

[CONTRIBUTION FROM THE FOOD RESEARCH DIVISION, CHEMICAL AND TECHNOLOGICAL RESEARCH, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

STUDIES ON GOSSYPOL. VI. THE ACTION OF BOILING HYDRIODIC ACID AS USED IN THE ZEISEL METHOD UPON GOSSYPOL AND SOME OF ITS DERIVATIVES. A SEMI-MICRO ZEISEL METHOXYL METHOD

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In the preceding report² it was stated that difficulties arose in determining the methoxyl content of various methylated derivatives of gossypol, especially tetramethoxypseudogossypolone. When treated in the usual manner with hydriodic acid in a Zeisel apparatus, this substance gave practically no silver iodide. The addition of acetic acid or acetic anhydride to the hydriodic acid was also without appreciable effect. For example, in one experiment about 4% of methoxyl was obtained after the substance was boiled for four hours; whereas, if there are 4 methoxyl groups in the compound, as other evidence indicates, the theory demands a methoxyl content of 22.7%. In another experiment, phenol was added to the hydriodic acid, as recommended by Weishut.³ When this was done, the white double compound of silver nitrate and silver iodide immediately formed. Since the first experiments indicated the advisability of running the determination for a longer time than is usually required, boiling was continued for three and one-half hours. When the final result was obtained, it was found rather unexpectedly that the methoxyl content corresponded to five groups more closely than to four.

In view of the combustion results and other information available, it seemed improbable that the compound had more than four methoxyl groups and, therefore, some other cause was responsible for the high value. Further investigation revealed that, when phenol was used with hydriodic acid in the Zeisel method, all gossypol derivatives tried, as well as gossypol itself, gave upon prolonged boiling a small quantity of silver iodide. When the silver iodide was calculated to methoxyl, an average of 81% of that required for one methoxyl group was obtained. This result was unusual since the oxygen in all the substances investigated was accounted for in forms other than alkoxy groups. It was then demonstrated that the silver iodide was not derived from methyl iodide or from the reagent and, further, that the apparatus was not responsible for it.

These facts were proved by running blank determinations on the reagents

¹ This work was done under a research fellowship supported by the Interstate Cottonseed Crushers' Association.

² Clark, *THIS JOURNAL*, **51**, 1475 (1929).

³ Weishut, *Monatsh.*, **33**, 1165 (1912).

and by using the method of Willstätter and Utzinger⁴ in which the alkyl iodide was caught in trimethylamine and the solubility of the addition product was studied. In performing this experiment, hexa-acetylapogossypol was employed. From analysis it was known that this material gives silver iodide equivalent to 3.9% of methoxyl. With this in mind, a quantity of the acetyl derivative equivalent to 7 mg. of methoxyl was treated in the Zeisel apparatus as described under "Experimental," and the alkyl iodide was collected in trimethylamine solution. No insoluble addition product formed, however, although in a control experiment with a quantity of a known compound equivalent to 3 mg. of methoxyl, sufficient tetramethylammonium iodide was precipitated to make it possible to obtain 10 mg. of the material in a pure dry condition. These facts showed, although indirectly, that the alkyl iodide was not methyl iodide and hence was not derived from a methoxyl group. This does not in itself exclude the possibility of the presence of other alkoxy groups, but their existence seems impossible since all of the oxygen in the gossypol derivative used in this experiment is accounted for as acetyl groups.

In view of these results, the most plausible explanation of this unusual situation is that prolonged heating of these compounds with the reagents used causes the gossypol nucleus to decompose in such a manner as to yield an alkyl iodide. One process by which this could be accounted for is the formation of formaldehyde which, with hydriodic acid, would yield methylene iodide, finally resulting in the production of silver iodide.

In this work it was not feasible to use the ordinary macro-Zeisel method on account of the lack of a plentiful supply of the material. Neither was a strictly micro-Pregl methoxyl determination possible because of not having the necessary equipment, especially a micro-balance. A method based largely upon Pregl's work, however, was adopted in which samples of about 20 mg. were successfully analyzed, employing an ordinary analytical balance sensitive to 0.1 mg. Since this procedure was developed for this investigation, the following description of the apparatus and the technique employed in the determination is given.

Incidentally, by this method the methoxyl content of apogossypol hexamethyl ether, previously reported⁵ as entirely resistant to boiling hydriodic acid, has been determined.

Experimental

Determination of Methoxyl Groups on Samples of Approximately 20 Mg.—The apparatus employed for this purpose is shown with all necessary specifications in Fig. 1.

