

[CONTRIBUTION FROM THE PHYTOCHEMICAL LABORATORY OF THE BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

## THE DETECTION OF METHYL ANTHRANILATE IN FRUIT JUICES.<sup>1</sup>

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At a meeting of the Committee on Referees of the Association of Official Agricultural Chemists during the annual convention in 1919 it was considered that a method for the detection of artificial flavoring materials, especially methyl anthranilate, was needed, and the author was requested to investigate the subject.

Methyl anthranilate, or the methyl ester of *o*-amidobenzoic acid,  $C_6H_4(NH_2)(COOCH_3)$ , although a well-known compound, has only in recent years been observed to be somewhat widely distributed in nature. The first observation of its occurrence in an essential oil was in 1895, when it was found by Walbaum in the oil of neroli or orange flowers.<sup>2</sup> Although present in the oil in relatively small proportion, it is the constituent which imparts to it the characteristic or distinctive aroma and also the violet fluorescence. In the meantime small amounts of this ester have been found in several other fragrant oils, such as those of the tuberose, jasmine, and gardenia, and also in the oils of ylang-ylang, sweet orange peel, and bergamot leaves. A methyl derivative of methyl anthranilate,  $C_6H_4(NH(CH_3))(COOCH_3)$ , has been observed to occur in the fruit and leaves of the mandarin.<sup>3</sup>

The general characters of methyl anthranilate may be noted as follows. M. p. 24.5°; b. p. 127°, at 11 mm. pressure. It is readily soluble in dil. mineral acids, alcohol, ether, chloroform, light petroleum and other organic solvents, and to some extent in water. It is volatile in steam, as is evident by its previously mentioned occurrence in several essential oils. Its solution in various solvents shows a more or less intense bluish fluorescence, depending upon the amount of ester present. The substance imparts to a pine shaving moistened with hydrochloric acid an intense orange color.

Among the derivatives of methyl anthranilate there may be mentioned the picrate, which was first obtained by Freundler<sup>4</sup> by mixing hot alcoholic solutions of the ester and picric acid. It crystallizes in handsome yellow needles, melting at 103.5° to 104°, but on account of its ready solubility in alcohol it is not adapted for the detection of small amounts of the ester. The same author<sup>5</sup> has also obtained a compound by condens-

<sup>1</sup> Read before the Association of Official Agricultural Chemists at its annual convention in Washington, D. C., November 16, 1920.

<sup>2</sup> "Die aetherischen Oele," by Gildemeister and Hoffmann, 2nd. edition, Bd. III, p. 101.

<sup>3</sup> *Ibid.*, Bd. I, p. 563.

<sup>4</sup> Freundler, *Bull. soc. chim.*, [3] 31, 882 (1904).

<sup>5</sup> *Loc. cit.*

ing equal molecular proportions of methyl anthranilate and phenyl-*iso*-thiocyanate, but this compound, a thiophenylketo-tetrahydro-quinazoline,<sup>1</sup> which melts above 300°, is also not suitable for the detection or estimation of small amounts of the respective ester. The same objection may be considered to apply to the benzoyl derivative of the ester, which was obtained by Erdmann<sup>2</sup> by treatment with benzoyl chloride. This derivative, which is sparingly soluble in cold alcohol, and crystallizes therefrom in long, white needles, melts at 99° to 100°.

The isolation of methyl anthranilate from an essential oil is usually effected by shaking the oil with dil. sulfuric acid.<sup>3</sup> When the ester is present in sufficient amount the sulfate will crystallize from its acid solution on cooling, and may be purified by recrystallization from alcohol. By subsequent treatment with sodium carbonate the ester may be regenerated. If the amount of methyl anthranilate contained in an essential oil is very small, and only the detection of its presence is required, the acid solution of the ester may be directly employed for the usual tests by diazotization and combination with a suitable amine or phenol. These tests and their applications will subsequently be more fully considered.

A method for the quantitative determination of methyl anthranilate in an essential oil, which depends upon the formation of the above-mentioned sulfate, has been devised by Hesse and Zeitschel.<sup>4</sup>

This method, which is stated to permit of the quantitative determination of so small an amount of the respective ester as 0.1% in an oil, is conducted by dissolving the oil in 2 to 3 parts of dry ether, cooling the solution in a freezing mixture to at least 0°, and then adding, drop by drop, a cooled mixture of one volume of conc. sulfuric acid and 5 volumes of ether until no further precipitate is produced. The precipitate is collected on a filter, and washed with dry ether until odorless, after which it may be dissolved in water, with the addition, if necessary, of a little alcohol, and titrated with 0.5 *N* potassium hydroxide solution. For further details reference should be made to the original publication.

