistry and Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.* Accepted for publication November 29, 1979. *Present address: Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

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

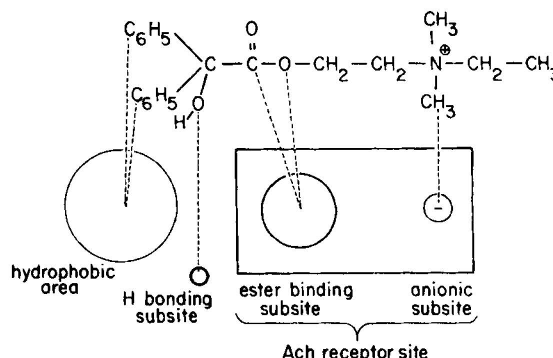


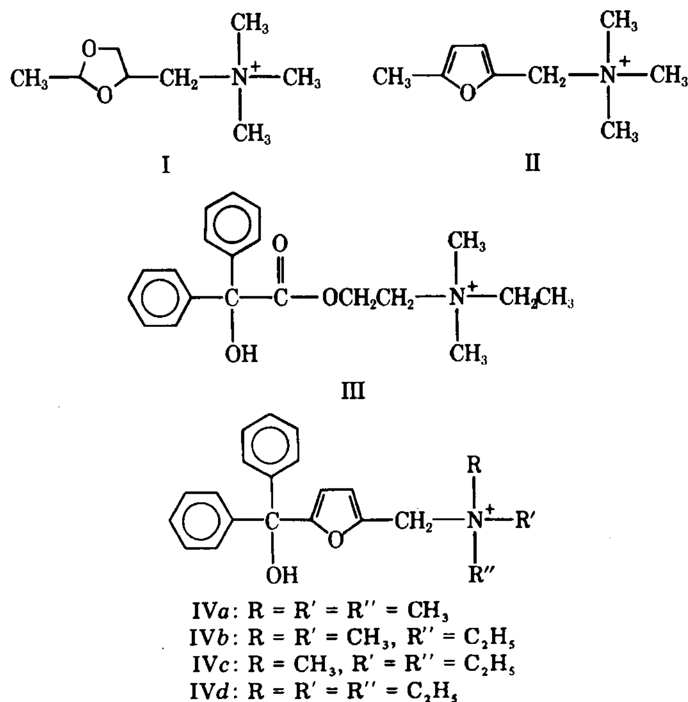
Figure 1—*Classical view of binding of lachesine to the muscarinic receptor (Ach = acetylcholine).*

0022-3549/80/0500-0534$01.00/0
© 1980, American Pharmaceutical Association

Table I—Properties of New 5-(Dialkylaminomethyl)-α,α-diphenyl-2-furfuryl Alcohol Alkiodides

Compound	Melting Point[a]	Yield, %[b]	Formula	Analysis, %[c] Calc.	Found	$pA_2 \pm SE$[d]
IV*a*	205–206°	41	$C_{21}H_{24}INO_2$	C 56.13	55.92	6.35 ± 0.13 (29)
				H 5.38	5.41	
				N 3.11	3.04	
IV*b*	193–194°	57	$C_{22}H_{26}INO_2$	C 57.02	57.20	6.24 ± 0.14 (31)
				H 5.65	5.69	
				N 3.02	2.98	
IV*c*	191–192°	70	$C_{23}H_{28}INO_2$	C 57.86	57.97	6.14 ± 0.12 (32)
				H 5.91	5.97	
				N 2.93	2.92	
IV*d*	184–186°	34	$C_{24}H_{30}INO_2$	C 58.66	58.77	5.75 ± 0.11 (26)
				H 6.15	6.29	
				N 2.85	2.79	
Lachesine						7.70 ± 0.17 (28)

[a] Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. [b] Reported yields are from single experiments with no attempt to recover additional compound from filtrates. [c] Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. [d] The $pA_2 + SE$ value is the negative log of the concentration causing a twofold shift in the response to acetylcholine plus or minus the standard error of the mean. The figure in parentheses is the number of points used in determining the pA_2 value.



not cover the ester binding subsite. The lipophilic and hydrogen bonding moieties in the antagonist may bind to other subsites as shown in Fig. 2.

