TABLE II
PROPERTIES

No.	Color	M. p., °C.	Behavior with concd. H_2SO_4 Cold	Hot	Dilute	Analyses Weight Subs.	Ag hal.	Percentage halogen Calcd.	Found
1	Chestnut-brown	112	Red-brown	Dark brown	Pale straw	0.1856	0.0972	22.96	22.38
2	Buffy-brown	235	Red-brown	Deep brown	Pale straw	.3916	.2238	24.21	24.32
3	Morocco-red	280	Red-purple	Deep purple	Pale pink	.1696	.0882	21.88	21.52
4	Liver-brown	192	Garnet	Dark red	Insol.	.2130	.1263	20.01	20.48
5	Mikado-brown	188	Ruby-red	Deep red	Straw	.2208	.1679[a]	31.46	31.09
6	Deep chrome	+300[b]	Dull brown	Dark brown	Light orange	.2374	.1460[a]	25.71	25.62
7	Diamine brown	189	Deep purple	Deep red	Red-purple	.3162	.2512[a]	32.62	32.48

[a] AgCl + AgBr.
[b] Decomp.

Summary

1. The nitrate, hydrobromide and trichloro-acetate of 2-amino-*p*-cymene were prepared.

2. Aminocymene gave dyes with sulfanilic acid and with *p*-nitroso-dimethylaniline, the latter being a Eurhodine.

3. The sulfanilic acid dye is an excellent raw material for making cymylene-2,5-diamine.

4. The sulfate of 2-amino-5-bromo-*p*-cymene was prepared.

5. New azo dyes were prepared by coupling 2-amino-5-bromo-5-cymene with *o*-cresol, catechol, phloroglucinol, carvacrol and *p*-toluidine. Sulfanilic and anthranilic acids were diazotized and coupled with amino-bromocymene.

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[CONTRIBUTION FROM LABORATORIES OF THE NEW YORK STATE COLLEGE OF FORESTRY AND THE CHEMICAL DEPARTMENT OF SYRACUSE UNIVERSITY]

A NOTE CONCERNING A NEW METHOD FOR THE PRODUCTION OF CELLOBIOSE FROM CELLOBIOSE OCTA-ACETATE

BY F. C. PETERSON AND C. C. SPENCER

RECEIVED JUNE 22, 1927 PUBLISHED NOVEMBER 5, 1927

Cellobiose (or cellose as it was formerly called) is a disaccharide sugar obtained by partial hydrolysis or acetolysis of either cotton or wood cellulose. It is also the product of the action of certain microörganisms on cellulose.

Cellobiose is at present attracting considerable interest in the field of bacteriology. It has been found that cellobiose may be used as a test for the separation of two types of bacteria commonly found in water. Previously the identification of these two types of organisms has been the result of data compiled from correlated tests. Cellobiose, however, is utilized by *A. aerogenes* with acid and gas formation, whereas no changes

are brought about by any strains of *B. coli*. This test is, therefore, specific in differentiating these two types of organisms. When applied to public health work it may thus be used to indicate the presence or absence of fecal pollution.

The literature at present reveals two procedures for the preparation of this sugar. The method given by Skraup and König[1] and published in 1899 is the older of the two, and the second, described by Maquenne and Goodwin[2] and published in 1904, is really a modification of the former method. Both of them depend on the saponification of the octa-acetate of the sugar by means of alcoholic potassium hydroxide, with subsequent isolation of the sugar from the potassium salt by means of an acid (aqueous acetic acid was used by Skraup and König; perchloric acid was used by Maquenne and Goodwin). In either case the solutions after acid treatment were concentrated *in vacuo* to a sirup or solid and the sugar was purified by repeated crystallization from methanol or ethanol.

Experiments in our Laboratories have shown that either of the methods gives satisfactory yields in the preparation of this sugar. Yield figures of purified cellobiose up to 75% were obtained. The procedures involved are, however, long and tedious and cellobiose if prepared commercially in this manner would necessarily have to be quoted at $3.00 or $4.00 per gram. In order that the time factor might be reduced and the yield increased, if possible, other means of preparation were sought.

A procedure has been developed which reduces the time of preparation to approximately one-half that formerly required and yields up to 85% of purified cellobiose have been obtained. Quantitative studies of the method have been confined to the preparation of several batches of this sugar.

The procedure for the newly developed method was as follows.

A 10% solution of sodium ethylate was prepared in 95% alcohol. Ten grams of finely pulverized cellobiose octa-acetate were incorporated into 85 cc. of this solution during a period of one hour. The mixture was constantly agitated by a mechanical stirrer. The reaction took place rapidly, as evidenced by the strong odor of ethyl acetate which was given off almost immediately after the addition of the first portions of the acetate, and was complete in less than one hour. The time limit of one hour was allowed in order that any small lumps of the acetate (resulting from improper pulverization) might be completely saponified. The sodium salt was then collected by filtration, washed with absolute alcohol and dissolved in a minimum volume of water and again filtered. To the resulting aqueous solution glacial acetic acid was slowly added until a precipitate began to form.

