A study was made of quinidine stability at room temperature in the extraction solvent methylene chloride by two chromatographic methods. This study was prompted by the report (19) that pharmaceutical dosage forms extracted with methylene chloride degrade rapidly to give several additional TLC spots. First, a stock solution of methylene chloride was evaluated by the proposed GLC method using multiple samples at 1-hr intervals for 6 hr. No decomposition of the quinidine was noted, as evidenced by complete recovery of the quinidine added. Second, a previously reported (19) TLC system was used to evaluate two stock solutions of quinidine in methylene chloride and methanol at 1-hr intervals for 6 hr, followed by a final 12-hr sample. A small unknown spot appeared in the 12-hr sample of the methylene chloride stock solution.

Thus, some decomposition of quinidines apparently can occur in methylene chloride, but it is not rapid at room temperature. Any potential decomposition of the quinidines can be avoided easily by immediately evaporating the extracting solvent and placing the enclosed residue under refrigeration until analysis.

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Synthesis of Histamine Analogs

CHARLES M. DARLING [x] and E. K. ROSE

Abstract □ Nineteen histamine analogs were synthesized, and their biological actions were compared to those of histamine in blood pressure, gastric secretion, and nasal decongestant screens. The analogs include *N*-substituted 4-aminoethylimidazoles, *N*-substituted 2-pyridylethylamines, 2-pyridylcyclohexylamines, and 2-pyridylcyclopropylcarbamates. None of the compounds showed appreciable histamine agonist or antagonist properties.

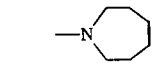
Keyphrases □ Histamine—19 analogs synthesized, biological actions compared, blood pressure, gastric secretion, and nasal decongestant screens □ Structure–activity relationships—19 histamine analogs □ Imidazole derivatives—synthesis of 19 histamine analogs, structure–activity relationships □ Pyridylethylamine derivatives—synthesis of 19 histamine analogs, structure–activity relationships

The biological properties of the naturally occurring autacoid, histamine, are well known. A molecule that selectively mimics or antagonizes a single action of histamine is a potentially useful agent in a broad range of pharmacological activities, including effects on vascular, bronchial, and intestinal musculature as well as inflammatory and secretory mechanisms. The classical antihistamines have only a narrow spectrum of histamine antagonism, blocking some histamine-stimulated secretions and intestinal smooth muscle contractions.

An excellent review of the actions of certain histamine analogs was reported (1), and the effect of structural modification on histamine agonist and antagonist actions was reviewed (2). A qualitative assessment suggests that the histamine molecule can be modified in many ways with retention of agonist activity so long as the length of the side chain remains unchanged. These modifications include replacement of the imidazole ring with other heterocyclic rings, alkyl substitutions on the side chain, and various substituents on the basic nitrogen of the side chain. The cyclopropyl derivative, 2-(4-imidazolyl)cyclopropylamine, was reported (3) to be almost devoid of activity.

The work reported here was designed to determine whether the selectivity of action could be increased by altering any of the three structural components of histamine: the ring, side chain, or *N*-substituent. The imidazole ring was substituted by a pyridine ring

Table I—Analytical Data for Pyridylethylamine Derivatives

Compound	R	Melting Point	Yield, %	Analysis, % Calc.	Found
XI		189–190.5°	47	C 56.31 H 7.99 N 10.11	56.56 7.90 10.15
XII	$-N(CH_3)-CH_2CH_2OH$	172–177°	31	C 47.44 H 7.17 N 11.06	47.50 7.21 10.94
XIII	$-N(CH_3)-CH_2CH_2Cl$	190–192°	51	C 44.22 H 6.31 N 10.31	44.43 6.30 10.20
XIV	$-N(CH_2CH_2OH)_2$	98–101[a]	24	C 53.12 H 6.29 N 7.29	53.29 6.35 7.20
XV	$-N(CH_2CH_2Cl)_2$	174–176.5°	59	C 41.28 H 5.67 N 8.75	41.47 5.70 8.65

[a] Sesquifumarate salt.