Moran and Triggle proposed a third possible view of the antagonist binding to the receptor (7). They proposed a distinct site on the receptor for the antagonists that is near but does not overlap the acetylcholine binding site. Occupation of the antagonist site results in a perturbation of the acetylcholine binding site so that the affinity is lowered and the antagonism is still competitive.

The most potent muscarinic antagonists derived from acetylcholine generally are obtained by replacement of the acetyl group by benzilyl or similar groups containing two hydrophobic rings and a hydroxyl group attached to the α-carbon atom such as is found in lachesine (III) (8). If methylfurtrethonium and lachesine both bind to the anionic and ester binding subsites utilized by acetylcholine, in accord with the classical view, then substitution of two phenyl groups and a hydroxyl group on the terminal carbon atom of methylfurtrethonium (II) to give Type IV compounds should produce potent antagonists. In the present study, a series of such compounds with different substituents on the cationic nitrogen was prepared and examined for antimuscarinic activity.

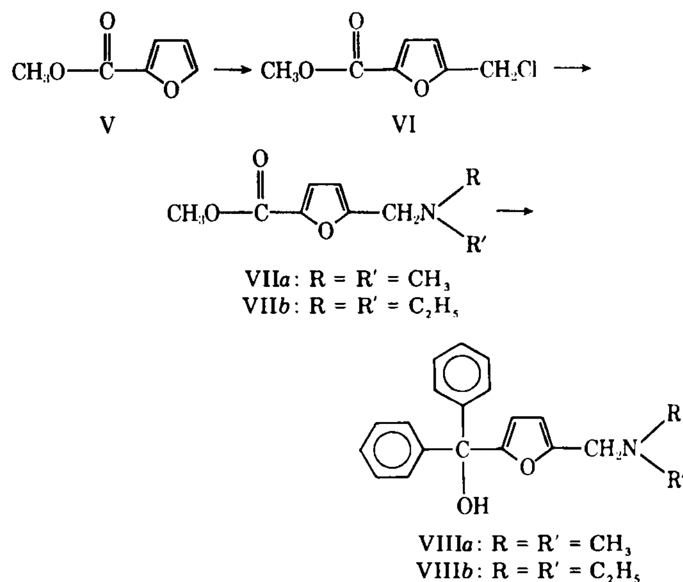
RESULTS AND DISCUSSION

Chemistry—The desired compounds were prepared by a method used (9) to prepare the bromide salt of IV*c* as illustrated in Scheme I, except that the methyl ester rather than the ethyl ester was the starting material. Chloromethylation of methyl 2-furoate (V) with paraformaldehyde, zinc chloride, and anhydrous hydrogen chloride yielded VI. Treatment of VI with dimethylamine or diethylamine gave VII*a* or VII*b*, respectively. An attempt to prepare VII*a* directly from V by a Mannich-type reaction, utilizing conditions employing 2-methylfuran (10), was unsuccessful. The ring-inactivating effect of the ester substituent apparently is too strong in this case.

Reaction of VII*a* or VII*b* with commercially prepared phenylmagnesium bromide gave the 5-(dialkylaminomethyl)-α,α-diphenyl-2-furfuryl alcohols (VIII*a* and VIII*b*). Alkylation of VIII*a* and VIII*b* with methyl iodide and ethyl iodide produced the desired alkiodide compounds. Properties of the new compounds are presented in Table I.

Pharmacology—To evaluate the affinity of the compounds for the muscarinic receptor, pA_2 values were measured by standard techniques. The method used was described previously in detail (11). Briefly, segments of rat jejunum were placed in an isolated organ bath containing Tyrode's solution at 25° and aerated with 95% oxygen-5% carbon dioxide. Cumulative dose–response curves to acetylcholine were obtained on each segment. These curves then were repeated in the presence of three or four different concentrations of the compound being evaluated. Segments of rat jejunum from six to eight animals were used with each compound. Linear regression analysis of the data according to the method of Arunlakshana and Schild (12) yielded the pA_2 values.

