temperature overnight the solvent was evaporated in a stream of air, leaving a clear, colorless sirup (1.20 g.), $[\alpha]^{22}\text{D}$ 37.7°, *c* 2.600 in methanol. Reacetylation of this sirup with 10 ml. of acetic anhydride and 5 ml. of pyridine at room temperature overnight yielded 1.96 g. (96% yield) of the triacetate, m. p. 102–103°.

Periodate Oxidation of 1,2-(1-Methylpentylidene)-D-glucose.—The details of this method have been described elsewhere.[10] The sample was weighed as the triacetate. Wt. of sample, 0.3141 g.; vol. of 0.0442 *N* KIO_4 soln., 50.00 ml.; vol. of 0.1000 *N* sodium arsenite soln., 50.00 ml.; vol. of 0.1152 *N* I_2 soln., 38.20 ml.; KIO_4 consumed, 0.8065 millimole; subst. oxidized, 0.808 millimole; subst.: KIO_4, 1.000: 0.997; acidity, negligible.

Acid Hydrolysis of 1,2-(1-Methylpentylidene)-D-glucose.—One gram of the crystalline triacetate was deacetylated with a trace of sodium methoxide in methanol. After evaporation of the methanol the residue was dissolved in 20 ml. of 1.0 *N* hydrochloric acid and the solution was heated on a steam-bath for two hours. At the end of this time the solution was cooled and neutralized. The neutral solution was extracted with three 10-ml. portions of ether. Distillation of the ether from the extract left about 0.5 ml. of colorless liquid. Treatment with a solution of 2,4-dinitrophenylhydrazine in concentrated hydrochloric acid and alcohol yielded 0.62 g. (88% yield) of 2-hexanone 2,4-dinitrophenylhydrazone, m. p. 108–110°. Melting points reported for this hydrazone ranged from 106–109°.

The neutral aqueous solution remaining after the ether extraction was heated with phenylhydrazine hydrochloride and sodium acetate. After two recrystallizations from alcohol, the resulting fine yellow crystals of D-glucose phenylosazone decomposed at 208°.

1,2-(1-Benzylethylidene)-3,4,6-triacetyl-D-glucose.—Following chromatographic fractionation of the crude reaction product (9.10 g.), the triacetate (2.52 g., 30% yield) was isolated in the form of white rectangular prisms, purified by recrystallization from mixtures of ethyl ether and pentane. The product melted at 78–79°; $[\alpha]^{22}\text{D}$ 29.4°, *c*, 3.566 in chloroform.

Anal. Calcd. for $C_{21}H_{26}O_9$: C, 59.71; H, 6.21; CH_3-CO—, 30.57. Found: C, 59.34; H, 6.20; CH_3CO—, 30.57.

The remaining sirupy fractions of the reaction products were not investigated further. All were optically active; $[\alpha]\text{D}$ ranged from 18–25° in chloroform.

Periodate Oxidation of 1,2-(1-Benzylethylidene)-D-glucose.—The sample was weighed as the triacetate: Wt. of sample, 0.3459 g.; vol. of 0.0442 *N* KIO_4 soln., 50.00 ml.; vol. of 0.100 *N* sodium arsenite soln., 50.00 ml.; vol. of 0.1152 *N* I_2 soln., 38.40 ml.; KIO_4 consumed, 0.818 millimole; subst. oxidized, 0.819 millimole; subst.: KIO_4, 1.000:1.00; acidity, negligible.

Acid Hydrolysis of 1,2-(1-Benzylethylidene)-D-glucose.—Hydrolysis of the sirupy compound in 1.0 *N* hydrochloric acid yielded phenylacetone and D-glucose. The ketone was identified by means of its *p*-nitrophenylhydrazone, m. p. 145–145.5°. D-Glucose was isolated from the hydrolysis mixture and acetylated with acetic anhydride and sodium acetate at 100°. β-D-Glucose pentaacetate, m. p. 129–130°, did not depress the melting point of an authentic sample.

Tetraacetyl-α-D-mannosyl Bromide and Dibutylcadmium.—A yellow colored sirup (5.30 g.), $[\alpha]^{28}\text{D}$ 6.2°, *c* 2.0 in chloroform, resulted from the reaction of the acetohalogenomannose and dibutylcadmium under the usual conditions. No crystalline products could be isolated.

