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Synthesis of Some Glycidic Hydrazides and Amides as Potential Psychotropic Agents and Anticholinergic Agents

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Abstract □ A series of glycidic hydrazides and amides was prepared by hydrazinolysis or aminolysis of glycidic esters obtained *via* a modified Darzens condensation. The hydrazides thus obtained were subjected to suitable acylating or alkylating reagents to obtain *N*-substituted hydrazides. The results of a preliminary pharmacological evaluation are summarized. The synthesized compounds were tested for their ability to reverse reserpine hypothermia in mice. Compounds synthesized as potential anticholinergics were evaluated for their spasmolytic activity using isolated rabbit ileum.

Keyphrases □ Glycidic hydrazides, amides—synthesis □ Hydrazides, glycidic—synthesis □ Amides, glycidic—synthesis □ IR spectrophotometry—identity □ Pharmacological screening—glycidic hydrazides, amides

Appropriately substituted hydrazides and amides have been of interest to the medicinal chemist for various reasons. Monoamine oxidase-inhibitory hydrazides have proved to possess dynamic pharmacological properties (*i.e.*, antitubercular and/or antidepressant properties). Amides that are analogous to bioactive esters (*e.g.*, procaine *versus* procainamide) offer models for structure–activity analysis on the basis of the predicted greater metabolic stability of amides due to their greater resonance stabilization.

Since the epoxide group affects the physicochemical properties (*e.g.*, lipid–water partition coefficient) of a compound, this function might also influence pharmacologic properties. Hence, it was decided to prepare α,β-epoxy hydrazides and amides for pharmacologic evaluation and to provide a basis for the study of the effect of the epoxide moiety on bioactivity.

Literature reports and reviews (1–4) substantiate the utility and applications of the Darzens glycidic ester condensation. Consequently, this reaction was applied to the synthesis of α,β-epoxy esters which, when exposed to hydrazinolysis and/or aminolysis, yield potentially active hydrazides and amides.

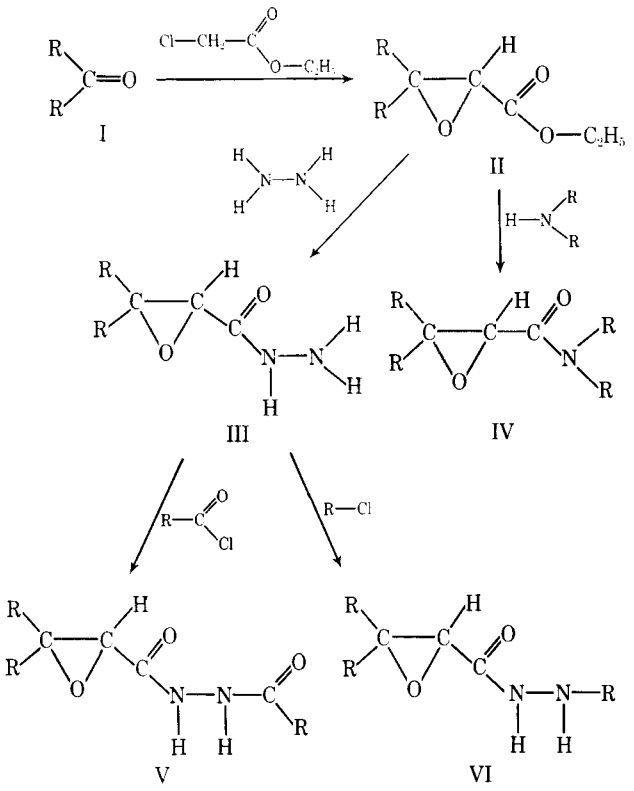
α,β-Epoxy amides and hydrazides have been of interest to the authors and others (5) as potential therapeutic agents. In appropriately substituted glycidic hydrazides, the presence of the epoxide function may affect the distribution of compounds possessing monoamine oxidase-inhibiting pharmacophores. In addition, the epoxide function may affect the susceptibility of such compounds to metabolic degradation. Conceivably, the drug–receptor interaction would be influenced as well, depending upon the degree of hydrolysis of the hydrazide linkage. Zeller (6) and others (7) have postulated that certain substituents play an important function in the bioactivity of appropriately substituted hydrazine derivatives. Several basic structural features appear to be associated with optimal monoamine oxidase inhibitory activity. These can be summarized as follows: (*a*) there should be at least one alkyl substituent on the hydrazine moiety, and (*b*) *N*-1 alkylation and *N*-2 acylation yield compounds with increased activity and decreased toxicity. On the other hand, disubstitution of either hydrazine nitrogen leads to inactive compounds. The alkyl and aralkyl substituents contribute to electronic, steric, and hydrophobic factors involved in the inhibitor–enzyme interaction, whereas the acyl moiety has been implicated as affecting the distribution and should be of such a nature that it can be easily hydrolyzed (8). Accordingly, the choice of the acyl moiety presumably is important with regard to tissue selectivity and susceptibility to metabolic cleavage to yield the postulated active moiety.

