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Some Properties of the *N*-Cinnamylephedrines, and the Analysis of Aspirin, Caffeine and Cinnamylephedrine in Admixture*

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The isomers of 1-phenyl-2-methylcinnamylaminopropanol-1, $C_6H_5CHOHCH(CH_3)N(CH_3)CH_2CH = CHC_6H_5$, are included in a group of substances described as "alkamines" or "phenylalkylolamines" which are the subjects of United States (1) and British (2) patents. These patents attribute anesthetizing and spasmolytic properties to the molecule of the above structure, and it has been indicated elsewhere in the literature (3) that 1-phenyl-2-methylcinnamylaminopropanol-1 (cinnamylephedrine) has a vasodilatory action. Aside from the references cited, no further information concerning the *N*-cinnamylephedrines has been encountered in the literature. Since at least one cinnamylephedrine isomer has been used in pharmaceuticals, it seemed desirable to supplement the meager information available in order to make possible the satisfactory identification of these substances.

The *N*-cinnamylephedrines may exist in two enantiomorphic forms corresponding to *d*- and *l*-ephedrine, and also in a racemic modification.[1] The three forms were prepared in good yields by the action of cinnamyl chloride on the corresponding ephedrines. As recorded in the patent literature, the precipitation of unsubstituted ephedrine as the hydrohalide accompanied the substitution of the cinnamyl radical. This was obviously due to the combination of excess ephedrine with the hydrogen chloride liberated during the replacement of amino hydrogen by the cinnamyl radical. It seems probable that the deposition of ephedrine salts reported as occurring in solutions of the base in chloroform (4) and other reactive organic halides (5) was the result of a similar reaction, the nature of the radical substituted for the amino hydrogen being dependent upon the particular halide involved.

* The portion of this paper concerning the analysis of the drug mixture was presented at the Pre-convention Meeting of the U. S. Pharmacopœial Convention, Washington, D. C., 1940.

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[1] The patents referred to cover substances having a certain chemical structure and, therefore, include not only compounds having an ephedrine-like configuration, but also those corresponding to the pseudoephedrines. This paper is concerned only with compounds spatially related to the ephedrines.

It does not appear likely that the deposition of ephedrine salts in the cases cited was due to the ability of the base to abstract hydrogen halide from the halide solvent (5).

Replacement of the amino hydrogen atom of ephedrine by a cinnamyl radical produced a product which rotated the plane of polarized light in a direction opposite to that characteristic of the ephedrine employed. Thus, the product from *d*-ephedrine was lævorotatory, and that from *l*-ephedrine was dextrorotatory in alcohol or benzene solutions. Such an effect has been observed in the synthesis of other *N*-substituted ephedrines, *e. g.*, benzylephedrine (6), a vinylog of cinnamylephedrine.

The optically active and inactive free bases were obtained easily in beautifully crystalline condition. The inactive hydrochloride was sufficiently well crystallized to permit the estimation of optical crystallographic properties, but the corresponding active salts were obtainable only in a microcrystalline condition. The crystallographic data which were determined are enumerated later.

As evidenced by the action of several typical alkaloidal precipitants on solutions of the racemic hydrochloride (Table I), the cinnamylephedrines respond to such reagents at considerably lower concentrations than do the ephedrines. Under the conditions of these tests, no crystalline products were formed. The precipitates were of an oily, resinous or amorphous nature.

One characteristic of the hydrochlorides of the *N*-cinnamylephedrines and, apparently, of the sulfates of these bases, is their solubility in chloroform. This solubility is relatively high, particularly in the case of the optically active hydrochlorides, when compared with the ability of most alkaloidal salts to dissolve in this solvent. The distribution of the cinnamylephedrines between diluted mineral acids (hydrochloric, sulfuric) and chloroform is of such a nature as to render impracticable their quantitative retention in the aqueous phase during extraction with chloroform. This is due to solubility of the salts in chloroform rather than to their hydrolysis. For this reason, the usual procedure for the quantitative separation of caffeine from other alkaloids and organic bases is not applicable to mixtures of caffeine and a cinnamylephedrine.

A procedure has been developed for the assay of a preparation containing aspirin, caffeine and cinnamylephedrine which permits the use of a single weighed portion of the mixture for the estimation of these three ingredients. Briefly, the method is to hold back the aspirin with cold, dilute bicarbonate, extract the caffeine and cinnamylephedrine base with chloroform and determine gravimetrically the combined weights of these two substances. The titer of the mixed residue gives directly the amount of cinnamylephedrine present, since caffeine has no titratable basicity under the conditions of assay. Aspirin is determined gravimetrically after extraction from the acidified bicarbonate layer. The gravimetric determination of aspirin, as such, in pharmaceutical preparations was originally described by Hitchins (7), and has subsequently been applied by Berman (8) and Grove (9). The assay is open to the objection that the value obtained for caffeine is subject to any errors arising from the titration procedure.

EXPERIMENTAL

Synthesis of Cinnamylephedrines.—These compounds were prepared by a method essentially that described in the patent literature, except that cinnamyl chloride was used instead of the bromide. Under these conditions, it was necessary to reflux the reaction mixture for six hours in order to obtain a maximum yield. Six Gm. (0.0363 mole) of the anhydrous ephedrine and 2.92 Gm. (0.0191 mole) of cinnamyl chloride in 23 cc. dry benzene were refluxed on the steam bath. After about five minutes the ephedrine hydrochloride began to precipitate. After six hours of refluxing, the ephedrine salt was filtered off, washed several times with a total of 75 cc. of benzene, and dried. The recovered salt amounted to 0.017–0.018 mole; melting point 216–217° (uncorr.) for the active hydrochlorides and 188–189° (uncorr.) for the racemic substance. The combined washings and filtrate from the precipitate where shaken with an excess of dilute sulfuric acid. A heavy, oily, orange-yellow layer (probably the sparingly soluble sulfate of cinnamylephedrine) separated and was drained off along with the aqueous phase into a separatory funnel. The benzene solution was shaken with three additional 100 cc. portions of dilute acid which were combined with the first extract. Sufficient water was added to dissolve

the oily layer (the volume at this point was about 600 cc.), and the acid solution was washed with benzene, made ammoniacal and extracted with chloroform. After evaporation of the chloroform, the residue was dissolved in 160 cc. hot alcohol-water mixture (53% by volume of ethyl alcohol) and cooled slowly, with shaking and seeding, to 5° C. The white needles were filtered off and dried in vacuum over sulfuric acid. Yields of 70–75% (based on the halide) were obtained. The melting point of the crystals was not appreciably changed by subsequent recrystallizations from alcohol-water or 30–60° petroleum ether. In reactions involving larger quantities of reactants, it was found more convenient, instead of using the extraction with acid, to concentrate the filtrate and washings from the precipitated ephedrine hydrochloride, and precipitate the cinnamylephedrine by gradual addition of 30–60° petroleum ether. This treatment removed most of the unreacted cinnamyl chloride and ephedrine base, and the crude product thus obtained was recrystallized from a suitable quantity of alcohol-water mixture.

The cinnamylephedrines were easily soluble in benzene, acetone, ether, chloroform and ethyl acetate; less soluble in alcohol and carbon tetrachloride; slightly soluble in petroleum ether and very slightly soluble in water. They were recrystallized twice more from alcohol-water to obtain pure samples for analysis, and the thrice recrystallized substances used in determining properties and constants.

N-Cinnamyl-l-ephedrine.—A sample of the substance gave a neutralization equivalent of 281 (calculated 281.4 for $C_{19}H_{23}NO$), and had the following properties: melting point[2] 85.6–85.7° (softened at 85.4°); $[\alpha]_D^{20}$ +24.6° (in benzene, $c = 10, l = 2$; preliminary work on 2% alcoholic solutions gave values of +10–11°). In ordinary light, under the microscope, the substance consisted of needles and rods (Fig. 1). Refractive indices were determined by the immersion method in potassium-mercuric iodide solutions: n_α = 1.582 (shown lengthwise on rods and needles); n_β = indeterminate; n_γ = 1.733 (shown crosswise on rods and needles; methylene iodide could be used for this determination); both values ±0.002. Immersion media consisting of suitable mixtures of potassium-mercuric iodide, glycerol and water were found most satisfactory for the optical study of this substance, it being somewhat soluble in organic mixtures consisting of monochlornaphthalene and methylene iodide, although for rapid determinations the latter series was suitable. Most of the needles and rods yielded n_α lengthwise and n_γ crosswise. The birefringence was very strong. In parallel polarized light (crossed nicols) the extinction was parallel and the sign of elongation was negative. The rods and needles invariably extinguished sharply with crossed nicols. No interference figures were observed in convergent polarized light (crossed nicols).

