

DIETHYLPHTHALATE IV.*

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I. INTRODUCTION.

Data appearing in the literature since our last paper, Diethylphthalate III (1),¹ was presented have been reviewed, and the study of the detection of this ester has been extended to include particularly essential oils and perfumes.

II. REVIEW OF THE LITERATURE.

Smith (2) reports several tests on the toxic properties of diethylphthalate and states that when given, *per ora*, to rats and pullets in amounts up to 0.5% and 1.5% of the body weight, respectively, that there was no permanent injury though pain and weakness persisted in some cases for 12 hours. Smith notes that workmen in factories making the ester show no ill effects from it even though their clothing may be saturated with it for several hours. Smith concludes that the ester is probably non-toxic to humans.

Harrison (3) found diethylphthalate in a sample of tincture of iodine examined by him and identified the ester by its taste.

Leffmann (4) describes an unsuccessful attempt to improve the delicacy of the detection test by resorcinol, *i. e.*, the fluorescein reaction, by examining the final alkaline solutions in a dark room with ultraviolet light.

Vuilleumier, (5) and Raleigh and Marie (8) describe confirmatory tests for diethylphthalate in alcohol which depend on determination of specific gravity and boiling point of the isolated ester. (Such tests are of course of little value in detecting traces of the ester in complex mixtures—such as beverages and perfumes.)

Hepburn (6) in an article containing the proposed text of the new Pharmacopœia on whisky gives a detailed procedure for the detection of diethylphthalate in whisky suspected to contain it which is practically that recommended for its detection in beverages in Diethylphthalate II. (JOUR. A. PH. A., 13, 600–609, 702–710 (1924).)

Thoms (7) describes a modification of the Handy-Hoyt method which he found the most satisfactory for detecting diethylphthalate in alcohol. Thoms claims to have improved the method by carrying out the condensation at 80° C., instead of 160° C. and states that his modification will detect 0.5 mg. of the ester in 50 cc. of sample. In addition this author outlines a procedure for the qualitative isolation of diethylphthalate from a sample by sublimation as phthalic anhydride after saponification and extraction.

Breithut and Apfelbaum (9) describe a method for the detection of diethylphthalate in perfumes in which the perfume oils are salted out with saturated salt solution and the ester detected in the aqueous solution by the phenolphthalein test. No data is given by them as to the sensitiveness of this test.

Brown (10) describes the use of the dialkyl esters—particularly ethyl and butyl—of phthalic acid as plasticizers in pyroxylin lacquers. His article includes an excellent bibliography which shows that the use of these esters in the pyroxylin industry dates back over 30 years.

* Scientific Section, A. PH. A., Des Moines meeting, 1925.

¹ NOTE: Numbers in parentheses refer to similarly numbered references in the bibliography.

Levinson (11) describes a modified fluorescein test for phthalates in which a sample is evaporated nearly to dryness, followed by the addition of one gram of boric acid, with evaporation to complete dryness and fusion of the residue with one gram of resorcinol. According to Levinson this method gives negative blanks and he states that a distinct fluorescence is obtained when only 1 cc. of a hair tonic containing 60% of 39B Specially Denatured Alcohol is used. (Such a 1-cc. sample would contain approximately 15 milligrams of diethylphthalate, and several fluorescein type tests previously described in the literature would easily detect 0.5 milligram in such a 1-cc. sample.)

Note.—The articles of Raleigh and Marie, Breithut and Apfelbaum, and Levinson state that these authors had found the official test for the detection of diethylphthalate to be unreliable. This official test was published as *Pro. Mimeograph Col.* No. 2840 9/23/21, but has been superseded for over a year by the revised test of Appendix to Regulations No. 61, Formulæ for Completely and Specially Denatured Alcohol, revised Sept. 1924, in which the condensation of the phthalate with resorcinol is carried out in acid solution. We wish to point out that the original procedure was founded on incorrect chemical principles since the formation of fluorescein by the condensation of phthalate with resorcinol cannot take place in an alkaline fusion. As a matter of fact if pure fluorescein is heated with caustic soda, it decomposes, yielding carbon dioxide, resorcin, and benzoic acid (Beilstein, "Handbuch der Organischen Chemie," 3rd ed., Vol. II, p. 2060). We found by actual test that pure fluorescein heated with caustic soda effervesced and left a residue entirely devoid of fluorescence. It is true that a dark reddish brown fluorescent product is formed in the caustic soda fusion of resorcin and a phthalate but it is not fluorescein.