Since this work involves the synthesis of selected α,β-epoxy hydrazides as potential psychotropic agents, it is fundamental to the study of the effect of the epoxide function on absorption, distribution, and metabolic fate of hydrazine derivatives.

Additionally, glycidic amides were prepared modeled after classical anticholinergic agents. Such compounds possessing the α,β-epoxy amide function, in addition to the potential ammonium group and appropriately positioned bulky, semirigid, and hydrophobic moieties, should exhibit anticholinergic spasmolytic activity; those without a potential ammonium function should be less active. Accordingly, it was decided to compare Compound X with the classic anticholinergic atropine as well as with the analogous amides: Compounds VII and VIII, which do not possess potential ammonium functions. The epoxide cycle should affect distribution as well as the receptor interaction; hence, α,β-epoxy amides were chosen for this study.

The compounds described herein were prepared by the application of the following reactions: Darzens

condensation, hydrazinolysis, aminolysis, *N*-acylation, and *N*-benzylation.



Scheme I

The carbonyl compounds (I) (Scheme I) employed as starting materials included pyridine-3-carboxaldehyde, pyridine-4-carboxaldehyde, diphenylacetaldehyde, benzophenone, dibenzosuberone, and xanthone. The Darzens glycidic ester product (II) of each of the foregoing carbonyl compounds was subjected to aminolysis (IV) and/or hydrazinolysis (III). The hydrazides (III) obtained then were treated with either benzyl chloride, benzoyl chloride, or 3,4,5-trimethoxybenzoyl chloride to prepare the substituted hydrazide derivatives (V and VI). The compounds prepared, together with their respective melting points and analytical data, are listed in Table I.

EXPERIMENTAL[1]

Preparation of Esters—The glycidic esters, starting materials for the synthesis of the glycidic hydrazides and amides, were prepared according to the method of Omodt and Gisvold (3) with suitable modifications. The yield was better from pyridine-3-carboxaldehyde (60–70%) than from pyridine-4-carboxaldehyde (40–50%). Additionally, among the ketones, dibenzosuberone gave higher yields (40–50%) of the glycidic ester than did xanthone (20–30%). IR spectrophotometry was utilized in the characterization of the esters described. The IR spectra showed characteristic peaks for the α,β-epoxy ester function: 1720 cm.$^{-1}$ ($\rangle$C=O); 1260 and 880 cm.$^{-1}$ (epoxide).

Ethyl 3-(4-Pyridyl)glycidate—Pyridine-4-carboxaldehyde (10.7 g.; 0.1 mole) and ethyl chloroacetate (12.6 g.; 0.1 mole) dissolved in anhydrous ether (150 ml.) were placed in a 3-neck, round-bottom flask equipped with mechanical stirrer, reflux condenser, calcium chloride drying tube, nitrogen inlet, and dropping funnel. Sodium hydride (4.8 g.; 0.1 mole of a 51% mineral oil dispersion) was added slowly to prevent excessive effervescence. Ethanol (4.8 g.; 0.1 mole) was added dropwise while cooling the solution to 0–10°. The mixture was stirred for 18 hr. while it was allowed to come to room temperature. All the while, nitrogen was passed through the reaction mixture. At this time the ether was removed under reduced pressure and replaced with an equal volume of chloroform. The mixture was then refluxed for 4 hr. Water (50 ml.) was added. The resulting two layers were separated. The chloroform layer was washed with a saturated solution of sodium chloride (three 50-ml. portions), a saturated solution of sodium bicarbonate (three 50-ml. portions), and a saturated solution of sodium bisulfite (three 50-ml. portions), and it was dried over anhydrous sodium sulfate. After filtering, the solvent was removed using a Rinco rotatory evaporator. The residue which resulted was characterized as the picrate salt, m.p. 101–102°.

