TABLE IV

PHYSICAL CONSTANTS AND YIELDS OF OLEFINS

Olefin	Yield, %	B.p.,[a] °C.	Press., mm.	$n^{20}D$	d^{20}_4	MR_D Obsd.	MR_D Calcd.
1-Hexene[b]	27	63	742	1.3862	0.6740	29.29	29.44
1-Heptene[b]	77	93	748	1.3990	.7004	33.84	34.06
4-Octene[c]	66	127.0	746	1.4128	.7184	38.86	38.68
3-Nonene[c]	74	147.4	750	1.4173	.7294	43.49	43.30
5-Decene[c]	77	169.6	746	1.4249	.7385	48.47	47.91
7-Me-3-octene[c]	77	140.7	746	1.4168	.7278	43.37	43.30
8-Me-4-nonene[c]	70	163.2	746	1.4229	.7400	48.16	47.91
5-Undecene[c]	86	191.2	750	1.4295	.7516	52.88	52.53
Isostilbene	87	133	8	1.6083	1.0143	60.73	59.18
2-Me-2-hydroxy-3-octene	50	99.4	50	1.4427	0.8387	44.84	45.92
β-Me-styrene	50	166.7	746	1.5420	.9088	40.89	39.69

[a] All boiling points except that for isostilbene were determined by means of the micro-Cottrell apparatus of Willard and Crabtree, *Ind. Eng. Chem., Anal. Ed.*, **8**, 79 (1936). [b] Schmitt and Boord, THIS JOURNAL, **54**, 751 (1932), record the following data: 1-hexene, b. p. 64° (760 mm.), $n^{20}D$ 1.3858, d^{20}_4 0.6732; 1-heptene, b. p. 95° (760 mm.), $n^{20}D$ 1.3999, d^{20}_4 0.6993. [c] These olefins have not been described previously.

and chloroplatinic acid were investigated in the reduction of amylacetylene and dibutylacetylene, as typical examples of mono- and dialkylacetylenes. In the case of amylacetylene, there was no promoting effect when chloroplatinic acid alone was used as promoter, but rather a slight retarding effect was noticed. When alkali and chloroplatinic acid were used together, there was some promotion observed (Fig. 2). In the case of dibutylacetylene, the use of alkali and chloroplatinic acid together resulted in no significant promotion (Fig. 2). The amounts of promoter, catalyst and unsaturated compound used were the same as in the work of Reasenberg, Lieber and Smith,[5] but the pressure was about four atmospheres, rather than one atmosphere.

TABLE V

ANALYTICAL DATA FOR THE NEW OLEFINS AND ACETYLENES

Compound	Calculated % C	Calculated % H	Found % C	Found % H
Et-*i*-Am-acetylene	87.01	12.99	86.95	13.13
Pr-*i*-Am-acetylene	86.87	13.13	86.71	13.31
Bu-*n*-Am-acetylene	86.77	13.23	86.58	13.45
4-Octene	85.62	14.38	85.45	14.61
5-Decene	85.62	14.38	85.80	14.55
3-Nonene	85.62	14.38	85.39	14.47
7-Me-3-octene	85.62	14.38	85.76	14.58
8-Me-4-nonene	85.62	14.38	85.89	14.42
5-Undecene	85.62	14.38	85.73	14.57

Summary

1. The reduction of mono- and disubstituted acetylenes in the presence of Raney nickel has been studied, and a relation shown to exist between the symmetry of the acetylene molecule and the course of the reduction.

2. Several new olefins have been prepared by the half reduction of dialkylacetylenes. The half and complete reduction of acetylenes by means of Raney nickel is a feasible method of preparing certain olefins and saturated hydrocarbons.

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, UNITED STATES PUBLIC HEALTH SERVICE]

The Conversion of Starch to Crystalline Dextrins by the Action of a New Type of Amylase Separated from Cultures of *Aerobacillus macerans*[1]

BY E. B. TILDEN AND C. S. HUDSON

It was first shown by Schardinger[2] that the cultivation of *Aerobacillus macerans* upon starch solutions produces a mixture of water-soluble dextrins, from which two distinct, non-reducing, crystalline compounds may be readily isolated. Subsequent work by other investigators has not demonstrated with certainty whether these crystalline dextrins represent comparatively simple components of starch itself, or whether they are formed as the result of synthetic activity of the living organism. In the latter case they would seem to be of little importance to the study of the constitution of starch. We now find, however, that when *Aerobacillus macerans* is grown for several weeks upon a potato medium, and the culture fluid is then filtered through a Berkefeld N filter to remove the microörganisms, the filtrate contains an enzyme which will produce the Schardinger dextrins from starch rapidly, and in greater yield than has been previously reported. We infer from this fact that the crystalline dextrins are components of the starch structure, or are closely related to such components.