(Table I) in some compounds for comparison. The *N*-substituents were chosen because they appear in molecules that exhibit antagonist activity in other test systems. For example, an *N*-allyl appears in some narcotic antagonists, a 2-chloroethylamino group occurs in phenoxybenzamine (an adrenergic blocking agent), and bulky lipophilic groups are characteristic of some cholinergic blocking agents (Table II). The side-chain length was retained but placed in constrained three- and six-membered rings.

EXPERIMENTAL[1]

General Preparation—Compounds I–IV and VII were prepared by refluxing a solution of 4-(2-chloroethyl)imidazole hydrochloride (X) (4) (8.4 g, 0.050 mole) in an excess of the appropriate amine with no solvent. The solution was refluxed for from 15 min to 7 hr, and excess amine was removed by distillation *in vacuo*. The residue was treated with excess saturated sodium carbonate solution, and the mixture was reduced to dryness *in vacuo*. Then the residue was triturated with absolute ethanol and filtered. The filtrate was saturated with dry hydrogen chloride and filtered. The residue was recrystallized from a mixture of absolute ethanol and ethyl acetate with the aid of activated charcoal.

The respective yields, melting points, and elemental analyses are reported in Table II.

4-[2-(2-Chloroethylmethylamino)ethyl]imidazole Dihydrochloride (V)—A solution of III (1.6 g, 0.0066 mole) in thionyl chloride (25 ml) was refluxed for 1 hr. The mixture was concentrated *in vacuo*, and the residue was recrystallized from absolute ethanol with the aid of activated charcoal, 1.0 g (59%), mp 189.5–191°.

4-[2-(2-Phenylethyl)aminoethyl]imidazole Dihydrochloride (VI)—To a refluxing solution of phenethylamine (51.0 g, 0.420 mole) in 1-propanol (50 ml) was added X (15.4 g, 0.090 mole) in 1-propanol (50 ml) during 30 min. Reflux was continued for 70 min, and the solution was cooled and filtered. The filtrate was concentrated *in vacuo* to an oil, and about three volumes of dry ether was added.

The cooled solution was filtered, and the filtrate was concentrated *in vacuo*. Excess phenethylamine was removed by vacuum distillation, and the residue was dissolved in acetone (200 ml). After the addition of concentrated hydrochloric acid (10 ml), the product was removed by filtration and recrystallized from absolute ethanol with the aid of activated charcoal, 5.3 g (21%) mp 240–243°.

4-[2-(3-Azabicyclo[3.2.2]nonyl)ethyl]imidazole (VIII)—A stirred mixture of X (8.4 g, 0.050 mole), 3-azabicyclo[3.2.2]nonane (6.3 g, 0.050 mole), and triturated potassium carbonate (20.7 g, 0.15 mole) in 1-butanol (50 ml) was refluxed for 4 hr. The hot mixture was filtered, and the residue was washed twice with absolute ethanol. The combined filtrates were concentrated *in vacuo* and dissolved in dilute hydrochloric acid (100 ml).

The acidic solution was washed with ether and made basic with saturated sodium carbonate solution. Then the product was extracted into ether and crystallized from ether on cooling. The filtered product was recrystallized three times from toluene with the aid of activated charcoal and twice from isopropyl ether, 2.2 g (18%), mp 122–126°.

4-[2-Bis(2-hydroxyethylamino)ethyl]imidazole Dihydrochloride (IX)—A mixture of X (8.4 g, 0.050 mole) and diethanolamine (21.0 g, 0.20 mole) in 2-butanol (50 ml) was refluxed for 29 hr. The mixture was decanted, and the decantate was concentrated *in vacuo* and dissolved in a mixture of methyl ethyl ketone and methanol. The solution was saturated with hydrogen chloride gas, and most of the methanol was removed by boiling. The mixture was cooled and decanted, and the black oily residue was recrystallized from a mixture of absolute ethanol and ethyl acetate with the aid of activated charcoal, 1.0 g (8%), mp 122.5–124.5°.

