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THE IDENTIFICATION OF VOLATILE OILS AND THEIR DERIVATIVES IN THE ANALYSIS OF MEDICINAL PREPARATIONS.

BY E. K. NELSON.

In the analysis of medicinal preparations the chemist is obliged to rely to some extent on his sense of smell. This is especially true in the detection of the essential oils. The odor is one of the most characteristic properties of a volatile oil, yet the nose is not always able to distinguish the presence of different oils in a mixture, especially if some of them are weak in odor, or present in small proportions. Also some substances of different classes possess quite similar odors as, for example, camphor (a ketone), and borneol (an alcohol).

As a number of volatile oils, or derivatives of them, are used in medicine for their therapeutic or antiseptic value, it is quite important that their detection be rendered as certain as the present state of our knowledge will permit.

The use for which the preparation is recommended will often be a guide to the analyst in determining what volatile oils are likely to be present.

If, by chemical methods, we can separate the volatile oil mixture into several groups or classes of compounds, such as phenols, aldehydes, etc., the odor of the products of such a separation affords a fairly safe means of determining its composition, even when there is insufficient material available to make any further examination.

The methods herein described are of a general character and are designed to assist in the examination of the volatile oil mixture obtained by the distillation of a medicinal preparation with steam. In case volatile oils are used merely as flavoring agents their identification is not so important, and they will be present in small quantity. But if used for their medicinal or antiseptic effect it will be desirable to obtain as large an amount as possible for the examination. A liberal sample of the preparation, neutralized if acid or alkaline, is submitted to steam distillation and the undissolved oily layer separated from the distillate.

The physical constants of the volatile oil mixture are first determined. The density is taken with a small Sprengel tube. The optical rotation and index of refraction are determined, and the boiling temperature is taken, keeping the fractions separate for each 10° difference and noting the amount and odor of each fraction. This will often afford a clue to the nature of the mixture and perhaps direct attention to some of the components.

ALDEHYDES (AND SOME KETONES).

Separation.—The oil (or a suitable fraction) is shaken with an equal volume of a saturated solution of sodium hydrogen sulphite in a separatory funnel and allowed to stand with occasional shaking for from 8 to 12 hours. If crystals separate they are filtered off; the aqueous layer is separated and the crystals added. To this solution add sufficient sodium carbonate to neutralize the acid sulphite, and distil with steam. Aldehydes will pass over into the distillate and will usually be recognized by their odor.

Benzaldehyde will indicate oil of bitter almonds; cinnamic aldehyde, oil of

cassia; pulegone, oil of pennyroyal; methyl nonylketone, oil of rue; thujone, oils of tansy, wormwood, or sage. (The last three are ketones which react like aldehydes with sodium hydrogen sulphite.)

ALDEHYDES: IDENTIFICATION.

Benzaldehyde: Liquid, b. p. 733 mm. 177°; d_{15}^{15} , 1.050–1.055; m. p. of semicarbazone, 214°; easily oxidized to benzoic acid.

Cinnamic aldehyde: Liquid, b. p. 252°; d_{15}^{15} , 1.054–1.058; m. p. of semicarbazone, 208°; oxidized by cold potassium permanganate to benzaldehyde and benzoic acid.

Citral: Liquid with lemon-like odor; b. p., 228–229°; m. p. of α -citryl β -naphtho cinchoninic acid, 200°.

ALDEHYDES: DETERMINATION.

Aldehydes and certain ketones (pulegone, carvone) can be estimated by the neutral sulphite method of Burgess.¹

PHENOLS: SEPARATION.

The oil left in the separatory funnel after treatment with sodium hydrogen sulphite, is shaken with two or three times its volume of a five percent solution of potassium hydroxide. After the undissolved oil has separated the aqueous layer is filtered through a wet filter and a slight excess of dilute hydrochloric acid is added. A turbidity at this point will indicate the presence of phenols. Methyl salicylate separates with the phenols.