Attempted Coupling of 2-Thiophenemercuric Chloride with Tetraacetyl-α-D-glucosyl Bromide.—2-Thiophenemercuric chloride was prepared in 55% yield.[13] A solution containing 4.00 g. of 2-thiophenemercuric chloride, 4.12 g. of tetraacetyl-α-D-glucopyranosyl bromide and 200 ml. of toluene was refluxed for twenty-four hours. Toluene was then distilled from the mixture until about 50 ml. of solution remained. On cooling to room temperature, 3.96 g. of 2-thiophenemercuric chloride, m. p. 180–183°, crystallized out of solution.

Analyses.—Microanalyses were performed by M. Hines. Analysis for acetyl was made by dissolving the acetate in alcohol, refluxing with alcoholic alkali, then titrating the excess alkali.

Summary

As a result of the generally lower reactivity of organocadmium reagents as compared to that of Grignard reagents, diphenylcadmium coupled with tetraacetyl-α-D-glucopyranosyl bromide and with tetraacetyl-α-D-mannopyranosyl bromide in refluxing toluene solution to yield the respective tetraacetyl-D-glycosylbenzenes in fair yields without attack of the ester functions.

Dibutyl- and dibenzylcadmium behaved differently and unanticipated products of an acetal-like structure resulted. On the basis of alkaline hydrolysis, periodate oxidation and acid hydrolysis data the products were 1,2-alkylidene-triacetylglucoses.

(13) Steinkopf and Killingtad, *Ann.*, **532**, 288 (1937).

EVANSTON, ILLINOIS RECEIVED NOVEMBER 4, 1949

[INVESTIGATION CONDUCTED IN THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF SOUTH DAKOTA, MEDICAL SCHOOL, VERMILLION]

Carbohydrate Tests Using Sulfonated α-Naphthol

BY ARTHUR W. DEVOR[1]

It has been known for a long time that when furfural or hydroxymethylfurfural is treated with sulfuric acid and α-naphthol, a bluish-violet colored condensation product is formed[2–6] (Molisch reaction). Since furfural and hydroxymethylfurfural are formed, respectively, from pentoses and hexoses when these are treated with strong acids, similar color reactions occur when carbohydrate materials are acted upon directly by sulfuric acid in the presence of α-naphthol.

It is probable, moreover, that under the conditions employed normally for carrying out the Molisch reaction, there is also action of sulfuric acid upon α-naphthol so that colored condensation products are actually composed of various sulfonated naphthols in combination with furfural derivatives.

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(1) Present address: Department of Chemistry, South Dakota State College, Brookings.

(2) W. Alberda Van Ekenstein and Blanksma, *Ber.*, **43**, 2355–2361 (1910).

(3) Blanksma, *Chem. Weekblad*, **6**, 1047 (1910).

(4) F. Angelico and A. Cappola, *Gazz. chim. ital.*, **42**, II, 583 (1912).

(5) Bredereck, *Ber.*, **64B**, 2856 (1931).

(6) Bredereck, *ibid.*, **65B**, 1110 (1932).

The four objectives of the present work were: (1) to make a more precise spectrophotometric study of the colored products formed in the Molisch reaction as carried out in the conventional manner with various carbohydrate materials and furfural; (2) to compare, spectrophotometrically, the products formed by such conventional methods with those formed when the naphthol was *presulfonated* by standing in contact with sulfuric acid before use; (3) to compare, spectrophotometrically, the products formed as described in (1) and (2) with the colored products obtained when pure preformed α-naphthol-2-sulfonate, α-naphthol-4-sulfonate, or α-naphthol-5-sulfonate was employed; (4) to determine by such means the feasibility of using some modification of the Molisch reaction for identification and quantitative estimation of small amounts of carbohydrate materials. It was considered probable that such presulfonation of naphthol would eliminate variations due to minor differences in concentrations of reagents, temperatures, times of heating, etc., and thus produce more strictly reproducible results.

Apparently there is no previous report of the use of sulfonated α-naphthol in carbohydrate tests and few investigators have considered the use of the Molisch reaction for quantitative determinations.[7,8]

Experimental

Materials.—The α-naphthol and the specific sulfonates of α-naphthol were purchased from the Eastman Kodak Company. The α-naphthol was C. P.; the 2- and 4-sulfonates were purified products with sodium chloride as the principal impurity while the 5-sulfonate was purified by the author. Chemically pure Pfanstiehl carbohydrates were used and the furfural was a high quality product furnished by the Quaker Oats Co. of Chicago.