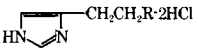
1-[2-(2-Pyridyl)ethyl]perhydroazepine Dihydrochloride (XI)—A mixture of 2-vinylpyridine (5.3 g, 0.050 mole) and hexamethylenimine (10.0 g, 0.10 mole) was refluxed for 18 hr. The solution was concentrated *in vacuo*, and the residual oil was distilled, 8.1 g, bp 80–84°/0.07 mm. The free base was converted to the hydrochloride salt with ethereal hydrogen chloride. Then the product was recrystallized from isopropanol, 6.6 g (47%) mp 189–190.5°.

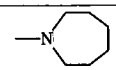
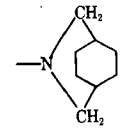
2-[2-(2-Hydroxyethylmethylamino)ethyl]pyridine Dihydrochloride (XII)—A mixture of 2-vinylpyridine (21.0 g, 0.20 mole) and 2-hydroxyethylmethylamine (15.0 g, 0.20 mole) was refluxed for 2.5 hr. The mixture was concentrated *in vacuo*, and the residue was distilled, 18.6 g, bp 111–115°/0.5 mm. Five grams of the distillate was treated with ethanolic hydrogen chloride, and the precipitate was recrystallized from absolute ethanol, 2.2 g (31%), mp 172–177°.

2-[2-(2-Chloroethylmethylamino)ethyl]pyridine Dihydrochloride (XIII)—To a solution of thionyl chloride (40 ml) in chloroform (50 ml) was added dropwise the free base of XII (9.0 g, 0.050 mole), followed by another portion of chloroform (50 ml). Refluxing conditions were continued for 1.5 hr, and then the solution was concentrated *in vacuo*. The residue was recrystallized from absolute ethanol with the aid of activated charcoal, 7.0 g (51%), mp 190–192°.

[1] Melting points are uncorrected. The NMR and IR data are consistent with the proposed structures. Analyses were performed by Analytical Research Laboratories, A. H. Robins Co., Richmond, Va.

Table II—Analytical Data for Imidazole Derivatives



Compound	R	Melting Point	Yield, %	Analysis, % Calc.	Found
I		244–247°	26	C 49.63 H 7.95 N 15.78	49.54 8.20 15.54
II	$-NHCH_2CH{=}CH_2$	140.5–143°	40	C 42.87 H 6.74 N 18.75	42.82 6.80 18.80
III	$-N(CH_3)-CH_2CH_2OH$	164–167.5°	13	C 39.68 H 7.08 N 17.35	39.54 6.91 17.38
IV	$-NH-CH(CH_3)(CH_2)_4CH_3$	190–193°	14	C 51.06 H 8.93 N 14.89	50.39 8.77 15.08
V	$-N(CH_3)-CH_2CH_2Cl$	189.5–191°	59[a]	C 36.87 H 6.19 N 16.12	36.82 6.20 16.00
VI	$-NHCH_2CH_2C_6H_5$	240–243°	21	C 54.18 H 6.64 N 14.58	54.07 6.80 14.55
VII	$-N(CH_3)-CH_2CH{=}CH_2$	186–189°	15	C 45.39 H 7.19 N 17.64	45.26 7.13 17.52
VIII		122–126°[b]	18	C 71.19 H 9.65 N 19.16	71.04 9.60 19.12
IX	$-N(CH_2CH_2OH)_2$	122.5–124.5°	8	C 39.72 H 7.04 N 15.44	39.69 7.25 15.36

[a] Yield calculated from Compound III. [b] Free base.

2-[2-Bis(2-hydroxyethyl)aminoethyl]pyridine Sesquifumarate (XIV)—A solution of 2-vinylpyridine (20.8 g, 0.198 mole) and diethanolamine (20.0 g, 0.190 mole) in 1-butanol (100 ml) was refluxed for 48 hr. The hot solution was poured into two volumes of cold water, and the separated organic layer was washed with water. The combined aqueous solutions were made basic with dilute sodium hydroxide solution and extracted with chloroform. The organic layer and chloroform extract were combined and concentrated *in vacuo*.