If the odor indicates the presence of methyl salicylate take up the separated phenols in a little ether; separate the ether solution and transfer it to a small flask; add from 5 to 10 Cc. of 5 percent potassium hydroxide solution and warm on a water bath under a reflux condenser to saponify the ester. Then pass in carbon dioxide to saturation and extract the phenols (free from methyl salicylate) with ether. Acidify the aqueous solution and extract with ether; if methyl salicylate is present a residue of salicylic acid will be left on evaporating the ether, which can be identified by its melting point and by the violet color its solutions give when treated with ferric chloride solution.

If methyl salicylate is not present the saponification is omitted. Evaporate the ethereal solution containing phenols at room temperature. The phenols which may be encountered include thymol and carvacrol (from oil of thyme), eugenol (from oil of cloves), and diosphenol (from oil of buchu). Observe whether the separated phenol shows any tendency to crystallize (thymol, diosphenol). Thymol and diosphenol may be separated from the more "acidic" phenols as follows: Dissolve the mixture in 5 percent potassium hydroxide solution and distil with steam. Thymol and diosphenol will come over from the alkaline solution while ordinary phenol and most of the eugenol will remain in the distilling flask and can be recovered with ether.

PHENOLS: IDENTIFICATION.

Thymol: Crystalline, m. p. 50.5–51.5°. Identify by U. S. P. test (greenish blue color on adding one drop each of sulphuric and nitric acids to its solution in glacial acetic acid).

¹ *Analyst*, 29, 78, 1904; "The Volatile Oils," Gildemeister and Hoffman, 2nd edition, Vol. I, English translation, pp. 581–590.

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Carvacrol: Liquid isomer of thymol, odor like thymol.

Diosphenol: Crystalline, m. p. 83° , peculiar minty odor. With ferric chlor its alcoholic solution gives a dark green color. Its solutions reduce ammonia silver nitrate and Fehling's solution.

Eugenol: Liquid, odor of cloves, m. p. of benzoate, $69-70^{\circ}$. Its alcohol solution gives a blue color with ferric chloride.

PHENOLS: DETERMINATION.

The shrinkage in volume on shaking a measured quantity of the oil with 5 percent sodium hydroxide solution will indicate the proportion of phenols present (See U. S. P. under *Oleum Thymi*.)

KETONES: SEPARATION.

The oil remaining after the extraction of aldehydes and phenols is now to be used for the separation of ketones. Advantage is taken of the property which ketones have of combining with semicarbazide to form crystalline, more or less difficultly soluble, and difficultly volatile semicarbazones. From $\frac{1}{4}$ to 1 gramme of semicarbazide hydrochloride and an equal amount of sodium acetate are dissolved in the least possible amount of water. The oil or its fraction (not over 5 Cc.) is added and enough alcohol is stirred in to give a clear solution. (Some NaCl may be precipitated.)

Let the mixture, which should be in a small stoppered flask, stand 12 to 24 hours and then dilute with water. If much ketone is present the oil which separates will soon crystallize more or less completely. If crystals separate, filter. In any event separate the oil, transfer it to a distilling flask, and distil with steam until the volatile oil is removed. If any ketone is present a crop of crystals should now separate from the residue left in the distilling flask if it is cooled and shaken. Filter off the crystals in a Büchner funnel and unite with any that may have separated previous to distillation.

To recover the ketones from their semicarbazones, transfer the semicarbazones to a saponification flask, reserving a portion for melting point and other determinations. Add from 5 to 10 Cc. of 25 percent sulphuric acid, stopper the flask, and heat on the steam bath until the crystals are decomposed. If camphor was present alone or in preponderating amount, it can be seen sublimed into the neck of the saponification flask. Cool and open the flask and note the odor.

KETONES: IDENTIFICATION.

Carvone: Liquid, from caraway and spearmint oils, b. p. $230-231^{\circ}$; m. p. of oxime, 72° .

Pulegone: Liquid, from oil of pennyroyal, minty odor, b. p. $222-223^{\circ}$; m. p. of semicarbazone, 168° .

Menthone: Liquid, from peppermint, pennyroyal, and buchu oils, minty odor, b. p. $207-208^{\circ}$; m. p. of semicarbazone, 184° .