In our opinion the Levinson method, which is unique in the use of boric acid as a condensing agent, is simple and rapid. However, it gives highly colored olive-green blanks; and unless the fusion is properly carried out, condensation of small amounts (*i. e.*, 1 mgm. or less) of phthalates, to form fluorescein, may not take place. It makes no provision for the elimination of interfering substances by extraction or other means. Our experience with this method showed it to be less sensitive and positive than the present Internal Revenue Bureau methods.

III. EXPERIMENTAL PART.

The idea of using a lower temperature for the condensation of resorcinol and diethylphthalate such as the 80° C. advocated by Thoms was tried out. Comparative tests were made using the same amounts of reagents but heating at different temperatures: 0.5-cc. portion 39-C Denatured Alcohol (equivalent to approx. 5 milligrams diethylphthalate) was evaporated to dryness after adding 1 cc. 10% NaOH, 2 cc. 1.84 H₂SO₄ containing 25 mg. resorcin per cc. were then added. The mixtures were heated (*a*) 10 minutes on the steam-bath, (*b*) 2 hours on the steam-bath, (*c*) 3 minutes at 125° C., and (*d*) 3 minutes at 150° C. The reaction mixtures were poured into 50-cc. distilled water and made alkaline. It was found that the intensity of fluorescence of (*a*) and (*b*) was practically the same and only a fraction of (*d*), heated at the higher temperature. (*c*) was intermediate in intensity. This test which confirms similar ones we have previously made indicates very clearly that the formation of fluorescein (which is a condensation of resorcinol

and phthalic acid in which water is eliminated) is much more complete at a temperature of 150° C. than at 80° C. or 100° C. Heating for a long time at 100° C. appears not to increase the "yield" of fluorescein.

Consequently in the following tests by the fluorescein reactions we have used a temperature of 150° C. in order to increase the amount of fluorescein formed even at the risk of decomposing the sample itself with the hot acid. A temperature of 150° C. is by actual trial less liable to bring about such decomposition than the 160° C. temperature formerly used.

DETECTION IN PERFUME RAW MATERIALS AND PERFUMES.

Diethylphthalate finds its way into perfumes chiefly from two sources, *viz.*: (1) the alcohol used in making the perfume is often Specially Denatured Alcohol 39B or 39C containing 2½% and 1%, respectively, of the ester, and, or (2) the essential oils or other perfume ingredients may have contained the ester added legitimately as a vehicle, fixative, or adjusting agent, or in some cases added in excessive proportion as a diluent.

If either 39B or C Denatured Alcohols have been used the detection of diethylphthalate in the perfume is very simple. The perfume will contain such a relatively large amount of the ester that a 1-cc. sample made alkaline, evaporated and the residue condensed with resorcinol and 1.84 H₂SO₄ will give a fluorescein test of such intensity that there can be no mistake about its presence. However, if the perfume has been made without these 39B and C Denatured Alcohols, from essential oils or synthetics which contain only a few per cent of the ester (and hence amounting to only a few hundredths of 1% in the final perfume), its detection is more difficult.

After a careful preliminary study of various methods, including extraction methods with pure water and brine, with and without alcohol, of essential oils to which the ester was added a procedure was adopted suitable for detecting diethylphthalate or other phthalates in perfumes and perfume raw materials.

Commercial samples of 25 of the more costly essential oils and synthetics used in perfumes were subjected to this test, according to the following procedure, with the results shown in Table I.

Procedure.—Qualitative Fluorescein Detection Test for Diethylphthalate or other Phthalates in Essential Oils and other Raw Materials for Perfumes.

Reagents: (1) *Alcoholic Potash.*—Dissolve 1.5 Gm. AgNO₃ in 3 cc. water, add to 1 liter of No. 30 Denatured Alcohol, and mix thoroughly. Dissolve 3 grams KOH in 10–15 cc. warm alcohol. After cooling, pour slowly into alcoholic AgNO₃. Do not shake. Let stand over night. Filter and distill. To the distilled alcohol so obtained add 3–5 grams *m*-phenylene-diamine hydrochloride per liter, boil under reflux for several hours. Distill and preserve the aldehyde-free alcohol in brown bottles. To this aldehyde-free alcohol add 40 grams per liter of C.P. KOH, heat until dissolved, cool and filter. The resulting alcoholic potash should be water white, or at most pale yellow, and should give an entirely negative and colorless blank in the fluorescein reaction test for phthalates (*i. e.*, 10-cc. alcoholic KOH evaporated to dryness, residue heated with 0.05 Gm. resorcin and 1 cc. 1.84 H₂SO₄ for 3 minutes at 150° C., resulting mixture added to 50 cc. of a 2% solution of NaOH).