The residue was distilled, 9.7 g (24%), bp 155–170°/0.5 mm. Then 4.2 g of the free base in methyl isobutyl ketone was mixed with a hot solution of fumaric acid (2.4 g) in absolute ethanol (25 ml). After cooling, the product was filtered and recrystallized from absolute ethanol, 1.7 g, mp 98–101°. NMR data and elemental analysis suggest that the product is the sesquifumarate salt.

2-[2-Bis(2-chloroethyl)aminoethyl]pyridine Dihydrochloride (XV)—To a solution of XIV (5.6 g, 0.026 mole) in chloroform (30 ml) was added slowly thionyl chloride (30 ml), and the mixture was refluxed for 1 hr. The mixture was concentrated *in vacuo*, and the residue was recrystallized from absolute ethanol with the aid of activated charcoal, 5.0 g (59%), mp 174–176.5°.

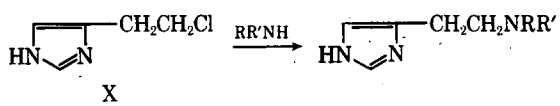
2-(2-Aminocyclohexyl)pyridine Dihydrochloride (XVI)—A mixture of 2-(2-pyridyl)cyclohexanone (5) (17.5 g, 0.10 mole) and ammonium formate (25.2 g, 0.40 mole) was heated at 145–150° until distillation ceased and was then heated at 185–196° for 4 hr. The mixture was partitioned between water and benzene and the aqueous portion was extracted further with benzene. The combined benzene solutions were washed with water and concentrated *in vacuo*. Then the residue was distilled, retaining the *N*-formyl fraction, 4.0 g (20%), bp 143–155°/0.5 mm. This fraction was combined with the same fraction of another run and redistilled.

Of the redistilled *N*-formyl compound, 4.2 g (0.02 mole) was dissolved in concentrated hydrochloric acid and the solution was refluxed for 30 min. The cooled solution was made basic with dilute sodium hydroxide solution and extracted into chloroform which was concentrated *in vacuo*. The residue was distilled, 2.3 g, bp 100–101°/0.5 mm, and converted to the hydrochloride salt with ethereal hydrogen chloride. The product was recrystallized from absolute ethanol, 1.3 g (5% overall yield), mp 249–255°.

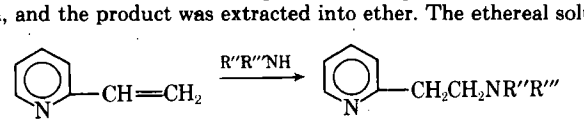
Anal.—Calc. for $C_{11}H_{16}N_2 \cdot 2HCl$: C, 53.02; H, 7.28; N, 11.24. Found: C, 52.83; H, 7.06; N, 11.19.

2-(2-Methylaminocyclohexyl)pyridine Dihydrochloride (XVII)—The redistilled *N*-formyl intermediate from the preparation of XVI was used without further purification. To a clear solution of lithium aluminum hydride (0.33 mole) in tetrahydrofuran was added ethyl acetate (29.1 g, 0.33 mole) under an atmosphere of nitrogen while a reaction temperature of 0–5° was maintained. The mixture was stirred for 10 min, and a solution of the *N*-formyl intermediate (16.3 g, 0.078 mole) in dry ether (70 ml) was added. The mixture was refluxed for 2 hr and stirred at ambient temperature overnight.