Camphor: Crystalline, from camphor and rosemary oils, m. p. $175-176^{\circ}$; m. p. of semicarbazone, $236-238^{\circ}$; m. p. of oxime, $118-119^{\circ}$.

Thujone: Liquid, from the oils of thuja, wormwood, tansy and sage, peculiar odor like wormwood, b. p. $200-201^{\circ}$; m. p. of α -thujone semicarbazone, $186-188^{\circ}$; m. p. of β -thujone semicarbazone, $174-175^{\circ}$; can be identified by the method of Enz.¹

¹ *Schweiz. Wochenschrift für Chem. u. Pharm.*, 49, 337, 1911.

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Methyl nonyl-ketone: Liquid, from oil of rue, odor like oil of rue, b. p. 226° , p. $+13.5^{\circ}$; m. p. of semicarbazone, $123-124^{\circ}$; m. p. of oxime, $46-47^{\circ}$.

KETONES: DETERMINATION.

The total quantity of ketones and aldehydes present can be estimated by the hydroxylamine titration method.²

This method will include those ketones which do not react with sodium sulphite or sodium hydrogen sulphite. In determining camphor by this method the results are found to be low.³

ALCOHOLS, ESTERS, ETHERS AND OXIDES.

The volatile oil remaining unacted on by the previous methods of treatment may contain alcohols (as menthol, sabinol, santalol, borneol and terpineol), esters (as menthyl acetate, and bornyl acetate), and phenol ethers (as methyl chavicol, safrol, anethol, and apiol), or oxides (as cineol).

Previous to the further examination of the oil it should be saponified by boiling with an excess of alcoholic potassium hydroxide in order to decompose any esters present. The alcoholic solution is then diluted with sufficient brine to precipitate the oil completely, and the brine solution can be used for the identification of organic acids derived from esters.

There is no good general method for separating the alcohols as a class, and the further examination will therefore be governed by the judgment of the analyst as to what alcohols are likely to be present.

The primary alcohols, such as geraniol, can be separated by the calcium chloride compounds or as acid phthalic esters, provided they are present in sufficient amount (at least 25 percent of the mixture).

The conversion of alcohols into esters difficultly volatile with steam will be successful in some cases. Thus by heating menthol with benzoic anhydride for two hours at $160-170^{\circ}$ menthyl benzoate is formed, and by distilling the mixture with steam the ester, being less volatile, remains in the distilling flask, is separated, and the menthol recovered by saponifying. The same method is, of course, applicable to any of the more stable alcohols provided they are esterified under these conditions and give benzoates slightly volatile with steam. The identification of the tertiary alcohols is even a more difficult matter, as they are more or less dehydrated on heating with acid anhydrides, but they are not often encountered in a medicinal preparation. When obtained in fairly pure form the alcohols may be characterized by the melting points of their phenyl urethanes. Sabinol is the alcohol occurring in oil of savin, and since this oil is frequently employed as an abortifacient it should not be overlooked. The best chemical method for identifying sabinol (which the writer has used successfully in one case) consists in oxidizing it by means of potassium permanganate to α -tanacetogen dicarboxylic acid (m. p. 140°). A mixture of the product with some of the known compound showed no depression in the melting point, which was considered sufficient proof of the presence of oil of savin in the preparation.

Safrol may be found in the higher boiling fractions of the oil, its boiling point being 233° . The characteristic odor of safrol will serve to direct attention to it,

² Nelson, *Jour. Ind. Eng. Chem.*, 3, 588, 1911.

³ U. S. Dept. Agr., *Bur. of Chem. Bull.* 152, 208, 1912.

and it can be identified by its oxidation product, α -homopiperonylic acid which melts at 127–128°. This is obtained by the oxidation of safrol with potassium permanganate. Another phenol ether which may be encountered in medicinal preparations is apiol. This boils at 294° and will therefore be found in the last fraction of the oil. Apiol has a faint parsely odor. On boiling with alcoholic potassium hydroxide apiol is converted into isoapiol which melts at 55–56°. Tri-brom apiol melts at 88–89°. Unless present in relatively large amount its identification, on account of its faint odor, is very difficult.