Preparation of Reagents

α-Naphthol-sulfuric Acid Solution.—Enough α-naphthol was dissolved in concentrated sulfuric acid to make a concentration of 0.400 g. per 100 ml. of solution and used within ten minutes after preparation.

Sulfonated α-Naphthol in Sulfuric Acid.—Prepared the same as the α-naphthol–sulfuric acid solution and allowed to stand in the dark at room temperature for at least eight hours before using.

Water Solution of Sulfonated α-Naphthol.—Four grams of C. P. α-naphthol was dissolved in 10 ml. of concentrated sulfuric acid and allowed to stand in the dark for eight days. At the end of this period sulfonation was usually complete and the reaction mixture was dissolved in water, diluted to 200 ml. and thoroughly mixed. If turbid, on account of incomplete sulfonation, the water solution was filtered through fritted glass.

Methods

Procedure I (action of α-naphthol).—Five ml. of freshly prepared α-naphthol–sulfuric acid solution was added to a test-tube containing 2 ml. of the carbohydrate solution. The reaction mixture was continually agitated during the addition of the α-naphthol reagent and was allowed to remain hot for one-half minute, then cooled in a mixture of crushed ice and water until the spectrophotometric readings were made. Within forty-five minutes after the tube was cool, the optical densities were measured between wave lengths of 340 and 850 mμ in a Beckman spectrophotometer with 1-cm. tubes. A blank using water in place of the carbohydrate solution was used as a reference mixture. At all times, the reaction mixture was kept in dim light and exposure to sunlight was avoided.

Procedure II (action of sulfonated α-naphthol) was the same as Procedure I except for the fact that the sulfuric acid–α-naphthol solution had been allowed to stand long enough for presulfonation to occur and heating of the reaction mixture was continued for ten minutes in a boiling water-bath.

Procedure III (action of sulfonated α-naphthol).—Five ml. of C. P. concentrated sulfuric acid was added to 2 ml. of water solution containing 20 mg. of the sulfonated α-naphthol and the carbohydrate (amount of carbohydrate given under description of figures). Otherwise, the procedure was the same as Procedure II.

Procedure III was used for comparing the action of the specific sulfonates with presulfonated α-naphthol while Procedure II was employed for comparative studies with the Molisch reaction.

Results and Discussion

The Action of α-Naphthol.—The optical densities of the colored products are plotted against wave length as shown in Figs. 1, 2, 3, 4, 5, 6 and 7. When treatment was with α-naphthol, two major absorption bands were observed for D(+)-galactose (Fig. 1), D(+)mannose (Fig. 2), L(+)-arabinose (Fig. 5), and D(+)-xylose (Fig. 6), while D(+)-glucose (Fig. 3), D(−)-fructose (Fig. 4) and furfural (Fig. 7) yielded products with one principal absorption band. The peaks for the hexoses were observed around 486 to 490 mμ and 572 to 573 mμ but for the pentoses, the peaks were around 488 to 496 mμ and 546 to 550 mμ. The absorption band at the shorter wave length was slight for fructose, glucose, starch, glycogen and furfural. Fructose gave the most rapid reaction (Fig. 4).

Action of Random-presulfonated α-Naphthol.—When sulfonated α-naphthol was used in place of α-naphthol, the reaction was smoother, slower and much more consistent. It should be noted in Figs. 1, 2, 5 and 6 that the sulfonated α-naphthol yielded products which do not give the absorption maximum at the shorter wave length and show greater absorption at the longer wave length than did the products from the α-naphthol reaction. The products from fructose and from furfural show almost the same absorption as did the products obtained from the action of α-naphthol. This is probably because intermediate compounds from fructose do not react with α-naphthol and, of course, furfural reacts directly.

The Action of Specific α-Naphthol Sulfonates.—The results obtained from the 2-, the 4- and the 5-α-naphthol sulfonates are compared with the results obtained from the random-presulfonated product and are illustrated in Fig. 8. Only the results from Procedure III are given here while all the other figures show the results from Procedures I and II. The lower absorption from the 2- and 4-sulfonates is due largely to the lower molecular concentration of the reagent. When sodium chloride was added to the sulfonated α-naphthol, little or no change was observed in the

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[7] Yamahuzi, Yosida and Hukuura, *Biochem. Z.*, **308**, 128 (1941).