One of the advantages of the method is stated to be that the methyl anthranilate may be completely removed from the oil without affecting its other constituents. This has been observed not to be the case when the oil is shaken with large amounts of a dil. mineral acid, since other non-basic constituents are then also brought into solution.

For the detection of very small amounts of methyl anthranilate recourse may be had to the previously intimated property which it has in common with all primary, aromatic bases of forming azo dyes. By this means Erdmann<sup>5</sup> has found that even a few milligrams of the ester may be quan-

<sup>1</sup> The compound described by Freundler is identical with one previously prepared by McCoy from anthranilic acid, *Ber.*, 30, 1688 (1897).

<sup>2</sup> Erdmann, *ibid.*, 32, 1216 (1899).

<sup>3</sup> Walbaum, *J. prakt. chem.*, [II] 59, 350 (1899).

<sup>4</sup> Hesse and Zeitschel, *Ber.*, 34, 296 (1901).

<sup>5</sup> Erdmann, *ibid.*, 35, 24 (1902).

titatively determined, and the method employed by him consists in titrating its diazotized solution with a standard alkaline solution of a phenol, such as  $\beta$ -naphthol.<sup>1</sup> The preliminary isolation of methyl anthranilate from an essential oil for the purpose of its colorimetric determination was conducted by Erdmann<sup>2</sup> by diluting the oil with ether and shaking the ethereal solution with dil. sulfuric or hydrochloric acid. It has been observed, however, by Hesse and Zeitschel<sup>3</sup> that the ester cannot be completely removed from the oil in this way, and they therefore prefer to precipitate it from the ethereal solution of the oil by means of sulfuric acid and titrate the precipitate with a 0.5 *N* alkali. A combination of the 2 methods has therefore been proposed, whereby the precipitate, consisting of the sulfate of methyl anthranilate, is dissolved in dil. sulfuric acid, and, after diazotizing, titrated according to the method of Erdmann with an alkaline solution of  $\beta$ -naphthol, when satisfactory results are said to be obtained.

With these preliminary observations, which may serve to elucidate the subject, consideration may now be given to the particular problem assigned to the author.

Although methods such as those described for the detection or quantitative determination of methyl anthranilate in essential oils have long been in use, the isolation of the substance from such products as fruit juices in a form sufficiently pure to permit of its identification was found to involve somewhat unexpected difficulties, but these were ultimately overcome.

In the beginning of the investigation it was deemed desirable to ascertain the delicacy of the reaction of diazotized methyl anthranilate with dimethylaniline and  $\beta$ -naphthol respectively, and for this purpose a large number of comparative experiments were made. It has thus been found that with the use of dimethylaniline 0.001 g. of methyl anthranilate may readily be detected, but the delicacy of the reaction is still greater with  $\beta$ -naphthol, which permits of the detection of so small an amount as 0.0001 g. of the anthranilic ester. The latter reagent, therefore, seems much to be preferred, especially when it is desired to ascertain the presence of such minute amounts of the substance.

#### Method for the Examination of Fruit Juices.

In an examination of grape juice and similar products for the presence of methyl anthranilate it was found that the preliminary isolation of this

<sup>1</sup> Attention may be called to an evident inaccuracy in the description of Erdmann's method, as given in Allen's "Commercial Organic Analysis," Fourth Edition, Vol. IV, p. 364. It is there stated that the diazotized solution of the ester is titrated with an "alcoholic solution of  $\beta$ -naphthol (rendered alkaline with caustic soda)", whereas the  $\beta$ -naphthol is required to be dissolved in a dilute solution of sodium hydroxide to which some sodium carbonate is subsequently added.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> Hesse and Zeitschel, *Ber.*, **35**, 2355 (1902).

substance in the requisite degree of purity could not be effected by direct extraction with ether. In the first place the ethereal liquid does not separate sharply owing to the tendency to form an emulsion, and, furthermore, other substances are extracted by the ether which interfere with the subsequent diazotization when the solvent is evaporated and the residue treated with dil. sulfuric acid and sodium nitrite. It is therefore best to first distil the fruit juice in a current of steam. For this purpose 500 cc. of the liquid may conveniently be employed, and about 200 cc. of distillate collected. The use of an alkali, such as sodium carbonate, for the neutralization of the acids before distillation is neither necessary nor desirable, since it not only causes the liquid to froth but gives rise to volatile decomposition products which contaminate the distillate. When methyl anthranilate is present in any appreciable amount the distillate exhibits a bluish fluorescence.