Approximately 20 mg. of substance upon a balanced piece of cigarette paper 2 by 3 cm. is weighed on an ordinary analytical balance sensitive to 0.1 mg. The paper containing the substance is placed in the bottom of the boiling flask, A, together with a

⁴ Willstätter and Utzinger, *Ann.*, **382**, 148 (1911).

⁵ Clark, *J. Biol. Chem.*, **78**, 165 (1928).

boiling rod.⁶ About 2.5 cc. of melted phenol and 5 cc. of constant boiling hydriodic acid solution of the quality employed in Zeisel determinations are added. The flask is connected by tension springs to the remainder of the apparatus, which consists of the trap B containing a small quantity of red phosphorus, suspended in water, and the receivers C, and D, containing alcoholic silver nitrate. The phosphorus is purified according to the directions of Pregl⁷ and is used in the same manner except that one charge may be employed for four or five determinations. The alcoholic silver nitrate solution is also prepared according to Pregl. Seven cc. are used in C and 4 cc. in D. A slow, uniform stream of carbon dioxide is passed through the capillary side arm of the boiling flask and the liquid is gently boiled by means of a mantled microburner at such a rate

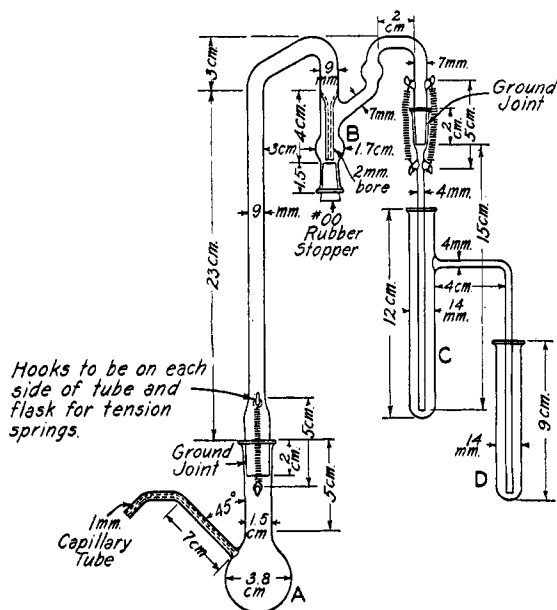


Fig. 1.—Semi-micro Zeisel methoxyl apparatus.

that the vapors of the boiling liquid rise to approximately 8 cm. from the first bend in the air condenser. After about three minutes the white double compound of silver nitrate and silver iodide appears in the first silver nitrate tube. For most substances thirty minutes is sufficient to complete the reaction and sweep out the apparatus. The contents of both the tubes C and D and any precipitate adhering to the adapter are washed into a 150-cc. beaker with approximately 75 cc. of water. The liquid is made acid with nitric acid and allowed to stand on a steam-bath overnight, or until all of the silver iodide has separated and the mother liquors are entirely clear.

⁶ The boiling rod used here is a glass tube approximately 60 mm. long, 3.5 mm. outside diameter, with a 1-mm. bore. It is sealed at one end and also closed about 10 mm. from the other. The open end is fire polished. When this is placed in the flask with the open end down, it will cause uniform boiling indefinitely so long as sufficient heat is constantly applied to the liquid.

⁷ Pregl, "Quantitative Organic Microanalysis," Translated by Fyleman, P. Blakiston's Sons and Company, Philadelphia, 1924, p. 155-156.

The precipitate is transferred to a sintered-glass Pregl filtering tube having a porosity less than 7, in accordance with the technique of Pregl.⁸ After the silver iodide is thoroughly washed, it is dried at 100° to constant weight. Usually half an hour is sufficient for this purpose. When the tubes are being weighed before and after collecting the silver iodide, another tube of the same type and size is always used as a tare.

The apparatus and procedure were tested on pure vanillin and α -methylglucoside. The results obtained are recorded in Table I.

TABLE I
RESULTS OBTAINED IN TESTING THE SEMI-MICRO ZEISEL METHOD

| Substance | Sample, mg. | | AgI, mg. | | Methoxyl, % | | |
|---------------------------|-------------|------|----------|------|-------------|-------|-------|
| | | | | | Calcd. | Found | Found |
| Vanillin | 21.2 | 20.3 | 32.3 | 31.4 | 20.40 | 30.23 | 20.44 |
| α -Methylglucoside | 21.4 | 20.6 | 25.6 | 24.9 | 15.98 | 15.81 | 15.97 |

When these experiments were repeated without phenol, the results were uniformly about 0.5% low.