Anal.—Calcd. for $C_{16}H_{14}N_4O_{10}$: C, 45.50; H, 3.31. Found: C, 45.54; H, 3.42.

Ethyl 3-(3-Pyridyl)glycidate—Pyridine-3-carboxaldehyde (10.7 g.; 0.1 mole) and ethyl chloroacetate (15.9 g.; 0.13 mole) in ethanol (4.6 g.; 0.1 mole) were added *via* two dropping funnels to a toluene dispersion of sodium hydride (4.8 g.; 0.1 mole) under nitrogen and cooled to 0–10°. The reaction mixture was allowed to come to room temperature and stirred for a period of 12 hr. Glacial acetic acid (3 ml.) and water (150 ml.) were added. The aqueous layer was separated and washed with ether, and the ether extracts were combined with the organic layer. The combined organic portions were washed as before, and the solvents were removed under reduced pressure. The crude epoxy ester was chromatographed on a silicic acid column developed with ether. The oil which was isolated was identified on the basis of its elemental analysis.

Anal.—Calcd. for $C_{10}H_{11}NO_3$: C, 62.15; H, 5.73. Found: C, 61.98; H, 5.95.

Ethyl 3,3-Diphenylglycidate—Benzophenone (18.2 g.; 0.1 mole) was dissolved in anhydrous ether (100 ml.) and placed in a 3-neck flask equipped as previously described. Ethyl chloroacetate (12.6 g.; 0.1 mole), dissolved in anhydrous ether (50 ml.), was added. While cooling the solution to 0–10°, freshly prepared sodium ethoxide (6.8 g.; 0.1 mole) was dissolved in anhydrous ether (150 ml.) and slowly added to prevent excessive effervescence. Stirring was continued for 18 hr. The ether was removed and replaced with an equivalent amount of anhydrous chloroform, and the mixture was refluxed for 18 hr. Glacial acetic acid (3 ml.) and water (150 ml.) were added cautiously. The organic layer was removed and washed as before and was concentrated under reduced pressure. The concentrate was distilled under reduced pressure; the product, distilling at 120–128° at 5 mm., solidified at room temperature and had a melting point of 46°.

Anal.—Calcd. for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 75.76; H, 5.92.

Ethyl 3-Xanthylglycidate—Ethyl 3-xanthylglycidate was synthesized in a manner analogous to that used for ethyl 3,3-diphenylglycidate, using xanthone and ethyl chloroacetate as the starting materials. On concentration of the organic layer, the residual material was characterized in the form of Derivatives IX and X, Table I.

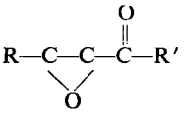
Ethyl 3-Dibenzosuberylglycidate—Ethyl 3-dibenzosuberylglycidate also was prepared according to the foregoing method, using ethyl chloroacetate and dibenzosuberone. Chromatography (silicic acid column) was used to purify the product.

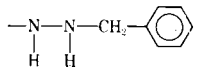
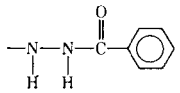
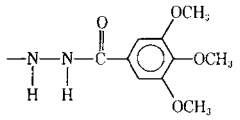
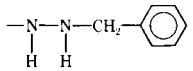
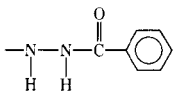
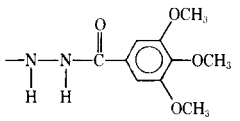
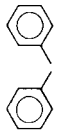
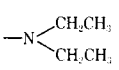
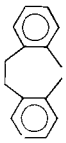
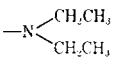
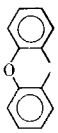
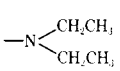
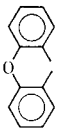
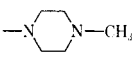
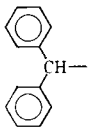
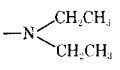
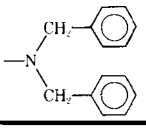
Anal.—Calcd. for $C_{19}H_{18}O_3$: C, 77.58; H, 6.16. Found: C, 77.86; H, 5.94.

Ethyl 3,3-Diphenylmethylglycidate—Ethyl 3,3-diphenylmethylglycidate was synthesized as previously described, employing diphenylacetaldehyde and ethyl chloroacetate. The product was purified by column chromatography on silicic acid and was characterized in the form of the corresponding *N,N*-diethylamide derivative (Compound XI, Table I).

[1] Reported melting points are uncorrected. A Thomas-Hoover Unimelt apparatus was used for the melting point determinations. Elemental analyses were conducted by the Schwarzkopf Micro-analytical Laboratory, Woodside, N. Y. IR spectral analyses were conducted with a Beckman IR-8 spectrophotometer.

Table I—Elemental Analysis for the Synthesized Compounds



Compd. No.	R	R′	Method of Purification	M.p.	Anal., % C Calcd.	C Found	H Calcd.	H Found
I			Alcohol–ether	Semisolid	66.90	66.77	5.57	5.38
II			Ethanol–water	195–196°	63.63	63.87	4.61	4.48
III			Ethanol	160–162°	57.90	57.43	5.10	4.93
IV			Isopropyl ether–ethanol	178–179°	66.90	67.21	5.61	6.02
V			Water–ethanol	187–189°	63.59	63.21	4.63	4.89
VI			Anhydrous ether	160–161°	57.91	58.24	5.09	4.87
VII			Ether	Oil	77.26	77.82	7.16	6.99
VIII			Chromatographed on silica	Oil	78.47	78.80	7.21	6.97
IX			Chloroform–ethanol	196–198°	73.76	74.00	6.19	6.38
X			Chromatographed on silica	Oil	71.41	70.98	5.99	6.18
XI			Chromatographed on silica	Semisolid	77.63	77.82	7.49	6.99
XII			Chromatographed on silica	Oil	76.72	77.05	5.85	5.43

Synthesis of α,β-Epoxy Hydrazides—The hydrazides were prepared by hydrazinolysis of the esters, and the yields were in the range of 25–30% of theory. IR spectral analyses were conducted on representative compounds, and the data substantiated the structures in Table I. The IR spectra showed characteristic peaks: 1600 cm.$^{-1}$ ($\rangle$C=O); 1260 and 880 cm.$^{-1}$ (epoxide).

1-[3-(4-Pyridyl)glycidyl]hydrazine—Ethyl 4-pyridylglycidate (4.8 g.; 0.025 mole) was dissolved in anhydrous benzene (80 ml.) and ethanol (15 ml.) and placed in a flask equipped with a Dean-

Stark separator. Hydrazine (anhydrous 95%) (0.8 g.; 0.025 mole) was added dropwise while stirring. After all of the hydrazine was added, the mixture was refluxed for 4 hr. At this time, most of the alcohol had been collected in the Dean-Stark separator, denoting approximate completion of the hydrazinolysis. The solvent was removed under reduced pressure, and the semisolid remaining was cooled to induce crystallization. The product was dried *in vacuo* and characterized.

Anal.—Calcd. for $C_8H_9N_3O_2$: C, 53.63; H, 5.06. Found: C, 53.97; H, 4.97.

1-[3-(3-Pyridyl)glycidyl]hydrazine—A procedure analogous to that employed for the 4-pyridyl isomer was used to synthesize and characterize the compound.

Anal.—Calcd. for $C_8H_9N_3O_2$: C, 53.62; H, 5.06. Found: C, 53.92; H, 5.54.

1-[3-(4-Pyridyl)glycidyl]-2-benzyl Hydrazine (Compound IV, Table I)—1-[3-(4-Pyridyl)glycidyl]hydrazine (2.2 g.; 0.0125 mole) was dissolved in anhydrous ether (40 ml.) and, while stirring, benzyl chloride (1.42 g.; 0.0125 mole) was slowly added. Additional ether (20 ml.) and 5% aqueous sodium hydroxide solution (10 ml.) were added to the mixture. The ether layer was separated, dried, and concentrated under reduced pressure. The resulting product was purified *via* crystallization from ethanol–isopropyl ether.

1-[3-(3-Pyridyl)glycidyl]-2-benzyl Hydrazine (Compound I, Table I)—The corresponding 3-pyridyl analog was prepared similarly to the 4-pyridyl isomer.

N-1-[3-(4-Pyridyl)glycidyl]-N-2-(3,4,5-trimethoxybenzoyl)hydrazine (Compound VI, Table I)—3-(4-Pyridyl)glycidyl hydrazine (4.4 g.; 0.025 mole) was dissolved in anhydrous benzene (60 ml.) with the aid of ethanol (10 ml.). Pyridine (2 ml.) was added. 3,4,5-Trimethoxybenzoyl chloride (3.7 g.; 0.025 mole) was added, and the mixture was refluxed for 6 hr. At this time, water (15 ml.) was added to dissolve the salt formed. The nonaqueous layer was separated, washed with a saturated solution of sodium bicarbonate, dried, and concentrated under reduced pressure. The resulting product was recrystallized from anhydrous ether.