The next step in the process consists in extracting the methyl anthranilate from the distillate, and experiments have shown that ether is not well adapted for this purpose, since even a carefully purified ether, when employed in such amounts as are necessary for the complete extraction of the ester, yields on evaporation a somewhat contaminated product, which is only apparent when treated with dil. sulfuric acid and subjected to diazotization. This difficulty is completely overcome by the use of chloroform.

The distillate was therefore extracted with 3 successive portions of chloroform of 10 cc. each, the united chloroform liquids passed through a dry filter, and carefully evaporated in a small beaker on a water-bath, a current of air being passed over the surface until the solvent was just completely removed. The residue is then immediately treated with 2 cc. of 10% sulfuric acid, and the solution transferred to a test-tube, when it is ready for the specific test. If a residue obtained by the evaporation of the chloroform be kept for any length of time exposed to the air before treatment with the dil. acid, volatilization of the ester will occur.

The above-mentioned acid liquid is cooled, one drop of a 5% solution of sodium nitrite added, and subsequently a few crystals of urea, in order to destroy any possible excess of nitrous acid.

One of the 2 following tests may then be employed, although, as previously stated, preference is given to the more delicate reaction with  $\beta$ -naphthol.

**I. Test with  $\beta$ -Naphthol.**—A mixture is first prepared of one cc. of a 0.5% solution of pure  $\beta$ -naphthol,<sup>1</sup> one cc. of a 10% solution of sodium hydroxide, and one cc. of a 10% solution of monohydrated sodium carbonate,  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$ . To this solution, contained in a test-tube, is added the above-mentioned 2 cc. of diazotized liquid, when if not less than 0.0001 g. of methyl anthranilate be present a yellowish-red precipitate will be produced.

<sup>1</sup> This solution may be prepared by dissolving 0.5 g. of pure  $\beta$ -naphthol in 2 cc. of a 10% solution of sodium hydroxide, and diluting the liquid to the measure of 100 cc. A solution of this phenolic strength, together with the requisite amount of sodium carbonate, is also well adapted for quantitative determinations of methyl anthranilate.

**II. Test with Dimethylaniline.**—To the 2 cc. of diazotized liquid is added one drop of dimethylaniline, and, when the latter has completely dissolved, a slight excess of a 10% solution of sodium hydroxide is added. If not less than 0.001 g. of methyl anthranilate be present a yellowish coloration will be produced, and on then slightly acidifying the mixture with dil. sulfuric acid the color will change to red, the intensity of these colors being naturally dependent upon the amount of ester subjected to the test.

If there should be evidence of an appreciable amount of methyl anthranilate in the material under examination it would be desirable to dissolve the residue from the chloroform extract of the distillate in 4 cc. of dil. sulfuric acid. Equal portions of the acid liquid could then be used for each of the above-mentioned tests, and confirmatory results would thus be obtained.

Having developed a practical and trustworthy method for the detection of methyl anthranilate in grape juice it is intended to examine both commercial samples of this product and those of known purity for the presence of the respective compound. The results of this more extended investigation must be reserved, however, for a future communication. It may finally be stated that in the experimental part of the present work the author has been assisted by Mr. V. K. Chesnut, to whom his thanks may here be expressed.

WASHINGTON, D. C.

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### NEW BOOK.

*Festschrift aus anlass des siebenzigsten Geburtstages von Cornelio Doelter*  
H. LEITMEIER, Editor. Theodor Steinkopff, Dresden and Leipzig, 1920. 96 pp.,  
9 fig., 16.5 × 25.5 cm. M.12.

As indicated by the title, this little book, which is really a collection of 5 papers, was published on the occasion of Doelter's birthday by some of his students in honor of his long continued labors, which have not yet ceased, in the field of mineral chemistry.

The various numbers of Doelter's chief work, the *Handbuch der Mineralchemie*, have been reviewed in previous numbers of *THIS JOURNAL*. The papers in the present pamphlet are: Monzonites, by F. Becke; Experiments on the formation of siliceous nickel ores, by E. Dittler; Skolecite and metaskolecite from the Hegeberge near Eulau, by H. Michel; Experiments on the origin of aluminum phosphates, by H. Leitmeier and H. Hellwig; and Determination of crystal structure by roentgenography, by H. Tertsch. The last paper is the longest but probably the most interesting, as it deals with periodic relations in the crystal symmetry of the elements and concludes with the statement that electrical forces must determine crystal structure no less than the structure of the atoms themselves.

All of the papers contain considerable discussion of previous literature.