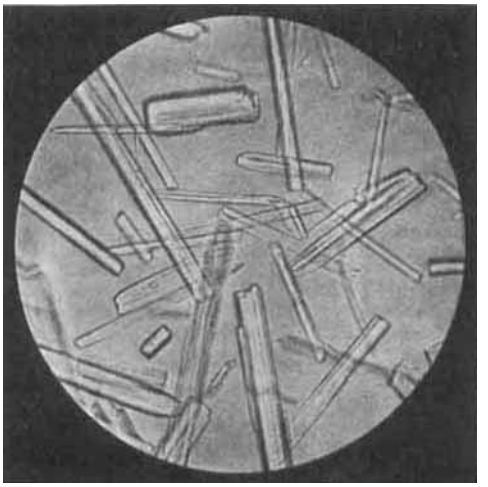

Fig. 1.—*N*-Cinnamyl-*l*-Ephedrine (×150).

N-Cinnamyl-dl-ephedrine.—This substance was of the same crystal habit as the active isomers (Fig. 1). A sample yielded a neutralization equivalent of 282 and melted at 66.7–67.7° (softened at 66.5°). Mixed melting-point determinations on mixtures of the racemic and active bases in several different proportions gave initial melting at temperatures within the melting range observed for the racemic base. This might indicate the inactive base to be a pseudoracemic mixed crystal or of eutectic composition rather than a true compound. In ordinary light, under the microscope, this substance consisted of small needles and rods. In determining refractive indices, the same immersion media used in the study of the *l*-ephedrine derivative were found suitable: n_α = 1.570 (invariably shown lengthwise; potassium-mercuric iodide-glycerol medium); n_β = indeterminate; n_γ = 1.733 (methylene iodide), shown crosswise on rods and needles; both values ±0.002. In parallel polarized light (crossed nicols), the extinction was parallel and the sign of elongation negative. The birefringence was quite strong. No interference figures were observed in convergent polarized light (crossed nicols).

N-Cinnamyl-d-ephedrine.—A sample gave a neutral equivalent of 282 and had the following constants: melting point 85.6–85.7° (softened 85.5°); $[\alpha]_D^{20}$ −24.7° (in benzene, $c = 10, l = 2$).

Cinnamylephedrine Hydrochlorides.—These compounds were prepared by the dropwise addition of ethereal hydrogen chloride to a solution of 2.00 Gm. of the purified base in 20 cc. benzene until a slight excess of acid was present. During addition of the

[2] Unless described as uncorrected, all melting points were taken using Anschutz-type thermometers previously tested by the National Bureau of Standards, and are corrected for stem exposure. They were observed while heating slowly (one-half degree or less per minute) from a temperature about 10 degrees below the point of fusion.

ether solution, the mixture was stirred and seeded to prevent deposition of the salt as a glassy film. After standing fifteen minutes, the white powdery hydrochloride was filtered off and washed with benzene. Yields were practically quantitative.

The optically active hydrochlorides were recrystallized by dissolving in 80 cc. boiling U. S. P. ethyl acetate and cooling slowly. Attempts to prepare these substances in a crystalline condition suitable for optical examination were not successful. Among methods tried were cooling of hot, saturated solutions in alcohol, water and chloroform, and precipitation from chloroform solutions by addition of ether or benzene. The racemic hydrochloride was recrystallized by dissolving in a boiling mixture of 150 cc. ethyl acetate and 45 cc. chloroform, and slowly cooling the solution.

These compounds were not appreciably soluble at room temperature in benzene, ether, acetone, carbon tetrachloride or ethyl acetate, but were somewhat soluble in alcohol, chloroform and water. The optically active salts were more easily soluble in chloroform, water and hot ethyl acetate than was the racemic salt. All tended to form supersaturated solutions from which they were slowly deposited, and rapid removal of solvent frequently left the hydrochloride behind as a resinous film which slowly assumed the state of a white solid. The substances had a bitter taste and exerted a strong anesthetic action on the tongue.

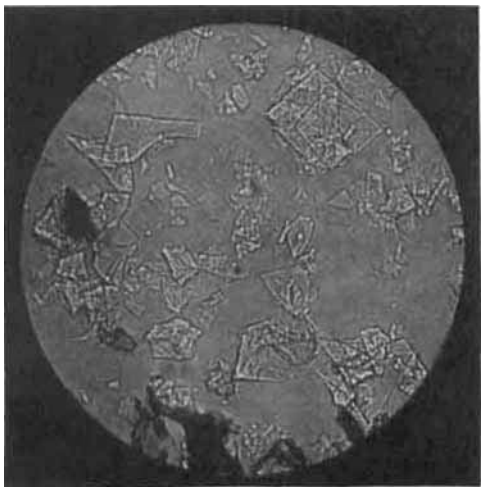

Fig. 2.—Racemic Cinnamylephedrine Hydrochloride (×150).

The racemic hydrochloride melted at 180.5–182°, with preliminary browning and shrinkage at 178°, and on analysis gave a value of 11.04% chlorine (calculated 11.16 for $C_{19}H_{24}ClNO$). In ordinary light, under the microscope, it consisted of irregular plates which broke up into angular fragments (Fig. 2). Its tendency was to occur in overlapping aggregates of plates. For determination of refractive indices, immersion media consisting of a series of mixtures of monochloronaphthalene and methylene iodide were most suitable; $n\alpha = 1.550$; $n\beta$ = indeterminate (probably shown on plates tipped on edge); $n\gamma = 1.733$; both ± 0.002. In parallel polarized light (crossed nicols), most of the fragments extinguished sharply, and the birefringence was very strong. In convergent polarized (crossed nicols), partial biaxial interference figures were occasionally shown.

The optically active salts consisted of microcrystals which tended to aggregate into small, irregular lumps. A sample of the *l*-ephedrine derivative gave an analysis of 11.03% chlorine and melted at 164–168°; its enantiomorph gave a value of 11.09% chlorine and melted at 163.5–168.5°. Before melting, both began to brown and shrink at 158–159°. Although the melting ranges were somewhat wider than that of the racemic salt, the active salts were, apparently, of the same degree of purity as that substance, since the residue from a chloroform solution of equal weights of the enantiomorphs melted at 180.5–182°, the temperature range recorded for the racemic salt.

Precipitation and Color Tests.—The following table lists results obtained in tests using several typical alkaloidal precipitants and a 1% solution of *dl*-cinnamylephedrine hydrochloride.

Table I.—Results of Precipitation and Color Tests

Reagent	Result	Approximate Minimum Concentration Responding to Reagent[a]
Saturated mercuric chloride	White ppt. consisting of oily droplets	1:350
Platinic chloride T.S.	Amorphous, yellowish ppt.; coagulates on shaking	1:2000
Gold chloride T. S.	Yellow ppt. consisting of oily or resinous material	1:10,000
Phosphotungstic acid, 1%	Heavy, flocculent light gray ppt.; coagulates slowly	1:10,000
Silicotungstic acid, 1%	Resembles phosphotungstic acid ppt.	1:10,000
Saturated alcoholic picric acid	Canary-yellow ppt.; deposits opaque resin on shaking	1:15,000
Mercuric potassium iodide T.S.	Resembles mercuric chloride ppt.	1:50,000
Iodine and potassium iodide T.S.	Red-brown ppt.; deposits dark brown droplets on standing	1:100,000

[a] The figures in this column represent the concentration in a solution of alkaloidal salt which yielded an easily visible turbidity or opalescence within a few second when ten drops of this solution were tested by adding from a fraction of a drop up to ten drops of the reagent. In some cases (*e. g.*, picric acid, platinic chloride, mercuric potassium iodide and iodine-potassium iodide), excess reagent seemed to destroy the turbidity, while in others (*e. g.*, gold chloride) the turbidity or opalescence was formed only at higher concentrations of the reagent.

When 0.01 Gm. of the cinnamylephedrine was dissolved in 1 cc. of concentrated sulfuric acid, there resulted red solutions having a faint orange tint. Addition of one crop concentrated nitric acid

to such solutions changed this color to amber, whereas addition of one drop of formaldehyde T.S. very greatly intensified the red color. One cc. of nitrosylsulfuric acid (prepared by dissolving 1.17 Gm. sodium nitrite in 15 cc. ice-cold concentrated sulfuric acid) when added to 0.01 Gm. of these compounds gave an intense brown color which was completely destroyed, with the attendant evolution of oxides of nitrogen, by addition of two volumes of water. With this reagent, ephedrine gave a light to medium brown color. Solutions of the hydrochlorides of the cinnamylephedrines (1%) gave the following reactions:

1. Addition of 10% potassium ferricyanide solution yielded a yellow precipitate (probably the ferricyanide of the base) which coagulated on shaking. The precipitate was dissolved on shaking with chloroform, and evaporation of the chloroform layer yielded a yellow, resinous film having a faint odor of benzaldehyde.

2. Dropwise addition of bromine water gave yellow precipitates which coagulated into small, orange resinous masses on shaking. Continued addition of reagent yielded additional precipitate which was increasingly difficult to coagulate.

3. Addition of 5% mercuric acetate drop by drop gave a white precipitate which dissolved in excess reagent. On boiling the resultant solutions, an odor resembling that of cinnamic aldehyde was observed.[3]

4. With cold potassium permanganate or boiling, alkaline potassium ferricyanide reagents, an odor of benzaldehyde was emitted. The odor was apparent even when 1 cc. of 1:20,000 solutions of the salts were used with an equal volume of reagent.

5. In the biuret test, modified according to Feng, the cinnamylephedrines gave results comparable with those recorded by that author for several other *N*-substituted ephedrines (6). To a mixture of 1 cc. of a 1% solution of the hydrochloride and an equal volume of 1% copper sulfate, 1 cc. of 20% sodium hydroxide was added. There resulted a violet color and voluminous precipitate. After shaking with ether, there separated a colorless ether layer and a clear aqueous layer having a faint bluish tint.

Analysis of Aspirin, Caffeine and Cinnamylephedrine in Admixture.—Racemic cinnamylephedrine base was found to undergo neither a detectable loss of weight or titer on drying one-half hour at 80°, although the substance melts somewhat below this temperature. Unlike ephedrine, a secondary amine, it does not react with chloroform under ordinary conditions. A solution of the base in chloroform, after one month of standing, suffered no loss of alkalinity such as would result from formation of the hydrochloride. Chloroform solutions of ephedrine deposit crystals of the hydrochloride on standing even for short periods of time.

The aspirin and caffeine used in the assay were of U. S. P. quality, the latter having been dried to constant weight, and the cinnamylephedrine hydrochloride employed was the optically inactive substance previously described. The powdered sample (consisting of 1.500 Gm. aspirin, 0.100 Gm. caffeine and 0.0500 Gm. cinnamylephedrine hydrochloride) was placed in a separatory funnel, 15 cc. cold water added and the mixture agitated until the powder was well wetted. Fifteen cc. of cold bicarbonate solution (prepared by dissolving 3 Gm. sodium bicarbonate in 45 cc. water at 15° or below and adding two or three drops dilute hydrochloric acid) were added, the separator swirled until the aspirin had completely reacted and the caffeine and cinnamylephedrine extracted with five 30 cc. portions of chloroform. Each extract was washed in a second separator containing 4.5 cc. water and 0.5 cc. strong ammonia water before filtering through cotton previously moistened with chloroform into a suitable vessel. The combined extracts were reserved for future use.

The determination of aspirin was carried out as soon as possible (to prevent undue hydrolysis) by the following procedure. The ammoniacal wash water was made acid with hydrochloric acid (1 + 1), added to the bicarbonate solution of aspirin, and the whole rendered acidic by adding more acid (approximately 10 cc. of 1 + 1 hydrochloric acid). The aspirin was completely extracted with chloroform, the extracts filtered through cotton into a tared container and evaporated on the steam bath in a current of air to a volume of 10 cc. The evaporation was continued to dryness in the air current, but without heat, and the residue allowed to stand in a desiccator for several hours or over night before being weighed. The residues could be checked by titration or bromination if desired. The aspirin residues obtained by this method were not distinguishable in melting point from U. S. P. aspirin, although tests for free salicylic acid were positive. Assuming 100% recovery of aspirin and the salicylic acid formed by hydrolysis, the difference between the weight of the residue weighed as aspirin and the amount of aspirin originally present indicated the hydrolysis of the ester to be less than 4% after remaining in the alkaline solution approximately one hour.