[8] Krainick, *Mikrochemie ver. Mikrochim. Acta*, **29**, 45 (1941).

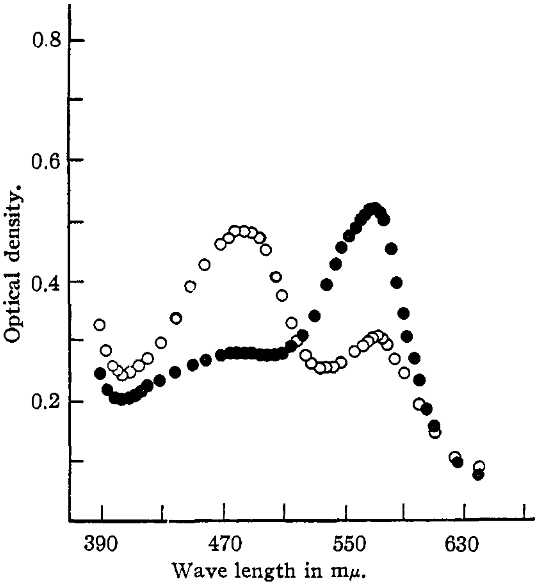

Fig. 1.—Absorption spectra (1-cm. cell) of the colored products formed when 50γ of D(+)-galactose was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

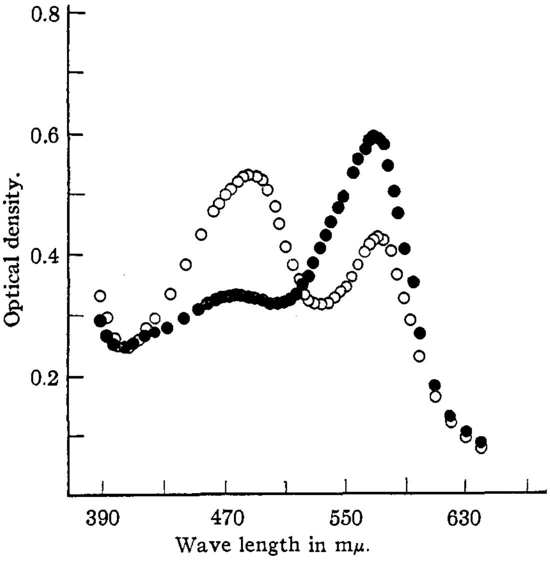

Fig. 2.—Absorption spectra (1-cm. cell) of the colored products formed when 50γ of D(+)-mannose was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

results. These results show that the reactions are probably with the 2- and 4-sulfonic acids as one might expect.

Use for Qualitative Tests.—From the results in Figs. 1 to 8, one can see that the random presulfonated α-naphthol would offer advantages when used *in* a general carbohydrate test according to the method designated as Procedure II.

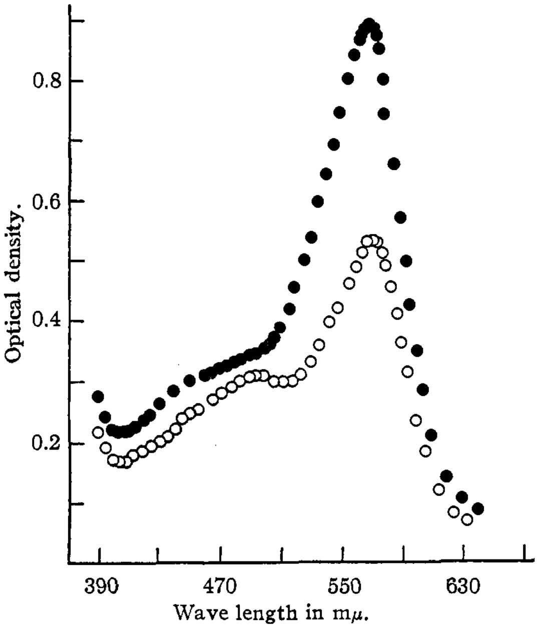

Fig. 3.—Absorption spectra (1-cm. cell) of the colored products formed when 50γ of D(+)-glucose was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