Cineol (b. p. 175°) is separated in the first fractions of the oil. Its odor, which suggests eucalyptus oil, may direct attention to it if there is not too much interfering material. Cineol is an important constituent of eucalyptus and cajeput oils and is often used in medicine in pure form, being more commonly known as eucalyptole.

It combines with phosphoric or arsenic acid giving unstable crystalline compounds from which cineol can be recovered by adding warm water. Its iodole compound (m. p. 112°) is characteristic, but rather difficult to prepare from impure cineol.

VOLATILE OILS OF THE U. S. P. (1916) 9TH REV.

Name.	Characteristic ingredient.
Bitter almond	Benzaldehyde
Anise	Anethol
Orange	Citral, citronellol, limonene
Cade	Sesquiterpenes
Cajuput	Cineol
Caraway	Carvone
Cloves	Eugenol
Cinnamon	Cinnamic aldehyde
Chenopodium	Ascaridol
Coriander	Linalol, pinene
Cubeb	Cadinen
Eucalyptus	Cineol
Fennel	Anethol, fenchone
Juniper	Cadinene, pinene
Lavender	Linalyl acetate
Lemon	Citral, limonene
Peppermint	Menthol, menthone, menthyl acetate
Spearmint	Carvone
Myristica	Terpenes, myristicin
Tar
Pimenta	Eugenol
Pine needles	Pinene, sylvestrene, etc.
Rosemary	Borneol, camphor, bornyl acetate
Sandalwood	Santalol
Sassafras	Safrol
Mustard	Allylisothiocyanate
Turpentine	Pinene
Thyme	Thymol, carvacrol

SULPHUR COMPOUNDS, MUSTARD OILS.

The esters of isothiocyanic acid, characterized by their penetrating odor, constitute a special group of sulphur compounds.

Volatile mustard oil obtained from black mustard, *Brassica nigra*, is mainly

allylthiocyanate, and as this boils at 151° it will be found in the first fraction of the oil and will be recognized by its pungent odor.

After completing the examination of the volatile portion of a preparation it is often impossible to say whether some of the compounds found were used in the pure form or as ingredients of volatile oils. This is especially true of compounds such as camphor, thymol, menthol, or cineol which are often used in medicinal preparations.

For the guidance of the analyst in determining from his results what volatile oils are probably present, a list of the oils of the Pharmacopoeia, with their characteristic ingredients, is given.

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THE ANALYSIS OF PROPRIETARY MEDICINES.

AN OUTLINE.

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For the examination of secret remedies there is not available any comprehensive work corresponding to the many good books on the subject of food analysis.

The number of substances which may be found in any mixture and the almost unlimited range of possibilities in the way of combinations of materials in different preparations sold for the same purpose makes it impossible to give any rigid procedure for the examination of such products. Nevertheless, certain tests and methods of examination which have been found useful can be described. The suggestions here presented were arranged primarily for the use of analysts in the Bureau of Chemistry who might have occasion to examine secret preparations sold for the treatment of various diseases.

There are certain determinations in the analysis of preparations of drugs which should always be made and other determinations which may be desirable, depending upon the medicinal claims or the use to which the preparation is to be put. Certain substances are very likely to be found in any such preparations, regardless of the use for which they are recommended. Suggestions as to the probable active constituents may be obtained by consulting the "Index to Diseases" in some Dispensary or *Materia Medica*. When possible, tests should be made for each drug so mentioned. The absence of a drug generally used in treatment of conditions for which the remedy is recommended is often as important to establish as its presence. Very often by referring to "*Die Pflanzenstoffe*," Wehmer (1911), or to articles appearing more recently in the journals, it will be found that there are substances characteristic of certain drugs for which tests may be made. The finding of a certain ingredient does not always prove that a particular drug is present, but the absence of this ingredient will show the absence of the drug. This fact is very important when considering the medicinal claims. Of course account must be taken of any change this ingredient could undergo during manufacture. Bearing in mind the limitations mentioned, the following outline is offered as a general guide for the examination of proprietary medicines: