in this table represent simultaneous but not independent duplicate determinations. Each distinct paired group (or single value) represents a determination independent of the other group. Intensities of the developed chromophores in each instance exhibited a mean average deviation of ±2% or less.

Color Stability.—In all instances the color of the steroid chromogen was stable during the 15-minute interval after the cooling step. Longer time intervals were not investigated.

Interferences.—Chloroform soluble carboxylic acids (acetylsalicylic acid and stearic acid, etc.) and esters (sorbitan monostearates, methyl, and propyl parabens, etc.) tended to inhibit color formation. Consequently, cream formulations were not capable of being assayed using the described reagent.

Reagent Blank.—The 1-cm. absorbance values at 485 and 620 mμ for the reagent blank were, respectively, 0.116 and 0.008.

General Aspects.—DTBPC reagent, oxidized by refluxing and aerating for 1 hour, gave identical color intensity for fluocinolone acetonide (6α,9α-difluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione-16,17-acetonide) as did untreated reagent, indicating that an oxidized form of DTBPC is not necessarily the reactant.

Paper chromatograms of fluocinolone acetonide, impregnated and heated with the combined TMAH–DTBPC reagent, indicated that this reagent is not suitable for the detection of steroid spots.

Fluocinolone acetonide color obtained by the original method (1) was lower by a factor of 7.5 relative to this method. The former method was, however, 1.6 times more sensitive for 3, 11-diketo (4-unsaturated) steroids than the present method.

From the data presented the following conclusions may be made: (*a*) that type A steroids (Table I) containing a 6α, 9α-difluoro moiety (fluocinolone acetonide) produced more color than their 6α-fluoro counterparts (paramethasone acetate), and (*b*) that the molar response of 11-unsubstituted-3-keto (4-unsaturated) steroids toward the DTBPC reagent was decreased by substitution of an 11-keto function and increased by the introduction of a 11-hydroxyl group.

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Assay for Methapyrilene in Complex Mixtures

By NATHAN SCOTT

A method is presented for the estimation of methapyrilene in complex mixtures which may include other antihistamines. Briefly, it consists of treating ninhydrin with methapyrilene which has been extracted from an alkaline solution with petroleum ether.

ANTIHISTAMINES have proved their therapeutic value over the years. Methapyrilene is one of the established antihistamines which has found increased usage by itself and in combination with other ingredients.

Many methods exist for the estimation of methapyrilene. The Association of Official Agricultural Chemists (1) and "The National Formulary" (2) describe methods employing an ultraviolet absorption. Clair and Chatten (3) describe a nonaqueous method and Cox *et al.* (4) employ chromatography and electrophoresis. Another method described by Celeste and Turczan (5) uses gas chromatography after a preliminary extraction. This article is concerned with a spectrophotometric method for its determination, particularly when it is present in pharmaceutical dosage forms with other materials.

EXPERIMENTAL

Materials.—Reagents used in the experiments were of analytical grade where available and used without additional purification. The following materials were used: ninhydrin (1,2,3 triketohydrindene) (Eastman Kodak); petroleum ether; sulfuric acid; polysorbate 80 U.S.P.; methapyrilene hydrochloride N.F.; sodium hydroxide; phenylephrine hydrochloride U.S.P.; dextromethorphan hydrobromide; chlorpheniramine maleate U.S.P.; and tripelennamine hydrochloride U.S.P.

Apparatus.—Beckman model DU spectrophotometer was used.

Procedure.—The method is applicable to liquid or solid dosage forms. A portion of liquid containing about 5 mg. of methapyrilene hydrochloride is transferred to a separator. The solution is rendered alkaline with either 1 *N* sodium hydroxide solution or sufficient sodium bicarbonate. The alkaline solution is then extracted successively with 15 ml. and 3 × 10 ml. of petroleum ether. The petroleum ether extracts are passed through a cotton plug moistened with petroleum ether into a 50-ml. volumetric flask. After rinsing the separator with about 3 ml. of solvent and filtering into the flask, the liquid is brought to the mark and mixed. One milliliter of the solution, equivalent to about 100 mcg. of antihistamine, is carefully transferred to a 10-ml. flask and the petroleum ether removed with a gentle stream of nitrogen for about 5 minutes. To the residue is then added 0.25 ml. of a 1% solution of 1,2,3 triketohydrindene (ninhydrin) in concentrated sulfuric acid. This solution should be freshly prepared, but is usable for several days. The flask is rotated so that the liquid covers its surface to insure complete contact with the residue. After about 5 minutes of intermittent rotation, 5 ml. of a 5% solution of poly-