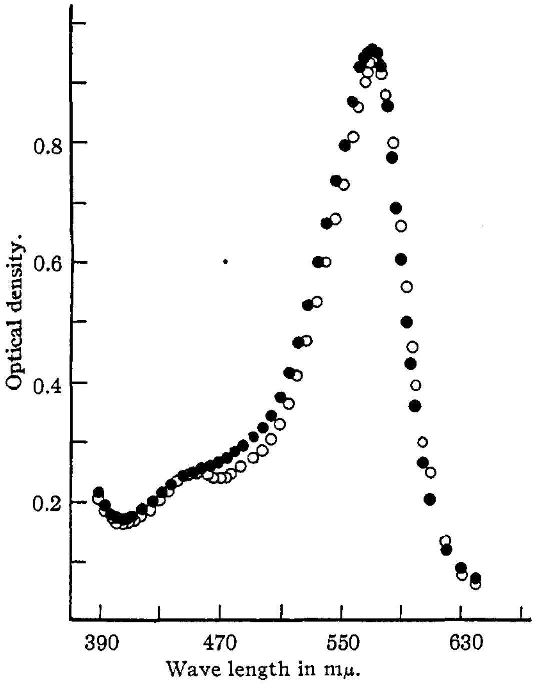

Fig. 4.—Absorption spectra (1-cm. cell) of the colored products formed when 50γ of D(−)-fructose was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

The advantages of this modified test over the usual Molisch reaction are: (1) sulfonated α-

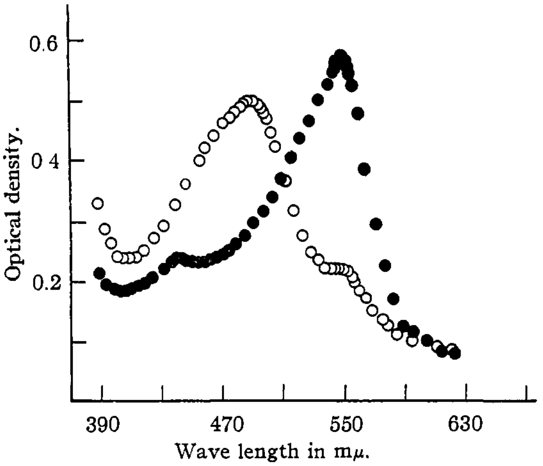

Fig. 5.—Absorption spectra (1-cm. cell) of the colored products formed when 40γ of L(+)-arabinose was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

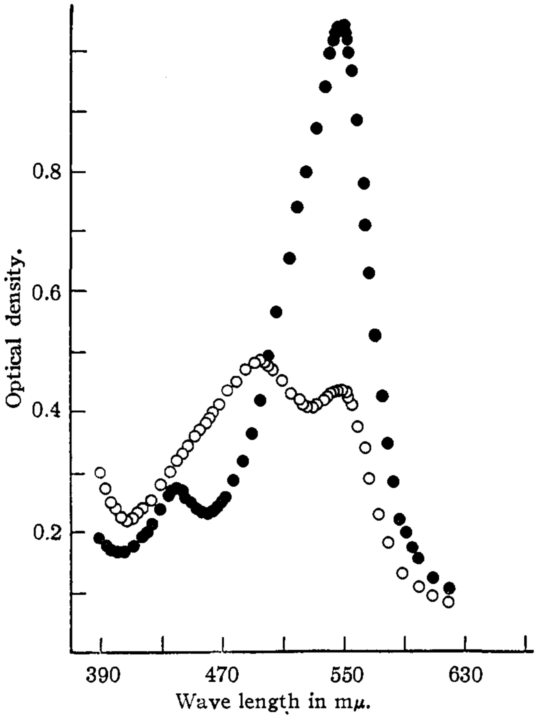

Fig. 6.—Absorption spectra (1-cm. cell) of the colored products formed when 40γ of D(+)-xylose was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

naphthol is soluble in water; (2) the color is more definite and one need not use the ring test; (3) an aqueous solution of sulfonated α-naphthol does not darken in the light as does an alcohol solution of α-naphthol; (4) water insoluble materials, such as cotton, dissolve as the sulfuric acid is added.