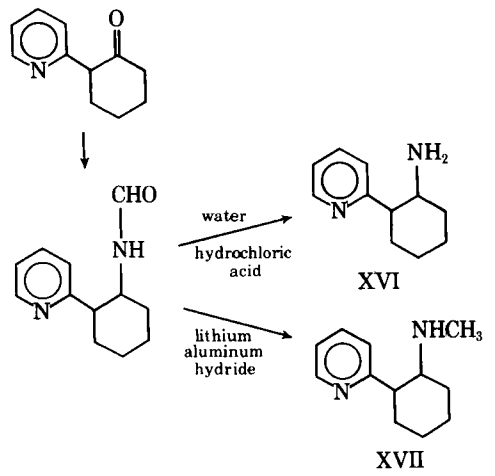
The mixture then was decomposed with magnesium sulfate solution, and the product was extracted into ether. The ethereal solu-



Scheme I



Scheme II



Scheme III

tion was concentrated *in vacuo*, and the residue was distilled, 10 g, bp 85–100°/0.1 mm. The distillate was converted to a salt with ethereal hydrogen chloride, and the product was recrystallized from isopropanol with the aid of activated charcoal. The hygroscopic crystals were dried *in vacuo*, 3.2 g (15.8%), mp 213–218°.

Anal.—Calc. for $C_{12}H_{18}N_2 \cdot 2HCl$: C, 54.76; H, 7.66; N, 10.64. Found: C, 54.48; H, 7.62; N, 10.48.

***trans*-2-(2-Pyridyl)cyclopropylcarboxyhydrazide (XXI)**—Ethyl *trans*-2-(2-pyridyl)cyclopropylcarboxylate (88.6 g, 0.5 mole), prepared by the method of Kaiser *et al.* (6), was added to a solution of anhydrous hydrazine (32.0 g, 1.0 mole) in absolute ethanol (200 ml), and the mixture was refluxed for 4 hr. The mixture then was concentrated *in vacuo*, and the residue was recrystallized twice from tetrahydrofuran, 71.6 g (80.8%), mp 116.5–118.5°.

Anal.—Calc. for $C_9H_{11}N_3O$: C, 61.00; H, 6.26; N, 23.71. Found: C, 60.93; H, 6.28; N, 23.88.

Ethyl *N*-*trans*-2-(2-Pyridyl)cyclopropylcarbamate Hydrochloride (XVIII)—To a cooled solution of *trans*-2-(2-pyridyl)cyclopropylcarboxyhydrazide (17.7 g, 0.10 mole) in a mixture of concentrated hydrochloric acid (50 ml) and water (100 ml) was added dropwise a solution of sodium nitrite (10.5 g, 0.15 mole) in water (30 ml) while the temperature of the reaction mixture was maintained below 0°. The cold mixture was made basic with sodium carbonate solution, and the azide was extracted into ether. The ether solution was dried over anhydrous magnesium sulfate.

The dried ether solution was added dropwise to refluxing absolute ethanol, allowing the ether vapors to escape. Then the mixture was refluxed for 18 hr and concentrated *in vacuo*. After acid and base washes, the residue was distilled, 10.1 g (49%), bp 130–155°/0.1 mm. For analysis, 2.5 g was converted to a hydrochloride salt, which was recrystallized from a mixture of absolute ethanol and ethyl acetate, mp 130–131.5°.

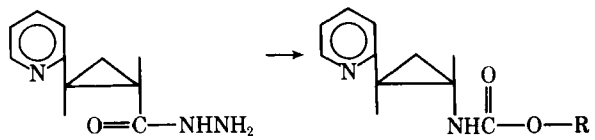
Anal.—Calc for $C_{11}H_{14}N_2O_2 \cdot HCl$: C, 54.44; H, 6.23; N, 11.54. Found: C, 54.43; H, 6.18; N, 11.47.

Benzyl *N*-*trans*-2-(2-Pyridyl)cyclopropylcarbamate (XIX)—The dry ether solution of the azide (0.187 mole), prepared as described for XVIII, was added rapidly to a solution of benzyl alcohol (40.5 g, 0.375 mole) in dry toluene (300 ml) at 85–90°, allowing the ether vapors to escape. The solution was heated at 90° for 30 min, and the mixture was concentrated *in vacuo*. The residue was eluted from a 1-kg column of magnesium silicate with 20% acetone in benzene (2.2 liters) and concentrated *in vacuo*. The residue was recrystallized from isopropyl ether, 22 g (44%), mp 85–85.5°.