During the course of the action of the enzyme upon starch there is no significant change in reducing power or optical rotation. In the present study, therefore, the progress of the reaction was followed by measuring the rapid and substantial decrease in viscosity, and also by means of a

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) F. Schardinger, *Zentr. Bakt. Parasitenk.*, Abt. II, **22**, 98 (1908); **29**, 188 (1911).

simple microscopic test based upon Schardinger's observation that the crystalline alpha and beta dextrins form characteristic crystalline compounds with iodine. By the use of these tests it has been found that the activity of the culture filtrate is usually such that 0.5 ml. will convert 30 mg. of starch in a total volume of 1.5 ml. in thirty minutes at 40°. At 30° the digestion requires twice this period and at 20° four times; at 50°, however, the time taken for digestion is more than half that required at 40°, which indicates that some inactivation has occurred at the higher temperature. The enzyme is active, under the conditions employed, over a *p*H range of 5.6 to 6.4. It is precipitated along with other material by 37.5% acetone in the cold, and can be extracted from this precipitate with one-tenth the original volume of water. Considerable purification is thereby effected, and the activity of the enzyme is unimpaired.

Experimental

Microscopic Iodine Test.—To a drop of 0.1 *N* iodine solution on a spot plate is added 3 drops of the digest. A small quantity of the mixture is transferred with a platinum loop to a microscopic slide. The crystalline iodine compounds may begin to form at once near the edge of the drop, but the progress of the conversion is indicated from the quantity of these crystals that are observed after complete evaporation of the droplet on the slide. Under the microscope the crystals are seen to be a mixture of the green needles and dark blue hexagons characteristic of alpha dextrin, together with the brown prisms formed from beta dextrin. In control tests made upon digests of starch with other amylases, *e. g.*, that of *Aerobacillus polymyxa*, from which the main products are reducing substances, these crystalline iodine compounds were not formed, and the microscopic appearances were entirely different from those observed in the case of the *macerans* digests. This iodine test therefore seems to be specific for the products of the latter enzyme action.

Preparation of the Enzyme.—The potato medium consists of 100 g. of sliced raw potato and 10 g. of calcium carbonate per liter of water. After autoclaving for about an hour at 125° it is cooled and inoculated with a few drops of an active culture of *Aerobacillus macerans*. The new culture is allowed to develop for several weeks at 38°, and the fluid portion is then passed through a Berkefeld N filter. A sample of filtrate which had been kept sterile and cold retained much of its activity for a period of three months.

Details of an Experiment.—A paste containing 57.2 g. of potato starch (equivalent to 50 g. of anhydrous starch) in 2 liters of water was autoclaved at 125° for one hour, cooled to 40°, 100 ml. of a sterile culture filtrate was added under aseptic conditions, and the mixture was diluted with sterile water to give a starch concentration of 2%. The entire experiment was carried out at 40°. Preliminary tests had shown that under the conditions described the digestion should be complete in eight hours. A 250-ml. portion of the mixture was removed aseptically for use in obtaining data on the progress of the reaction, leaving 45 g. of starch in the main body of the solution which was to be used for isolation of the crystalline dextrins.