(2) *Resorcinol—Sulphuric Acid.*—Dissolve 2.5 grams C.P. Resorcinol in 100 cc. 1.84 H_2SO_4 with the aid of heat. (*Note.*—Part of the resorcinol separates as a fine white ppt. on cooling but the reagent remains entirely satisfactory if kept in a stoppered container, to prevent absorption of moisture by the H_2SO_4).

Qualitative Test.—To 0.1 cc. (usually 5 small drops) of sample in a small beaker, flask or short test-tube, add 1 cc. alcoholic potash prepared as above. Heat on the steam-bath until the alcohol is completely removed. From a graduated pipette add 0.5-cc. resorcinol— H_2SO_4 reagent, rotating the container so that the acid thoroughly wets the entire residue, and heat for 3 minutes in an oil-bath at a temperature not over 150° C. Cool and pour the reaction mixture into 40-cc. distilled water in a small flask or an 8" × 1" test-tube. Make alkaline with 10 cc. of 10% NaOH solution. A yellowish green fluorescence persistent for 24 hours and longer is proof of the presence of diethylphthalate or some other phthalate in the sample.

TABLE I.

Lab. no.	Name.	Qual. test for phthalates.	Lab. no.	Name.	Qual. test for phthalates.
1.	Cinnamic Alcohol	—	14.	Patchouly Vieux	—
2.	Citronellol	—	15.	Petitgrain	—
3.	Clove Clair	—	16.	Phenylethylalcohol	—
4.	Oil Geranium	—	17.	Absolute Rose	—
5.	Hydroxycitronellol	—	18.	Absolute Rose S	+ (Strong)
6.	Alpha Ionone	—	19.	Rose Liq.	+ (Moderate)
7.	Methyl Ionone	—	20.	Santal Citrin	—
8.	Jasmine Liq.	—	21.	Tolu Clair	+ (Intense)
9.	Labdanum Clair	+ (Intense)	22.	Tuberose Liq.	—
10.	Methyl Anthranilate	—	23.	Vetiver Vieux	—
11.	Mousse de Chene (A), liquid	+ (Intense)	24.	Sauge Sclaree	+ (Moderate)
12.	Absolute Orange	—	25.	Mousse de Chene	
13.	Fleur d'Oranger	+ (Intense)		(B) thick, pasty	—

The samples of Labdanum Clair and Tolu Clair contained a very large proportion of diethylphthalate, Mousse de Chene (A), liquid and Fleur d'Oranger both contained a large amount and Absolute Rose S, Rose Liq. and Sauge Sclaree smaller quantities.

During the course of these tests the important and valuable observation was made that in samples containing an appreciable amount of diethylphthalate there occurred a separation of fine silky needle-like crystals of potassium phthalate a few minutes after the sample (5 drops) mixed with 1-cc. alcoholic potash was placed on the steam-bath. In none of the samples found to be free from diethylphthalate did any needle-like crystals form. With some samples, notably Clove Clair and Methyl Anthranilate a crystallization takes place just before the alcohol is completely evaporated but the crystals are entirely different from those of potassium phthalate.

The sensitiveness of this simple qualitative test was determined by adding known small amounts of diethylphthalate to the 18 samples shown to contain none by the qualitative fluorescein test and subjecting 0.1 cc. (or 5 drops) portions of such samples to saponification with 1-cc. alcoholic potash.

Preliminary control tests on 0.1 cc. of diethylphthalate-free sample to which 1-cc. alcoholic potash was added showed that in none of these samples did any needle-like crystals form during evaporation.

Series I.—It was found that in every one of the 18 samples so tested the addition of 5% of diethylphthalate was easily and unmistakably revealed by the formation of the needle-like crystals of potassium phthalate when 0.1 cc. of sample (*i. e.*, containing approx. 5 milligrams of diethylphthalate) was saponified on the steam-bath with 1 cc. alcoholic potash.

Series II.—The test was repeated with 0.1-cc. portions of these same 18 samples containing only 2% of diethylphthalate and in 13 of the 18 samples the needle-like crystals appeared. In the case of Clove Clair, Jasmine Liquid, Methyl Anthranilate and Absolute Orange the crystals failed to appear due of course to the fact that in the presence of these substances the 2 milligrams (approx.) of potassium phthalate formed was soluble.

DETAILS OF THE NEW POTASSIUM PHTHALATE CRYSTAL TEST FOR THE DETECTION OF PHTHALATES IN ESSENTIAL OILS AND OTHER RAW MATERIALS FOR PERFUMES.