Anal.—Calc. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.47; H, 5.92; N, 10.42.

***tert*-Butyl *N*-*trans*-2-(2-Pyridyl)cyclopropylcarbamate (XX)**—The dry ether solution of azide (0.10 mole), prepared as described for XVIII, was added rapidly to refluxing *tert*-butyl alcohol (150 ml), allowing ether vapors to escape. The solution was refluxed for 4 hr and concentrated *in vacuo*. The residue was recrystallized from toluene and then isopropyl ether with the aid of activated charcoal, 3.1 g (13%), mp 77–79°.

Anal.—Calc. for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.89; H, 7.80; N, 12.07.



Scheme IV

Table III—Pharmacological Profile of Histamine Analogs[a]

Compound	Blood Pressure[b]	Gastric Secretion[c]	Nasal Congestion[d]
I	–	0	–
II	+	0	+
III	+	0	+
IV	+	0	0
V	+	0	+
VI	+	+	+
VII	+	0	+
VIII	+		–
IX	–	0	0
XI	+		
XII	–		0
XIII	+	0	0
XIV	0	0	–
XV	–	0	–
XVI	–	0	0
XVII	+	0	–
XVIII	+	0	0
XIX	+		0
XX	+		–

[a] The + denotes histamine-like action, – denotes action dissimilar to histamine, and 0 denotes no observable effect. [b] In anesthetized dogs. [c] In dogs and/or rats (9). [d] In dogs (see footnote 2).

RESULTS

Chemistry—The imidazole and pyridylethylamines were prepared by well-known methods of treating X (as the free base) and 2-vinylpyridine with appropriate amines, as shown in Schemes I and II, respectively.

A modification of the Leuckart reaction (7) was used for the preparation of the 2-(2-pyridyl)cyclohexylamines as outlined in Scheme III. The *N*-formyl intermediate was used in each reaction without further purification.

The cyclopropyl derivatives were prepared by a modification of the Curtius reaction (8), using the hydrazide shown in Scheme IV. Several unsuccessful attempts were made to hydrolyze and reduce the carbamates to the corresponding primary and secondary amines. However, only unresolvable mixtures resulted.

Pharmacology—Three pharmacological tests were used to assess the qualitative action of the compounds in relation to the action of histamine.

The effect of the compounds on blood pressure was determined in anesthetized adult mongrel dogs, 7.6–13.3 kg, of either sex. Anesthesia was induced and maintained with a combination of pentobarbital and phenobarbital. Conventional methods and equipment were employed for monitoring blood pressure. The compounds were administered intravenously in an initial dose of 1 mg/kg, with each subsequent dose doubled to the lethal dose or to a maximum of 32 mg/kg. A deviation of at least ±20% from the pretreatment mean arterial pressure was considered a blood pressure effect (Table III).

Gastric secretion experiments were performed in dogs and rats (9). The nasal decongestant studies were done in anesthetized dogs[2], using doses of 0.1–0.8 mg/kg iv (Table III).

DISCUSSION

Structural changes associated with changes in type of activity are not apparent. All compounds except VI exhibited no observ-

[2] The methodology will be published by C. A. Leonard, B. V. Franko, and J. W. Ward, Research Laboratories, A. H. Robins Co.

able effect on gastric secretion at 5 mg/kg sc in dogs; VI showed slight histamine-like activity. Compounds II–VII, XI, XIII, XVIII, and XIX exhibited histamine-like effect on blood pressure and histamine-like or no effect on nasal volume. Compounds IX, XII, and XVI caused an increase in blood pressure (dissimilar effect) and no effect on nasal volume, while XIV produced no effect on blood pressure and caused an increase in nasal volume (dissimilar effect). Compounds VIII, XVII, and XX lowered blood pressure (similar effect) and caused an increase in nasal volume (dissimilar effect). Compounds I and XV exhibited effects dissimilar to those of histamine by causing an increase in blood pressure and nasal volume.