N-1-[3-(3-Pyridyl)glycidyl]-N-2-(3,4,5-trimethoxybenzoyl)hydrazine (Compound III, Table I)—This compound was prepared in a manner similar to that used for the 4-pyridyl isomer.

1-[3-(4-Pyridyl)glycidyl]-2-benzoyl Hydrazine (Compound V, Table I)—3-(4-Pyridyl)glycidyl hydrazine (5 g.; 0.028 mole) was dissolved in anhydrous pyridine (30 ml.) and anhydrous benzene (60 ml.). The benzoyl chloride (5 g.; 0.036 mole) was added as quickly as possible. The resulting mixture was refluxed for 6 hr. and then poured into 250 ml. of water. The aqueous layer was removed and washed with benzene. The combined organic solutions were washed with 5% aqueous sodium bicarbonate solution and dried. After filtering, the solution was concentrated under reduced pressure. The product was isolated by crystallization from a water–ethanol mixture and characterized.

1-[3-(3-Pyridyl)glycidyl]-2-benzoyl Hydrazine (Compound II, Table I)—This compound was prepared according to the procedure described for the 4-pyridyl analog.

Synthesis of α,β-Epoxy Amides—*α,β-Epoxy* N,N-*Diethyl Amides*—The compounds, together with their respective melting points and/or analytical data, are listed in Table I. Compounds VII–XII were prepared by treating the respective glycidic esters with diethylamine in a procedure according to that given for *N,N*-diethyl-3,3-diphenylmethyl glycidamide. The yields obtained ranged between 30–35%. IR spectral analyses were conducted on representative compounds, and the data substantiated the structures in Table I. The IR spectra showed characteristic peaks: 1650 cm.$^{-1}$ ($\rangle$C=O); 1260 and 880 cm.$^{-1}$ (epoxide).

N,N-Diethyl 3,3-Diphenylmethylglycidamide (Compound XI, Table I)—Ethyl 3,3-diphenylmethylglycidate (7.0 g.; 0.025 mole) was dissolved in anhydrous benzene (75 ml.) with the aid of anhydrous ethanol (15 ml.). Diethylamine (2.0 g.; 0.027 mole) was slowly added. The mixture was refluxed for 6 hr. At this time, it was cooled and a methyl halide was added to remove unreacted amine. The filtered solution was concentrated under reduced pressure. The oil which resulted was chromatographed on silica.

N-3-Xanthylglycidyl-N-methylpiperazine (Compound X, Table I)—Ethyl 3-xanthylglycidate (3.5 g.; 0.0126 mole) was dissolved in anhydrous benzene (80 ml.) with the aid of anhydrous ethanol (10 ml.) and heat. *N*-Methylpiperazine (1.3 g.; 0.0125 mole) was added, and the mixture was refluxed for 4 hr. The *N*-methylpiperazyl derivative was separated from the reaction mixture after concentration under reduced pressure by vacuum distillation The methyl bromide derivative was prepared by conventional methods.

PHARMACOLOGIC EVALUATION

This pharmacologic evaluation was undertaken to obtain a preliminary indication of some structure–activity relationships among the compounds synthesized. Considering that some of these compounds were synthesized as potential monoamine oxidase inhibitors and others as anticholinergic spasmolytics, it was decided to test for these bioactivities according to the following methods. Compounds I through XII were tested for their ability to reverse reserpine hypothermia in mice. Compounds VII, VIII, and X were evaluated for their spasmolytic activity.

Reversal of Reserpine Hypothermia—*Method*—The administration of reserpine to normal animals usually results in a gradual depression of the central and peripheral sympathetic systems and is characterized by conditions such as sedation, ptosis, hypotension, bradycardia, and hypothermia (9).

Pretreatment with a monoamine oxidase inhibitor results in a reversal of the reserpine effects. Symptoms of excitation, exophthalamus, piloerection, mydriasis, and hyperthermia are observed (10).

The method of Garrattini *et al.* (11) was adopted for the estimation of the effect of the test compounds on reserpine-induced hypothermia.