Because methylfurtrethonium is a more potent agonist than acetylcholine, it seems that the furan ring must bind well to a subsite on the receptor. If methylfurtrethonium binds to the same set of subsites as does acetylcholine, it would be reasonable to expect that the addition of two phenyl rings and a hydroxyl group to the terminal methyl group would produce a potent antagonist comparable to lachesine (III). An examination of Table I shows that the affinities of IV*a*–IV*d* are much less than those of lachesine; they are ~23–90-fold less potent as antagonists on rat



Scheme I

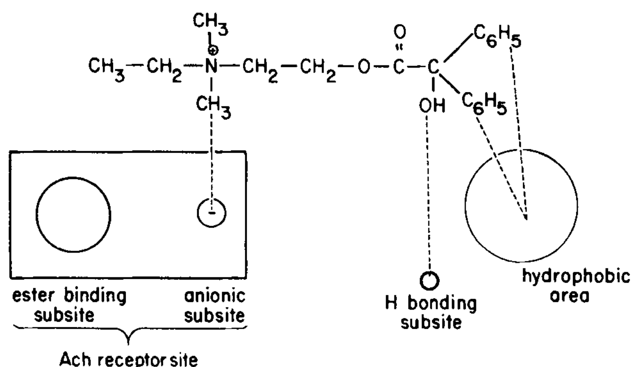


Figure 2—*Possible binding of lachesine to the muscarinic receptor according to Ariens' theory.*

jejunum. The furan ring clearly cannot be substituted for the ester group in the antagonists in the same manner as with the agonists. The classical view of antagonist binding to receptors does not explain the reason for this difference.

If antagonists bind in the manner proposed by Ariëns (5, 6) (Fig. 2), the relatively poor activity of the furan analogs could be explained. In such a case, the furan ring in the antagonists would not occupy a position on the receptor comparable to the furan ring in methylfurtrethonium. Earlier work by Stubbins and coworkers (11, 13, 14) was directed at finding evidence for the correctness of Ariëns' view. Since the ester binding subsite was not involved in binding antagonists according to this theory, it was proposed that addition of a properly placed agonist moiety on typical antagonists might increase the affinity and selectivity for the muscarinic receptor compared to antagonists lacking such a feature. A series of ester-type antagonists with moieties added to resemble acetylcholine, carbachol, or methylfurtrethonium was synthesized and compared in activity to lachesine. The binding of such compounds might be typified as shown in Fig. 3. In fact, the addition of the agonist moiety lowered the affinity in all compounds (13).

Ariëns' proposal might not apply to ester-type antagonists (which resemble acetylcholine more closely) but only to nonester antagonists. Therefore, a second series of 11 compounds having agonist moieties attached to typical nonester antagonist molecules was prepared and tested. A member of this series might bind as illustrated in Fig. 4. No consistent increase in affinity was found (14). Therefore, evidence in support of Ariëns' theory was not found, but this theory cannot be excluded.

Another possible way in which the difference could be explained is on the basis of the suggestion by Moran and Triggle (7) that methylfurtrethonium might bind to the receptor differently from acetylcholine. They suggested that the cationic head of all agonists binds to the anionic subsite but that the remainder of the molecule changes its position on the receptor depending on its relative lipophilic or hydrophilic character. According to this theory, methylfurtrethonium might bind as shown in Fig. 5. In this case, addition of two phenyl rings and a hydroxyl group to the two agonists would not be expected to affect affinity in the same way. Alternatively, the suggestion of Moran and Triggle that antagonists bind to entirely different sites than acetylcholine might account for the result.

In summary, the furan ring cannot replace the ester group in antimuscarinics without a significant loss in affinity for the receptor. The classical view of antagonist binding to the receptor is inadequate to explain this observation, but several alternative hypotheses might account for the results. More work is needed to determine the topography of the muscarinic receptor sites for agonists and antagonists.

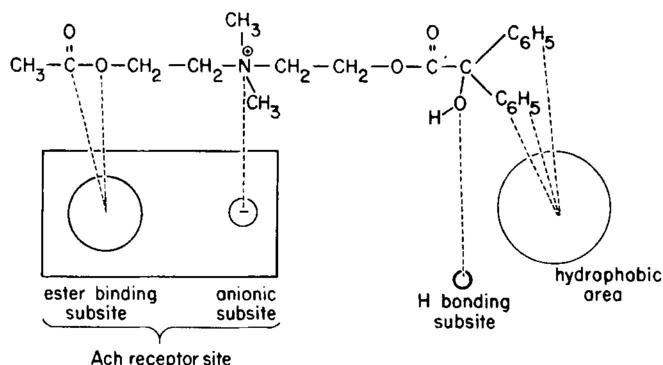


Figure 3—*Possible binding of ester antagonist with an additional agonist moiety to the muscarinic receptor.*

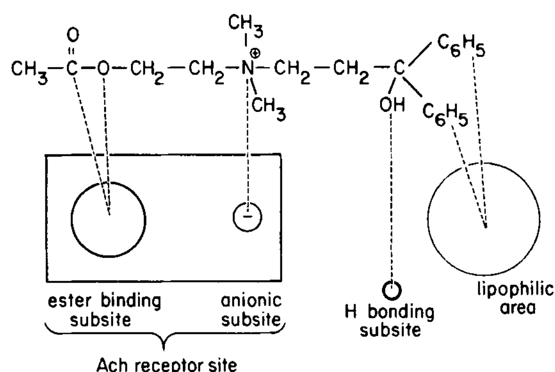


Figure 4—*Possible binding of nonester antagonist with an additional agonist moiety to the muscarinic receptor.*

EXPERIMENTAL

Methyl 5-Chloromethyl-2-furoate (VI)—Methyl 2-furoate (17.6 g, 140 mmoles) was dissolved in 50 ml of methylene chloride. Paraformaldehyde (6.0 g, 200 mmoles of formaldehyde) and zinc chloride (5.0 g, 37 mmoles) were added, and the mixture was warmed to 35°. Anhydrous hydrogen chloride gas was passed through the mixture until the exothermic reaction subsided. The mixture was poured into 300 ml of water. The organic layer was separated, washed twice more with water, and dried over potassium carbonate. The solvent was removed *in vacuo*, and the residual oil was distilled to give 16.2 g (66% yield) of VI, bp 86–88°/0.2 mm [lit. (15) bp 116–117°/2 mm].

Methyl 5-Dimethylaminomethyl-2-furoate (VII*a*)—Dimethylamine gas was bubbled slowly through a solution containing 15.0 g (86 mmoles) of VI in 130 ml of dry benzene for 2 hr. The mixture was allowed to stand overnight and was filtered. The solvent was evaporated from the filtrate, and the residue was distilled. Compound VII*a*, 11.5 g (73% yield), was obtained, bp 72–78°/0.2 mm.

Methyl 5-Diethylaminomethyl-2-furoate (VII*b*)—A solution of 16.2 g (93 mmoles) of VI in 35 ml of benzene was added dropwise to a stirred solution, heated to reflux, of diethylamine (17.4 g, 238 mmoles) in 30 ml of benzene. Heating was continued for 5 hr after addition was completed, and the mixture was allowed to stand at room temperature overnight. The solid by-product was removed by filtration, and the filtrate was evaporated. The residual oil was distilled to obtain 15.8 g (81% yield) of VII*b*, bp 94–98°/0.2 mm.

5 - Dimethylaminomethyl - α,α - diphenylfurfuryl Alcohol (VIII*a*)—Methyl 5-dimethylaminomethyl-2-furoate (11.1 g, 61 mmoles) dissolved in 30 ml of ether was added dropwise to a 2.07 *M* ethereal solution of phenylmagnesium bromide (120 ml, 241 mmoles) with stirring. After the addition was completed, the mixture was allowed to stand at room temperature for 1 hr and was heated to reflux for 2 hr. The mixture then was poured slowly into 350 ml of water containing 214 g of ethylenediaminetetraacetic acid and 117 g of sodium hydroxide. The ether layer was separated, and the aqueous layer was extracted three times with ether. The ethereal solutions were combined and dried over magnesium

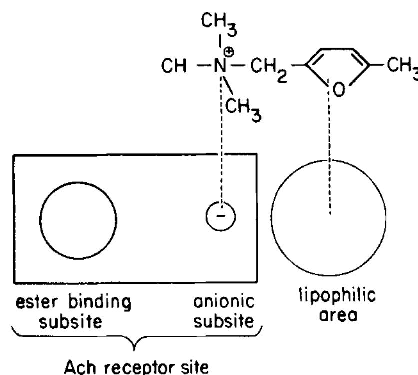


Figure 5—*Possible binding of methylfurtrethonium to the muscarinic receptor according to the proposal of Moran and Triggle (7).*

sulfate. Solvent removal left an oily residue, which crystallized upon trituration with petroleum ether. Recrystallization of the solid from benzene–petroleum ether yielded 7.6 g (41% yield) of VIII*a*, mp 122–124°.

5 - Diethylaminomethyl - α,α - diphenylfurfuryl Alcohol (VIII*b*)—Methyl 5-diethylaminomethyl-2-furoate (15.8 g, 75 mmoles) was reacted with phenylmagnesium bromide (300 mmoles) as in the preparation of VIII*a*. Compound VIII*b*, 10.0 g (40% yield), was obtained, mp 110–111.5° [lit. (9) mp 112–113°].

5-Dialkylaminomethyl-α,α-diphenylfurfuryl Alcohol Alkiodides (IV*a*–IV*d*)—Two grams of the tertiary amine (VIII*a* or VIII*b*) was dissolved in a minimum quantity of benzene. Methyl iodide or ethyl iodide (2 ml) was added, and the mixture was allowed to stand overnight. The product was removed by filtration and recrystallized from isopropanol. The physical properties of the compounds are given in Table I; NMR (dimethyl sulfoxide-d_6): 7.32–7.34 (s, 10H, phenyl), 6.83–6.88 (d, 1H, furan), 6.67–6.70 (s, 1H, OH), 6.17–6.27 (d, 1H, furan), and 4.59–4.68 (s, 2H, furfuryl methylene). In addition, the different compounds exhibited appropriate NMR peaks for the *N*-alkyl groups: 3.18–3.28 (m, NCH_2C), 2.91–3.04 (s, NCH_3), and 1.16–1.22 (t, CCH_3).

REFERENCES

(1) R. P. Stephenson, *Br. J. Pharmacol.*, **11**, 379 (1956).
(2) R. Schneider and A. R. Timms, *ibid.*, **12**, 30 (1957).
(3) J. M. van Rossum and E. J. Ariëns, *Arch. Int. Pharmacodyn. Ther.*, **110**, 349 (1957).
(4) E. J. Ariëns and A. M. Simonis, *Ann. N.Y. Acad. Sci.*, **144**, 842 (1967).
(5) E. J. Ariëns and A. M. Simonis, *J. Pharm. Pharmacol.*, **16**, 137 (1964).
(6) E. J. Ariëns, *Adv. Drug Res.*, **3**, 235 (1966).
(7) J. F. Moran and D. J. Triggle, in "Cholinergic Ligand Interactions," Academic, New York, N.Y., 1971, pp. 119–136.
(8) E. J. Ariëns and A. M. Simonis, *Arch. Int. Pharmacodyn. Ther.*, **127**, 479 (1960).
(9) C. L. Zirkle, U.S. pat. 3,097,206 (1963); through *Chem. Abstr.*, **59**, 13,950 (1963).
(10) R. F. Holdren and R. M. Hixon, *J. Am. Chem. Soc.*, **68**, 1198 (1946).
(11) J. F. Stubbins, P. M. Hudgins, J. Andrako, and A. J. Beebe, *J. Pharm. Sci.*, **57**, 534 (1968).
(12) O. Arunlakshana and H. O. Schild, *Br. J. Pharmacol.*, **14**, 48 (1959).
(13) P. M. Hudgins and J. F. Stubbins, *J. Pharmacol. Exp. Ther.*, **166**, 237 (1969).
(14) J. F. Stubbins, P. M. Hudgins, D. C. Murphy, and T. L. Dickerson, *J. Pharm. Sci.*, **61**, 470 (1972).
(15) British pat. 705,950 (1954); through *Chem. Abstr.*, **49**, 2233 (1955).