While the data indicate that structural changes can selectively minimize certain pharmacological actions of histamine, no correlation of structural changes to selectivity of action was observed.

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Multiliter Production and Immunochemical Cross-Reactivity of Plant Tissue Culture Antigens

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Abstract □ Cells of short ragweed suspension culture were successfully propagated in a multiliter fermentor. Proteins were isolated from tissue cultures (short ragweed, cantaloupe, marigold, and Ammi) and plants (short ragweed pollen, cantaloupe fruit, and marigold flowers) by ammonium sulfate precipitation, cross-linked dextran gel filtration, and anion-exchange chromatography. Some fractions elicited allergenic responses in ragweed-sensitive patients but not in ragweed-nonsensitive control patients. Double-immunodiffusion analysis of these fractions showed their nonidentity with short ragweed antigen E.

Keyphrases □ Plant antigens—isolated from tissue cultures, allergenic activity screened □ Antigens—proteins isolated from plant tissue cultures, allergenic activity screened

Ragweed pollen is a major causative agent of allergic rhinitis. Active allergens were isolated from short ragweed pollen, and antigen E was found to be the most active (1–3). In one study, ragweed-sensitive patients reacted positively to intradermal injections of the allergens isolated from short ragweed tissue cultures (4). In the present study, short ragweed tissue cultures were grown in multiliter fermentors to determine their growth characteristics in such systems and to produce an adequate amount of cells for further clinical investigation.

The relationship between short ragweed allergic rhinitis and melon or banana sensitivity was studied (5). All patients with melon- or banana-induced oral pruritus were also sensitive to pollen. Therefore, other plant (melon and marigold flowers) and tissue culture (short ragweed, melon, marigold, and Ammi) sources were examined for their antigenic cross-reactivities with short ragweed pollen extract and antigen E.

EXPERIMENTAL

Plant Materials[1]—Short ragweed (*Ambrosia elatior* L.) pollen was purchased[2]. Cantaloupe[3] (*Cucumis melo* var. *cantalupensis* Naud.) and marigold[3] (*Tagetes patula* L.) seeds were germinated and grown in the medicinal garden of the University of Minnesota. Cantaloupe fruit and marigold flowers were harvested in August 1973.

Plant Tissue Cultures—Cantaloupe and marigold seeds were surface sterilized in 2.5% sodium hypochlorite solution for 20 min under reduced pressure and then rinsed three times with sterile distilled water. Germinated seeds were transferred into sterile 30-ml (1-oz.) square vials containing 18 ml of revised Murashige and Skoog tobacco medium (6) supplemented with 1% agar and 1.0 ppm of (2,4-dichlorophenoxy)acetic acid ($RT_{1.0}$). After incubation at 25° in the dark for 5–6 weeks, sufficient light-yellow cantaloupe callus and brownish-green marigold callus were obtained for transfer. They were designated seedling calli and were subcultured every 4 weeks.

The short ragweed and Ammi [*Ammi visnaga* (L.) Lam.] tissue cultures used were approximately 2 years old in June 1973. The short ragweed tissue culture was initiated from the leaves of short ragweed (4), and the Ammi callus culture was initiated from Ammi fruit. Both short ragweed and Ammi tussue cultures were maintained on $RT_{2.0}$ [2 ppm of (2,4-dichlorophenoxy)acetic acid] and $RT_{0.1}$ [0.1 ppm of (2,4-dichlorophenoxy)acetic acid] media, respectively.

Suspension cultures were initiated by aseptically transferring

[1] Short ragweed antigen E was provided by Dr. R. V. Pennington, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

[2] Greer Labs, Lenoir, N.C. (1972 collection).

[3] George W. Park Seed Co., Greenwood, S.C.