Although only the hydrazides were prepared as potential monoamine oxidase inhibitors, it was decided also to evaluate the amides of the series.

Young male Swiss-Webster mice weighing between 20 and 35 g. were used throughout. The animals were given free access to food and water before and during the evaluation. The testing was scheduled so that no mouse was used more often than once a week.

Prior to the administration of any drug, the rectal temperature of each mouse was determined using a telethermometer with rectal probe. The rectal temperature then was measured at 4, 6, and 24 hr. after the intraperitoneal administration of reserpine, 2.5 mg./kg. One week later, the same group of mice was given a dose of the synthesized drug intraperitoneally, followed by intraperitoneal administration of reserpine 4 hr. later. The ability of the compound to reverse the reserpine-induced hypothermia 4, 6, and 24 hr. after the reserpine injection was noted.

Doses of the individual compounds were isomolar with respect to the standard: iproniazid. Most of the drugs were sufficiently soluble to be given either as a solution or as a stable suspension in propylene glycol.

Results—The experimental results are listed in Table II.

Discussion of Results—The pharmacologic data reveal bioactivity differences among the synthesized compounds in the test method utilized. Some of the more significant may be summarized. Among hydrazine derivatives, the 3-pyridyl series is less active than the 4-pyridyl. Also, among hydrazine derivatives, *N*-benzyl derivatives tend to be more active than the corresponding diacyl derivatives, but a significant exception is found in the case of the trimethoxybenzoyl derivatives which are more active than the benzyl derivatives. Actually, highest activity is found among the trimethoxybenzoyl derivatives. The hydrazine derivatives tend to be more potent than the amides, but some exhibit significant potency in the test.

On the basis of probable metabolic alterations, the bioactivity of the diacylhydrazines *in vivo* could be dependent upon three species. These are: the intact diacyl hydrazine, which should possess little activity; the monoacyl hydrazine, which might possess some activity; and hydrazine which should possess little activity, all to the extent that *in vitro* structure–activity relationships apply. In analogous fashion, the bioactivity of the alkyl acyl hydrazines would be dependent upon two species: the alkyl acyl hydrazine and the alkyl hydrazine. Again, to the extent that *in vitro* structure–activity relationships relate to activity in the intact animal, both of these might possess significant activity. In the case of amides, the likely species would be the amides and the amines. Significant inhibitory potency associated with either of these would not be expected. On the basis of the foregoing, the activity of the compounds synthesized and evaluated would be amides, diacyl hydrazines, and acyl alkyl hydrazines, in increasing order.

As the summary of the results indicates, the results anticipated are, in a general way, obtained, but there are important exceptions