TABLE II
METHOXYL VALUES OF SOME GOSSYPOL DERIVATIVES AS INFLUENCED BY THE DURATION OF BOILING

| Substance | Sample, mg. | AgI obtained, mg. | Period of boiling, hours | OCH ₃ found, % |
|--|-------------|-------------------|--------------------------|---------------------------|
| Tetramethoxy pseudogossypolone; mol. wt., 546.4; calcd. for 4 OCH ₃ groups, 22.7% of methoxyl | 20.9 | 37.6 | 0.5 | 23.77 |
| | 20.5 | 36.9 | 0.5 | 23.79 |
| | 21.8 | 44.5 | 3 | 27.0 |
| | 21.8 | 44.7 | 3 | 27.1 |
| | 25.4 | 52.7 | 6 | 27.4 |
| Apogossypol hexamethyl ether; mol. wt., 546.5; calcd. for 6 OCH ₃ groups, 34.07% of methoxyl | 21.1 | 54.8 | 0.5 | 34.32 |
| | 20.5 | 53.0 | 0.5 | 34.17 |
| | 20.4 | 58.4 | 4 | 37.83 |
| | 20.3 | 58.0 | 4 | 37.76 |

In Table III data are given showing the quantity of silver iodide obtained from several gossypol derivatives upon prolonged boiling (three to four hours). The oxygen in all of these substances is accounted for in forms other than alkoxy groups.

TABLE III
SHOWING THE YIELD OF SILVER IODIDE OBTAINED FROM SOME GOSSYPOL DERIVATIVES

| Substance | Mol. wt. | Sample, mg. | AgI obtained, mg. | Calcd. to methoxyl, % |
|------------------------------|----------|-------------|-------------------|-----------------------|
| Gossypol | 518.4 | 21.4 | 9.2 | 5.7 |
| Apogossypol hexa-acetate | 714.5 | 20.2 | 5.9 | 3.9 |
| Apogossypolone tetra-acetate | 548.4 | 20.7 | 8.1 | 5.2 |
| Hexa-acetylgossypol | 770.5 | 21.6 | 3.3 | 2.0 |
| Tetra-acetylgossypolone | 618.4 | 20.0 | 5.9 | 3.9 |

From the figures presented before concerning tetramethoxy pseudogossypolone and apogossypol hexamethyl ether, it will be seen that in the former

⁸ Ref. 5, p. 113.

instance three to six hours' boiling gave results from 4.4 to 4.7% high; whereas in the latter case the results were 3.7% high. These quantities, in excess of that demanded by theory, are of the same order of magnitude as those obtained on the products recorded in Table III.

Summary

In the Weishut-Zeisel methoxyl determination, gossypol and some of its derivatives, *although containing no alkoxy groups*, yield upon prolonged boiling a small quantity of silver iodide.

The silver iodide thus formed is responsible in some cases for the abnormally high values obtained in the Zeisel determination of the methoxyl groups in several oxymethylgossypol derivatives.

The explanation has been advanced that under the conditions of the Weishut-Zeisel determination the gossypol nucleus is disrupted in such a manner as to yield an alkyl iodide. This iodide, however, is not methyl iodide.

A description is given of an apparatus and a procedure for the determination of methoxyl groups in samples of the order of 20 mg. In this method an ordinary analytical balance sensitive to 0.1 mg. is employed.

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THE PREPARATION AND PROPERTIES OF THE ISOMERIC HEPTANES. PART I. PREPARATION¹

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I. Introduction

In connection with a study in this Laboratory of certain properties of aliphatic hydrocarbons, it was decided to investigate each of the nine isomeric heptanes. This group includes nearly every type of structure which an aliphatic hydrocarbon may have, and yet is not too large to make its synthesis impracticable.

Only one of the heptanes, the normal, was available in reasonable quantity, from Jeffrey pine oil.² The others were synthesized in considerable quantities by methods described below.

Inspection of the literature showed that the data on physical properties of even normal heptane leave much to be desired, although the work of Kremers and others in recent years has increased considerably our knowledge of this hydrocarbon. Few reliable data were available in the older

¹ Presented at the Symposium on Organic Chemistry, Columbus, Ohio, December 29, 1927.

² E. Kremers, *J. Am. Pharm. Asscn.*, 9, 857 (1920).