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TABLE I.—ABSORBANCE DETERMINATIONS

mcg.	Absorbance					Av. Absorbance	S.D.
20	0.086	0.089	0.094	0.083	0.094	0.089	0.004
40	0.174	0.177	0.176	0.169	0.174	0.174	0.002
60	0.265	0.259	0.264	0.254	0.268	0.262	0.004
80	0.346	0.349	0.350	0.347	0.347	0.348	0.001
100	0.435	0.438	0.439	0.447	0.441	0.440	0.003
120	0.520	0.531	0.525	0.538	0.529	0.529	0.006
140	0.611	0.609	0.611	0.604	0.619	0.611	0.005

TABLE II.—ASSAY RESULTS

	Soln.		Methapyrilene Added	Amount Recovered	% Recovered
I.	Dextromethorphan HBr	5 mg.	5 mg.	4.94 mg.	98.3
	Chlorpheniramine maleate	5 mg.		5.02 mg.	100.4
	Pyrilamine maleate	20 mg.			
II.	Phenylephrine HCl	12.5 mg.	5 mg.	4.98 mg.	99.5
	Pyrilamine maleate	5 mg.		5.03 mg.	100.6
	Chlorpheniramine maleate	1 mg.			
III.	Pyrilamine maleate	10 mg.	5 mg.	4.90 mg.	98.0
	Phenylephrine HCl	25 mg.		5.03 mg.	100.6
	Chlorpheniramine maleate	2 mg.			
IV.	Tripelennamine extract in petroleum ether	100 mcg.	100 mcg. in petroleum ether	100.5 mcg.	100.5
V.	Tripelennamine extract in petroleum ether	50 mcg.	100 mcg. in petroleum ether	101.6 mcg.	101.6

sorbate 80 in distilled water is added with gentle mixing. The solution is then read on a Beckman spectrophotometer or other suitable instrument at 525 mμ. A standard of the corresponding methapyrilene salt is prepared by a similar procedure, or an aqueous solution of methapyrilene hydrochloride is evaporated with the aid of heat and treated with the ninhydrin reagent. A reagent blank is prepared and is used to set the instrument. The red-purple solution may be read immediately, although it is stable for several hours. This color will vary somewhat. The higher the concentration, the more purplish the solution.

Powders, capsules, and tablets may be treated in the same way after first dissolving a portion of pulverized material in a specific volume. The solution is filtered where necessary, and an aliquot treated in the manner outlined above.

RESULTS

Table I gives the absorbance, average absorbance, and standard deviations of a series of experiments. Treatment of portions of a petroleum ether extract of methapyrilene hydrochloride containing 100 mcg./ml., reacted with ninhydrin according to the method outlined above, gave the results of Table I. The measurements were made at 525 mμ. Although no sharp peak is obtained with the colored solutions, a flattened peak is observable at about 525 mμ. If the results of the absorbances were graphed, a nearly straight line would result.

To determine the specificity of the method, several mixtures were prepared and tested. These mixtures simulated in composition some actual commercial products. The results are listed in Table II. As can be seen in Table II, satisfactory recovery results of added methapyrilene are obtainable by the method described.

Other solutions tested, not included in the table, but which gave good results contained vitamins, salicylamide, acetophenetidin, antibiotics, and phenylpropanolamine.

DISCUSSION

Great flexibility may be exercised in the amount of sample used, the number of extractions made, and the ultimate volume of the extracted material. Different solvents, such as chloroform, may replace the petroleum ether and solubilizing agents other than polysorbate 80 may be used.

Of the compounds tested, as shown in Table II, dextromethorphan interfered with the assay method. In instances where dextromethorphan hydrobromide is present, it may be removed by extracting the sample with several portions of chloroform after first acidifying the preparation with 5 ml. of 1 *N* hydrochloric acid. The sample is then made alkaline and extracted with petroleum ether. In general, due to the complexity of liquid preparations, it is desirable to pre-extract the acidified syrups to remove any potential interferences from these mixtures.

The accuracy and reproducibility of this procedure are satisfactory for analytical and control purposes. That the methapyrilene can be differentiated readily from the other components in complex mixtures contributes to its usefulness.

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