Table II—Effect of the Synthesized Compounds on Body Temperature

	Body Temperature (°F.)			
	0 hr.	4 hr.	6 hr.	24 hr.
Control with known drug				
Reserpine[a]	100	87(2.7)[b]	84(2.2)[b]	85(3.3)[b]
Reserpine & iproniazid[c]	102	93(2.7)[d]	90(2.6)[d]	92(2.5)[d]
I 1-[3-(3-Pyridyl)glycidyl]-2-benzyl hydrazine: (significantly active)				
Reserpine[a]	100	87.1(3.36)	76.6(2.47)	89.0(1.6)
Reserpine & exp. drug[c]	102	91.3(3.43)	89.5(1.76)[d]	87.1(2.17)
II 1-[3-(3-Pyridyl)glycidyl]-2-benzoyl hydrazine: (significantly active)				
Reserpine	101.1	88.7(1.99)	74.2(1.49)	83.1(2.61)
Reserpine & exp. drug	99	88.0(2.5)	84.2(1.92)[d]	84.6(1.48)
III *N*-1-[3-(3-Pyridyl)glycidyl]-*N*-2-(3,4,5-trimethoxybenzoyl)hydrazine: (signif.)				
Reserpine	103	88.1(2.56)	69.8(2.32)	84.2(2.99)
Reserpine & exp. drug	102	89.7(1.58)	89.5(2.7)[d]	89.6(2.26)[d]
IV 1-[3-(4-Pyridyl)glycidyl]-2-benzyl hydrazine: (significantly active)				
Reserpine	100	91(1.7)	81(3.1)	87(3.2)
Reserpine & exp. drug	100	96(0.95)[a]	91(1.5)[a]	89(2.0)
V 1-[3-(4-Pyridyl)glycidyl]-2-benzoyl hydrazine: (significantly active)				
Reserpine	100.6	90.2(2.35)	80.6(3.08)	86.8(3.33)
Reserpine & exp. drug	100.6	84.3(2.62)[d]	80.3(1.97)	83.4(3.09)
VI *N*-1-[3-(4-Pyridyl)glycidyl]-*N*-2-(3,4,5-trimethoxybenzoyl)hydrazine: (signif.)				
Reserpine	101	88.5(1.95)	70.3(2.06)	85.2(3.67)
Reserpine & exp. drug	100	85.5(2.97)[d]	83.8(2.89)[a]	85.4(2.07)
VII *N*,*N*-Diethyl-3,3-diphenylglycidamide:				
Reserpine	101	89.8(3.1)	83.2(2.94)	84.8(1.85)
Reserpine & exp. drug	101	89.3(2.65)	85.3(2.55)	87.6(2.76)
VIII *N*,*N*-Diethyl-3-dibenzosuberylglycidamide: (significantly active)				
Reserpine	100	84.8(2.21)	84.4(1.79)	80.2(1.85)
Reserpine & exp. drug	100	95.4(1.67)[d]	94.1(1.69)[d]	88.2(1.72)[d]
IX *N*,*N*-Diethylxanthylglycidamide: (significantly active)				
Reserpine	101	89.3(2.83)	80.0(3.48)	85.1(2.71)
Reserpine & exp. drug	100.6	88.1(2.5)	87.0(3.02)[d]	86.8(2.99)
X *N*-3-Xanthylglycidyl-*N*-methylpiperazine:				
Reserpine	102	93(2.3)	91(2.4)	93(2.8)
Reserpine & exp. drug	102	94(1.8)	93(2.1)	95(1.7)
XI *N*,*N*-Diethyl 3,3-diphenylmethylglycidamide:				
Reserpine	100	91.6(1.7)	90(2.3)	94(2.7)
Reserpine & exp. drug	100	93(1.7)	90(2.3)	93(2.6)
XII *N*,*N*-Dibenzyl-3-(4-pyridyl)glycidamide:				
Reserpine	101	89.0(2.69)	88.8(1.87)	88.2(1.92)
Reserpine & exp. drug	101	88.0(2.69)	88.2(2.33)	89.8(2.09)

[a] Induced rectal temperature depression with intraperitoneal reserpine, 2.5 mg./kg., in male Swiss-Webster mice. [b] Standard deviation in parentheses. [c] Reversal of reserpine-induced rectal hypothermia in male Swiss-Webster mice; both drugs were administered intraperitoneally. [d] Significantly active; $p = 0.01$.

such as the high activity of the trimethoxybenzoyl derivatives and the amides. In this connection, it is well recognized that there is not necessarily a direct relationship between *in vitro* potency and activity *in vivo*. Additionally, it has been demonstrated that imipramine can reverse reserpine-induced hypothermia, probably through some other mechanism than inhibition of monoamine oxidase. The possible operation of these factors might account for the deviations from anticipated results. The possible operation of these factors throughout the series also precludes a precise delineation of structural features associated with activity. Through further biological evaluations and the synthesis of related compounds, it is hoped that this more precise delineation can be made.

Spasmolytic Activity—*Method*—The method for the determination of spasmolytic activity was adapted from the method of Miller *et al.* (12). The method is simple and convenient and allows potency estimates of a new compound against an established reference drug such as atropine. The method adapted here was the ability of the synthesized compound to reduce the spasm induced by a spasmogen on an isolated rabbit ileum *versus* the ability of the atropine to reduce the spasm of the spasmogen in the rabbit ileum in a set amount of time.

A Tyrode's solution of the following composition in grams per liter of purified water was used as the bath fluid:

Sodium chloride	9.0
Potassium chloride	0.2
Calcium chloride	0.2
Magnesium chloride	0.1
Sodium bicarbonate	1.0
Sodium acid phosphate	0.05
Glucose	1.0

The smooth muscle employed as test tissue was a strip of ileum 1–2 cm. in length, quickly removed from a recently sacrificed rabbit. The strip was suspended in the 10-ml. chamber filled with Tyrode's solution maintained at 37°. The muscle contractions were amplified and recorded on an E. and M. physiograph through a myograph attachment.

The remaining smooth muscle not being used was placed in Tyrode's solution suitably aerated and kept at constant temperature at 37° for subsequent pharmacological evaluation.