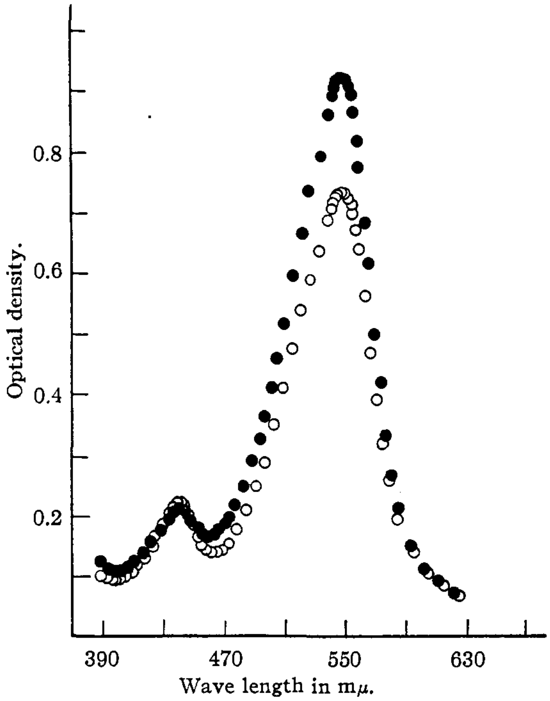

Fig. 7.—Absorption spectra (1-cm. cell) of the colored products formed when 19γ of furfural was treated with: ○, α-naphthol–sulfuric acid mixture (Procedure I); and ●, with random-presulfonated α-naphthol (Procedure II).

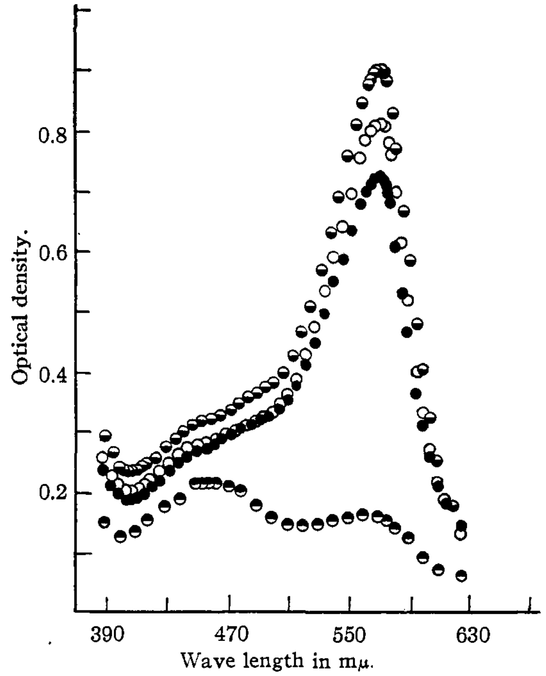

Fig. 8.—Absorption spectra (1-cm. tubes) of the colored products formed when 50γ of D(+)-glucose was treated as described under Procedure III: ◒, random-presulfonated α-naphthol; ○, 2-sodium sulfonate; ●, 4-sodium sulfonate; ◓, 5-sodium sulfonate (each of α-naphthol).

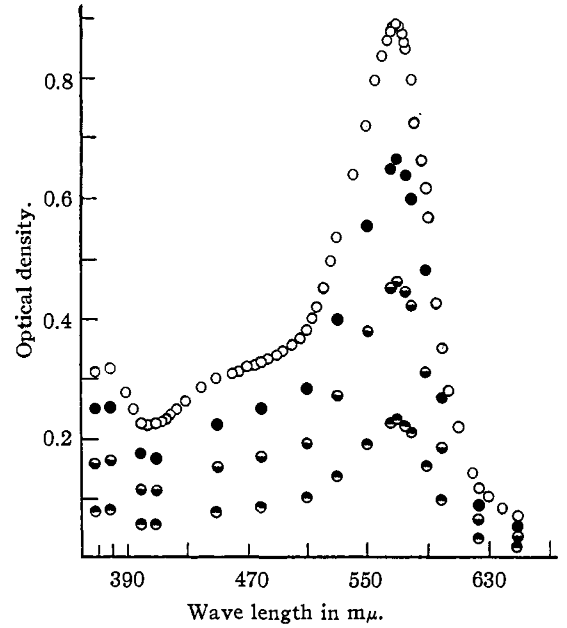

Fig. 9.—Absorption spectra (1-cm. tubes) of the colored products formed when glucose was treated according to Procedure II (using random-presulfonated α-naphthol): ○, 50γ; ●, 37.5γ; ◒, 25γ; and ◓, 12.5γ of glucose.