Apparatus.—A very small beaker (the 10-cc. beaker used with the Zeiss immersion refractometer is excellent) or a short glass vial about $\frac{3}{4}$ " to 1" in diameter is the most convenient container, although a short test-tube may be used if these are not available.

Reagent.—Alcoholic potash, 40 grams per liter, prepared from aldehyde free alcohol as previously described.

Procedure.—To 0.1 cc. or 5 small drops of sample in a suitable glass container add 1-cc. alcoholic potash reagent. Place on the steam-bath and allow to evaporate *slowly*. Observe the sample *frequently* by holding the container in front of a bright light (a pocket flashlight may be used for convenience). If the sample contains 5% of diethylphthalate the small silky needle-like crystals of potassium phthalate will be observed soon after the sample has been placed on the steam-bath (*i. e.*, usually within 5 minutes) and before the alcohol has entirely evaporated. In many cases as small an amount of diethylphthalate as 2% will be clearly shown by this test.

Notes.—(1) for comparison run a standard test on 5 milligrams of diethylphthalate (0.5-cc. 39C Denatured Alcohol plus 1-cc. alcoholic potash).

(2) It is important that the heating and evaporating be slow and that the sample be observed before the bulk of the alcohol has evaporated, since a small amount of the crystals are not as easily seen when the residue approaches dryness.

(3) In the case of samples containing gross amounts of diethylphthalate the solution soon after placing on the steam-bath will appear to almost solidify owing to the great mass of potassium phthalate crystals formed.

(4) The dried residue may be subjected to the fluorescein test if desired by adding 0.5-cc. resorcinol-sulphuric acid reagent, heating for 3 minutes at 150° C. and making alkaline.

(5) The crystals of potassium phthalate will also form if the sample to which alcoholic potash has been added is allowed to evaporate spontaneously at room temperature. If this procedure is used, the crystals tend to be larger and sometimes form in fan-shaped bundles.

Caution.—It should be noted that in this potassium phthalate crystal test the presence of water (*i. e.*, in excess of the water in 190° proof alcohol) will prevent the formation of the crystals. The method is applicable directly to denatured alco-

hols, perfumes, essential oils and other water-free liquids. In the case of toilet waters and beverages the sample should be extracted with petroleum ether and the test applied to the extract.

The Potassium Phthalate Crystal Test was also applied to 20 additional samples of more common essential oils which preliminary tests had shown to be free from diethylphthalate, using 0.1-cc. portions of each oil containing 2 mg. and 5 mg. respectively of diethylphthalate. The formation of potassium phthalate crystals in these tests is shown in Table II.

It will be noted that in each of the 20 samples, 5 mg. of diethylphthalate could easily be detected in 0.1 cc. of sample and that in over half of the samples 2 mg. could similarly be found.

TABLE II.

No.	Name of essential oil.	0.1-cc. sample containing 2 mg. diethylphthalate.	0.1-cc. sample, containing 5 mg. diethylphthalate.
1.	Anise	+	+
2.	Bay	+	+
3.	Bergamot	(-)	+
4.	Caraway	+	+
5.	Cedar Leaf	+	+
6.	Cedarwood	+	+
7.	Cassia	(-)	+
8.	Cloves	(-)	+
9.	Cumin	(-)	+
10.	Eucalyptus	(-)	+
11.	Geranium (African)	(-)	+
12.	Lavender	+	+
13.	Lemongrass	(-)	+
14.	Nutmeg	+	+
15.	Sweet Orange	(-)	+
16.	Lemon	+	+
17.	Peppermint	+	+
18.	Rosemary	+	+
19.	Rhodium	(-)	+
20.	Sandalwood	+	+

DETECTION OF DIETHYLPHTHALATE IN PERFUMES.

Diethylphthalate may be easily and unmistakably detected in perfumes made with either 39B or 39C Specially Denatured Alcohols, on a sample as small as 1 cc. by the fluorescein type test. The standard method of the Bureau of Internal Revenue (Appendix to Regulations 61, revision of Sept. 1924), the Andrew Method, or the procedure recommended in our paper Diethylphthalate II can all be used successfully. The test can be made on the perfume directly without extraction with petroleum ether, saturated salt solution or other solvents. The phenolphthalein method of Breithut and Apfelbaum gives satisfactory results on perfumes made from 39B or 39C Alcohols (in which the content of diethylphthalate of the finished perfume would be 1% or more). However, our experience has been that phenolphthalein test as applied to essential oils containing 5% or less of the ester and to perfumes made from such ingredients with diethylphthalate-free alcohol (in which finished perfumes the content of diethylphthalate would be only a few hundredths of 1%), is not as sensitive as either the fluorescein type test or our new potassium phthalate test. The phenolphthalein test was applied to 18

samples of essential oils to which 5% diethylphthalate had been added, and using the 0.1-cc. portions (in which the ester was unmistakably found in every case by both the fluorescein and potassium phthalate crystal tests) the phenolphthalein test was positive on only two samples.