Test samples of freshly prepared solutions of the glycidyl compounds were prepared in concentrations of 1.4×10^{-5} m*M*. An aqueous solution of atropine sulfate, 1.4×10^{-6} m*M*, was used as the standard spasmolytic agent throughout the study.

An aqueous solution of mecholyl chloride was used as the standard spasmogen in millimolar quantities (1.3 m*M*).

A glass cylinder of 10-ml. capacity, equipped with an air inlet and a drainage arrangement maintained in a constant-temperature bath, was used throughout. The muscle chamber was equipped with an aerating tube which effectively aerated the muscle strip and efficiently mixed the test drugs.

As soon as the suspended strips had recovered from the contraction caused by the operative manipulations, the testing was begun. The dose of the spasmogen was administered to produce virtually maximum contraction. The spasmolytic agent was introduced without washing out the spasmogen. The degree of relaxation produced in the first 2 min. was noted. Following the observation the drug containing Tyrode's solution was flushed out and replaced with fresh solution. When the strips had relaxed to the former tonus level, they were ready for another trial.

Results—The pharmacological data on the spasmolytic activity of Compounds VII, VIII, and X are represented in Table III,

Table III—Summary of Data Showing Concentration–Response Relationships for the Spasmolytic Effects of Compounds VII, VIII, and X and Atropine Sulfate Against Methacholine Chloride-Induced Spasm in Excised Rabbit Ileum

	Spasmolytic Agent			
	Compound X	Compound VIII	Compound VII	Atropine Sulfate
Ml. added to 10-ml. muscle bath	0.8 ml.	2.0 ml.	2.0 ml., 3.0 ml.	0.2 ml.
Concentration in muscle bath	1.15×10^{-4}	2.88×10^{-4}	2.88 and 4.32×10^{-4}	2.88×10^{-6}
Response: No. positive/No. tried	7/10	6/10	0/10 1/10	8/10
Relative potency	2.5	1.0	0	1×10^{2}

noting concentration in millimolar quantities as well as relative potency when compared to atropine sulfate.

Discussion of Results—Examination of Compound X reveals that it is about 2.5 times as active as Compound VIII. Compound VII was found to be inactive in the ability to reduce the spasms induced by mecholyl. While spasmolytic activity is present in Compounds X and VIII, the activity present is only about 1/40th and 1/100th, respectively, that of the standard spasmolytic agent, atropine sulfate.

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Dissolution of Macromolecules II: Dissolution of an Ethylene–Maleic Acid Copolymer

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Abstract □ Factors influencing the dissolution of an ethylene–maleic acid copolymer have been studied. Polymer swelling, hydrated layer thickness, and solvent pH were shown to influence the dissolution of the polymer. Linear dissolution rates were observed following an initial induction period. Hydrated layer thickness was found to be a controlling factor in the dissolution process. An immersion refractometry method was employed to measure aqueous polymer concentrations during dissolution.

Keyphrases □ Ethylene–maleic acid copolymer—dissolution □ Tablets, ethylene–maleic acid copolymer—dissolution study □ Dissolution test apparatus—diagram □ Refractometry, immersion—polymer determination

Polymeric materials are widely used in many pharmaceutical systems. In systems utilizing polymer films and particularly in dosage forms in which the polymer is compressed in tablets to produce controlled drug release, the dissolution of the polymer is an important parameter. Polymer systems are frequently sought which, based on their dissolution properties, will provide a particular type of drug release.

Previous investigators of polymer dissolution have studied the dissolution of polystyrene in organic solvents (1–3). However, a detailed investigation of the dissolution in aqueous solvents of polymers having reactive functional groups has not been reported. In an earlier report the surface phenomena associated with the dissolution of such polymers were described (4). The present investigation reports the dissolution of an ethylene–maleic acid (dicarboxylic acid) copolymer as affected by these surface parameters.

EXPERIMENTAL

The ethylene–maleic acid copolymer, referred to as EMA-22, and the polymer tablets used in this study were identical to those used in a previous investigation (4). The measurement of polymer swelling, solvent penetration, and hydrated layer thickness was also identical to that of the earlier study (4).

The dissolution apparatus employed for the study of polymer dissolution is shown in Figs. 1–3. Figure 1 describes the sample holder; Fig. 2 shows the dimensions of the Plexiglas dissolution cell. Figure 3 is a schematic representation of the dissolution unit and the component parts that made up the entire system. The lip of the