The test may be used along with the α-naphthol and orcinol tests[9,10,11] as an aid in distinguishing between various carbohydrates. This use was tried and proven successful with milk (lactose) and for glucose and galactose cerebrosides.

The speed of the reactions is an important factor in identifying a monosaccharide.

Use for Quantitative Estimation.—As illustrated in Fig. 9, the reaction is additive and the most accurate estimation can be made at the absorption maximum. The carbohydrates in milk, cerebrosides and in blood filtrates give excellent results. As little as 10 γ of carbohydrate can be accurately estimated by this simple, rapid procedure. The orcinol reaction[9,10,11] has proven valuable for some of these purposes but the sulfonated α-naphthol reaction will be of further aid. It is necessary to use a standard for each determination because the size of the test-tubes used and the room temperature are important factors.

Interfering Substances.—It has been previously reported[12] that α-naphtholsulfuric acid gives color reactions with aldehydes. All four of the aldehydes tried in the present investigation gave an absorption maximum in the violet region and very little absorption above 540 mμ. Blood filtrates show an increased absorption at the shorter wave lengths but there seems to be no interference at the absorption maximum for hexoses.

Summary

The use of random-presulfonated α-naphthol (Procedure II) offers a more satisfactory method for a general carbohydrate test than the usual Molisch reaction. This new test can be used as an aid in identifying carbohydrate groups and offers a simple, rapid procedure for estimating small amounts of carbohydrates. A solution of sulfonates of α-naphthol does not darken on standing and is made up in water solution which is not possible for α-naphthol itself.

[9] Sørensen and Haugaard, *Compt. rend. trav. lab. Carlsberg*, **19**, No. 12, 1 (1933).

[10] Sørensen, *Biochem. Z.*, **269**, 271 (1934).

[11] Sørensen, *Compt. rend. trav. lab. Carlsberg. ser. chim.*, **21**, No. 8, 123 (1936).

[12] Ekkert, *Pharm. Zentralh.*, **68**, 563 (1927).

BROOKINGS, SOUTH DAKOTA RECEIVED MARCH 4, 1949

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF SCHERING CORPORATION]

Quaternary Carbon Compounds. IV. N-Trisubstituted Alkyl Pyridine Carboxamides as Antispasmodic Agents[1]

BY NATHAN SPERBER, DOMENICK PAPA AND ERWIN SCHWENK

In several recent publications, the pharmacological action associated with quaternary carbon compounds has been demonstrated. Trialkylacetamides[2] ($R' = CONH_2$), trialkylethylamines[3] ($R' = CH_2NH_2$), trialkylacetic acids[4] ($R' = COOH$) and trialkylcarbinamines[5] ($R' = NH_2$) of the general formula R_3C-R' (I), wherein R_3 are alkyl groups of 3–5 carbon atoms and total 12–15 carbon atoms, have pronounced musculotropic, but rather feeble neurotropic, antispasmodic activity. In view of the known pharmacological and clinical action of pyridine carboxamides,[6] it appeared of interest to synthesize aryl and heterocyclic acid amides embodying the quaternary carbon moiety of I. This communication describes the synthesis and preliminary pharmacological data of a series of N-trisubstituted alkyl pyridine carboxamides[7,8] of the

[1] Presented in abstract before the Division of Medicinal Chemistry of the American Chemical Society at Atlantic City, September 20, 1949.

[2] Junkmann and Allardt, U. S. Patent 2,186,976, Jan. 16, 1940.

[3] Allardt and Junkmann, U. S. Patent 2,361,524, Oct. 31, 1944.

[4] Sperber, Papa and Schwenk, THIS JOURNAL, **70**, 3091 (1948).

[5] Sperber and Fricano, *ibid.*, **71**, 3352 (1949).

[6] Nicotinamide (Niacinamide) and N,N-diethylnicotinamide (Coramine) are representative of clinically effective pyridine carboxamides.

[7] Billman and Rendall, THIS JOURNAL, **66**, 540 (1944), prepared a series of N-substituted pyridine carboxamides and amides of pyrazine monocarboxylic and pyrazine 2,3-dicarboxylic acids. They reported that "the benzylamide of nicotinic acid possessed pronounced antispasmodic activity."

[8] Badgett, Prevost, Ogg and Woodward, *ibid.*, **67**, 1136 (1945),