The chloroform containing the caffeine and cinnamylephedrine base was evaporated to a small volume on the steam bath, transferred quantitatively to a tared 50-cc. beaker and evaporated to dryness. After drying the residue one-half hour at 80°, it was weighed as cinnamylephedrine base plus anhydrous caffeine. An excess of 0.02*N* sulfuric acid was added and the residue dissolved by the aid of warming. The judicious use of a small amount of chloroform greatly facilitated solution, but it was entirely expelled by warming before titrating the excess acid with 0.02*N* sodium hydroxide in the presence of one drop methyl red T.S. Each cc. of 0.02*N* acid is equivalent to 0.006357 Gm. cinnamylephedrine hydrochloride or 0.005628 Gm. of the free base. From the titration figure, the amount of free base weighed with the caffeine was calculated and the

[3] Private communication from M. W. Tapley, Sterling Products Company, New York, N. Y.

weight of caffeine computed by difference. Results obtained by the method are listed in Table II.

Table II.—Results of Analysis of Aspirin, Caffein and Cinnamylephedrine in Admixture

Aspirin		Caffeine		Cinnamylephedrine HCl	
Recovery, Gm.	Recovery, %	Recovery, Gm.	Recovery, %	Recovery, Gm.	Recovery, %
1.494	99.6	0.100	100	0.0498	99.6
1.491	99.4	0.101	101	0.0497	99.4
1.493	99.5	0.101	101	0.0488	97.6
1.492	99.4	0.101	101	0.0486	97.2
1.490	99.3	0.100	100	0.0493	98.6
1.487	99.1	0.100	100	0.0495	99.0
1.487	99.1	0.100	100	0.0496	99.2
1.487	99.1	0.100	100	0.0495	99.0
Av.	99.3		100		98.7

The direct titration of the cinnamylephedrine-caffeine residues may be carried out in 50% alcohol, but the indicator is so affected by the medium that results are reliable only when one is thoroughly familiar with the end-point under such conditions.

When applied to tablets containing lubricants, the method must be modified to obtain correct recoveries of caffeine. The residue of caffeine, cinnamylephedrine and lubricant may be taken up with acid, the grease or wax removed by shaking with petroleum ether or a little benzene, and the cinnamylephedrine and caffeine recovered by extraction from the aqueous layer after rendering it ammoniacal.

For qualitative purposes, cinnamylephedrine base may be separated from caffeine by leaching the mixture with carbon tetrachloride or ether, in which caffeine is but slightly soluble, filtering, evaporating the solvent and recrystallizing the residue from alcohol-water mixture.

SUMMARY

(1) The preparation of the *N*-cinnamylephedrines by the action of cinnamyl chloride on the corresponding ephedrines has been described, and some properties of the bases and their hydrochlorides have been observed and recorded.

(2) A method for the separation and estimation of aspirin, caffeine and cinnamylephedrine in admixture has been described.

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Toxicity of Red Squill Powder and Extract for Chickens, Rabbits and Guinea Pigs*

By J. A. Lubitz and C. R. Fellers

INTRODUCTION

It is believed that red squill powder is non-toxic to some animals (hogs, dogs, cats, chickens) due to an emetic principle present in the red squill powder. This principle causes emesis when red squill is taken in toxic quantities, thereby preventing poisoning. The belief behind the specific raticide action of red squill is that rats have no vomiting mechanism and so cannot rid their systems of squill. Therefore, they become poisoned when squill is ingested in considerable quantity. It is interesting to note that according to Dukes (1) the act of vomiting is not common to all species of animals. Rodents, ruminants and solipeds seldom or never vomit, whereas carnivores and omnivores (except such as are rodents) vomit readily.

This work was conducted to determine the effect of a red squill extract on guinea pigs, rabbits and chickens, for there was doubt expressed as to the supposed harmless character of red squill extract baits to animals other than rats.

REVIEW OF LITERATURE

References in the literature (2, 3, 4) indicate that red squill is not readily taken by animals other than rats, and if consumed the emetic principle in the

* Contribution No. 389, Massachusetts Agricultural Experiment Station, Amherst.