ACKNOWLEDGMENTS

Supported in part by National Institutes of Health Grant NS-07273.
The authors thank Ms. T. L. Dickerson for technical assistance.

Mode of Action of Sesquiterpene Lactones as Anti-Inflammatory Agents

I. H. HALL *x, C. O. STARNES, Jr. *, K. H. LEE *, and T. G. WADDELL ‡

Received July 29, 1979, from the **Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514*, and the ‡*Department of Chemistry, University of Tennessee at Chattanooga, Chattanooga, TN 37401.* Accepted for publication December 7, 1979.

Abstract ☐ Sesquiterpene lactones containing an α-methylene-γ-lactone moiety were shown to be potent inhibitors of carrageenan-induced edema and chronic adjuvant-induced arthritis in rodents at 2.5 mg/kg/day. The mode of action of sesquiterpene lactones as anti-inflammatory agents appeared to be at multiple sites; for example, at 5×10^{-4} *M*, the sesquiterpene lactones effectively uncoupled the oxidative phosphorylation of human polymorphonuclear neutrophils and elevated the cyclic adenosine monophosphate levels of rat neutrophils and rat and mouse liver cells. Free and total lysosomal enzymatic activity was inhibited by these agents at 5×10^{-4} *M* in both rat and mouse liver and rat and human neutrophils. Furthermore, the structure–activity relationships for the stabilization of lysosomal membrane for rat liver cathepsin activity followed the same structural requirement necessary for anti-inflammatory activity; *i.e.*, the α-methylene-γ-lactone moiety contributed the most activity, whereas the β-unsubstituted cyclopentenone and α-epoxycyclopentanone contributed only minor activity. Human polymorphonuclear neutrophil chemotaxis was inhibited at low concentrations (*i.e.*, 5×10^{-5} and 5×10^{-6} *M*), whereas prostaglandin synthetase activity was inhibited at a higher concentration (*i.e.*, 10^{-3} *M*) by the sesquiterpene lactones.

Keyphrases ☐ Anti-inflammatory agents—sesquiterpene lactones, mode of action determined *in vitro* and *in vivo* ☐ Sesquiterpene lactones—anti-inflammatory agents, antiarthritic agents, mode of action determined in rat and human neutrophils and rat and mouse liver cells ☐ Oxidative phosphorylation—uncoupling effect of sesquiterpene lactones, mode of action determined in inflammation process ☐ Lysosomal membrane—effect of sesquiterpene lactones on membrane stability, hydrolytic enzyme release, mode of action in inflammation process ☐ Cyclic adenosine monophosphate—effect of sesquiterpene lactones on *in vitro* levels, role in inflammation process ☐ Structure–activity relationships—effect of sesquiterpene lactones on inflammation process, several modes of action determined *in vitro* and *in vivo*

The anti-inflammatory activity of sesquiterpene lactones in rodents was reported previously (1). An α-methylene-γ-lactone moiety within the structure of the pseudoguaianolide and germacranolide derivatives was required for activity against edema-induced carrageenan inflammation. Saturation of the 11,13-double bond of the methylene group of the lactone resulted in a loss of activity (1).

In the chronic adjuvant-induced arthritic screen, compounds containing the α-methylene-γ-lactone, the β-unsubstituted cyclopentenone, and α-epoxycyclopentanone afforded significant inhibitory activity at 2.5 mg/kg/day (1). In addition, these derivatives also suppressed the writhing reflex, induced pleurisy, delayed hypersensitivity, and passive cutaneous anaphylaxis and were mild immunostimulants of immunoglobulins (1). The effects of the

0022-3549/80/0500-0537$01.00/0
© 1980, American Pharmaceutical Association