We have tried our new potassium phthalate crystal test on 20 samples of perfumes, including a variety of floral odors and bouquets, known to have been made with either 39B or 39C Specially Denatured Alcohols. To a 1-cc. portion of each perfume was added 1-cc. alcoholic potash and the mixture allowed to slowly evaporate. The characteristic needle-like crystals of potassium phthalate formed in every case. With some perfumes the addition of alcoholic potash caused a turbidity or precipitation of a gummy mass but this in no way interfered with the test.

To detect diethylphthalate in a perfume made with alcohol free from that ester but which contains essential oils or other perfume ingredients to which the ester has been added, it is necessary of course to use a larger sample. Experiments which we have made indicate that the crystal test will detect 5 milligrams of diethylphthalate (and in many cases 2 milligrams) in a 10-cc. portion of perfume.

PROCEDURE FOR DETECTING DIETHYLPHTHALATE IN PERFUMES BY THE POTASSIUM PHTHALATE CRYSTAL TEST.

(A) To 1 cc. of perfume in a small beaker add 1-cc. alcoholic potash (40 grams per liter). Evaporate slowly with gentle heat and observe frequently, holding the beaker in front of a light. Provided the 1-cc. sample used contains 5 milligrams or over of diethylphthalate (*i. e.*, 0.5%, and in many cases if only 2 milligrams are present) the characteristic silky needle-like crystals of potassium phthalate will be seen to form in the solution. If no characteristic crystals form, it is proof that some alcohol other than 39B or 39C has been used in the manufacture of the perfume.

(B) If no crystals form by Test A, repeat, using 10 cc. of sample and 1-cc. alcoholic potash. Evaporate and observe as in Test A. If the sample contains 5 milligrams or more of diethylphthalate (*i. e.*, 0.05%), crystals of potassium phthalate will form.

Notes.—If desired the presence of the ester may be confirmed by making the fluorescein test in the usual way on the residue evaporated to dryness.

The difference in the sensitiveness of detecting diethylphthalate by the crystal test varies somewhat owing to the fact that various essential oils influence the solubility of potassium phthalate.

SUMMARY.

1. Work on the fluorescein type test has been extended to include the detection of diethylphthalate in essential oils and perfumes.

2. A new qualitative test for diethylphthalate has been worked out based on the formation of the characteristic crystals of potassium phthalate when an essential oil or a perfume is heated with alcoholic potash. This method is simple, rapid and sensitive. It is applicable directly to essential oils, perfumes, denatured alcohols and other water-free liquids and may be applied to the petroleum ether extract of products such as toilet waters and beverages. Results of its application

to a great variety of essential oils, perfume ingredients and perfumes show that it will detect with certainty 5 milligrams (and often as small an amount as 2 milligrams) of diethylphthalate in a 0.1-cc. portion of an essential oil or in a 10-cc. portion of perfume.

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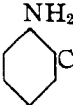
SOME STUDIES ON TASTE AND CHEMICAL CONSTITUTION.

BY THOMAS C. JALESKI.

There is, it seems, no constancy, or very little constancy, in the relation between chemical groups, structure and taste in organic compounds. Also it is apparent that there exists very little relation between physiological action of a substance and taste. If some sort of relation could be established between the above factors, the economic importance would be very great. Taste is an important consideration in the selection of a therapeutic agent.

A few rules have been observed to be important, however, and their application has brought forth some very good results. For instance, it is known that in the aliphatic alcohols increasing the hydroxyl groups augments the sweet taste. For example, glycerin with only three hydroxyl groups is moderately sweet while the hexoses with six hydroxyl groups are true sugars. Sternberg said there are only two groups which can produce a sweet taste. These are the hydroxyl-OH group and the amino-NH₂ group.

The replacement of an aliphatic group by an aromatic one tends to change the compound from a sweet tasting substance to an intensely bitter one. Butenyl glycerin CH₃.CH(OH).CH₂(OH) is sweet while phenyl glycerin C₆H₅.CH(OH).CH₂(OH) is bitter.

Also the proximity of an amino-NH₂ group and a carboxyl-COOH in the aromatic hydrocarbons affects taste. Anthranilic acid  is sweet while *p*-