Very frequently complete crystallization of the cellobiose took place

[1] Skraup and König, *Monatsh.*, **22**, 1011 (1901).

[2] Maquenne and Goodwin, *Bull. soc. chim.*, **31**, 854 (1904).

within 10 or 15 minutes after the addition of sufficient glacial acetic acid. In other cases it was necessary to allow the mixture to stand overnight in order that complete crystallization might be effected. Apparently somewhat decreased yields result when it becomes necessary to adopt the latter procedure.

The crude cellobiose was collected by filtration, washed with ether and dried at 65°. Purification of the crude sugar was accomplished by dissolving it in a minimum volume of water, filtering and adding acetone to the aqueous solution until reprecipitation was complete. A product of sufficient purity was usually obtained after two such recrystallizations from acetone. Approximately 80% of the acetone was recoverable by fractional distillation. Yields of crude cellobiose as high as 95% have been obtained by the use of this method. Yields of purified sugar as high as 85% have also been obtained. The purified sugar melted at 225° (uncorr.) and gave satisfactory results when analyzed for carbon and hydrogen. Its rotation (in water) was 24.4° at the end of fifteen minutes and 35.2° at the end of 27 hours.

The advantages derived through the use of this method over those previously employed for the preparation of cellobiose from its acetate are as follows:

(1) Yield figures of both crude and purified cellobiose are materially higher than those by the methods of Skraup and König and Maquenne and Goodwin. The yield of purified sugar melting at 225° (uncorr.) has been increased approximately 10%.

(2) The crude cellobiose as it crystallizes from glacial acetic acid is free from any gummy, foreign material. When using the methods employed by the former investigators some gummy material is always associated with the crude cellobiose. Contamination of the crude sugar by this gum increases the number of recrystallizations necessary in order that a product of sufficient purity may be obtained. Inasmuch as each recrystallization is accompanied by a decrease in yield and inasmuch as the number of recrystallizations is less by the use of our method than by those previously employed, this may explain in part the higher yields which we were able to obtain.

(3) The time consumed in the preparation of cellobiose is reduced approximately one-half and in some cases three-quarters of that formerly required.

(4) Needle-shaped crystals of purified cellobiose measuring approximately two millimeters or over in length are consistently obtained. Upon only one occasion during a period of two years have crystals of this size been obtained when following the methods of Skraup and König or Maquenne and Goodwin. These latter methods usually yield needle-shaped crystals of approximately $^1/_{10}$ of one millimeter in length.

(5) Both labor and cost of materials are reduced. This allows cellobiose to be prepared commercially at a figure which is not prohibitive to those laboratories desiring to use it in public health work.

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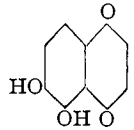
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

HYDROXYNAPHTHOQUINONE STUDIES. VII. THE BROMINATION OF NAPHTHAZARIN

BY ALVIN S. WHEELER AND B. G. CARSON[1]

RECEIVED JUNE 25, 1927 PUBLISHED NOVEMBER 5, 1927

The first study of the bromination of naphthazarin was carried out by one of us and Edwards.[2] The constitution of naphthazarin was regarded at that time as 5,6-dihydroxy-1,4-naphthoquinone but it has now been proved to be 5,8-dihydroxy-1,4-naphthoquinone. Dimroth and Ruck[3]



Old

New

have shown that pyroboro-acetate reacts with naphthazarin to form a diboro-acetate. Therefore, the two hydroxyl groups must be para to each other and not ortho, since this reaction occurs between carbonyl and hydroxyl groups ortho to each other. Pfeiffer[4] has shown that tin tetrachloride forms an addition product with loss of one molecule of hydrochloric acid, such a reaction occurring with hydroxyquinones where the carbonyl is ortho to the hydroxyl group. Many formulas in the literature are now affected by this new formula for naphthazarin.

Bromination of Naphthazarin

Naphthazarin takes up a maximum of four atoms of bromine in hot glacial acetic acid solution. Positions 2, 3, 6 and 7 are undoubtedly occupied (Formula II). This tetrabromo naphthazarin gives a diacetyl derivative (III) and a dianilide (IV) on boiling with aniline. In the latter reaction the aniline may have reacted with the bromine atoms of the quinone ring or with those of the phenol ring. The same doubt exists in regard to the relative mobility of the hydrogen atoms of the two rings in naphthazarin. When two chlorine or bromine atoms are taken up, which ring do

[1] This paper is an abstract of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, at the University of North Carolina, in June, 1927.

[2] Wheeler and Edwards, THIS JOURNAL, **39**, 2460 (1917).

[3] Dimroth and Ruck, *Ann.*, **446**, 123 (1926).

[4] Pfeiffer, *Ber.*, **60**, 111 (1927).