The change in viscosity was observed at 40° by means of an Ostwald viscosimeter which had an outflow time of ninety seconds for water at this temperature. The initial viscosity of the digest was assumed to be the same as that observed with a separate solution prepared from a culture filtrate which had been previously inactivated by boiling. The outflow time for this solution was 522 seconds, and by expressing viscosity in terms of the ratio t_s/t_w, where t_s is the outflow time for the digest sample, and t_w that for water, the initial viscosity of the digest was 5.80. In one hour the viscosity of the digestion mixture had dropped to 2.62, although the iodine test still showed a blue color, and no crystals were present. In two hours the viscosity was 1.95, the color with iodine was blue-violet, and a few crystals characteristic of Schardinger's iodine compounds had appeared. After four hours the viscosity was 1.11, the color with iodine was brownish-violet, and there was an abundance of the crystalline iodine compounds. By this time the solution had become water-clear, and its rotation in a 2-dm. tube was +8.4°. After six hours the viscosity had become constant at 1.02 and the rotation at +8.1°. The final specific rotation was therefore $[\alpha]D$ +203°. During the next eight hours there was no change except in the color with iodine, which gradually became nearly a pure brown. Tests for reducing power were made at hourly intervals upon 5-ml. samples of the digest by means of the Shaffer–Hartmann microtechnique[3] and negligible values were obtained at all times. The final reducing power was found to be less than that of a 0.01% solution of glucose. The *p*H, which was initially 6.4, remained constant.

Isolation of the Crystalline Dextrins.—The procedure was that of Schardinger as modified by Lange,[4] who introduced trichloroethylene as a precipitating agent for the crystalline dextrins. The yield of crude product, calculated to the dry basis, was 18 g. or 40%. From this mixture the beta dextrin has been isolated and identified; the remaining material apparently consists largely of the alpha dextrin.

Summary

It is shown that the aqueous fluid from cultures of *Aerobacillus macerans* contains an enzyme which converts gelatinized starch to a high rotating ($[\alpha]D$+203) mixture of non-reducing dextrins, of which the crystalline alpha and beta dextrins of Schardinger are components, without the production of maltose, glucose or other reducing sugar. We infer, therefore, that these dextrins are not products of the synthetic metabolism of

(3) P. A. Shaffer and A. F. Hartmann, *J. Biol. Chem.*, **45**, 365 (1920–21).

(4) H. Pringsheim, "Chemistry of the Monosaccharides and of the Polysaccharides," McGraw-Hill Book Co., Inc., New York, 1932, p. 280.

the organism but are either the true components of starch or are closely related to such true components. The research is being continued.

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Triarylcarbinols. VII.[1] 4'-Dimethylaminobiphenyldiphenylcarbinol and its Relation to the Theory of Color of Dyes

BY AVERY A. MORTON AND WILLIAM H. WOOD

This paper is concerned with the structure of triphenylmethane dyes, particularly with the effect of a dimethylamino group substituted in the 4'-position of a biphenyl nucleus. It will be recalled that Dilthey[2] and Wizinger[3] have abandoned the conventional quinoid formula for dyes in favor of a carbenium, frequently called carbonium, structure in which the central carbon atom is given preference over nitrogen as the seat of basicity. Contrasting models for crystal violet are shown below.

$$[((CH_3)_2C_6H_4)_3C^+]X^-$$

$$(CH_3)_2N^+=\langle\rangle=C(C_6H_4N(CH_3)_2)_2$$
$$X^-$$

The argument that the positions of nitrogen and carbon in the periodic table should favor a nitrogen base is met by claiming that the basic influence of the dimethylamino group is propagated through the benzene ring to a centrally located and basically enhanced carbon atom so that the latter becomes a superior base. In this scheme the coördinately unsaturated carbon atom is the chromophore which on conversion to the ionized state becomes colored. The dimethylamino group is the auxochrome.

We have applied this view to a system where the two competing, though vinylogously connected, points are separated by an additional phenyl nucleus in order to note, in a quantitative way, whether changes in basicity of the carbonium group are paralleled by changes in the properties of dyes. The sequence of reasoning underlying this work begins with the established fact that replacement of phenyl by a biphenyl nucleus enhances halochromism of triarylcarbinols and therefore by general opinion the basicity of the central carbon atom. This effect is well demonstrated with triphenyl- and trixenylcarbinol. While quantitative comparisons are difficult to make, the latter compound can be estimated qualitatively from colorimetric data of Ziegler and Boye[4] to be over five times stronger than triphenylcarbinol. Moreover, trixenylcarbinol becomes colored in sulfuric acid–acetic acid solution which is about one hundredth the concentration needed to produce color with triphenylcarbinol.[5] There is a definite shift in wave length from 414 to 542 mμ for the larger carbinol. As far as color can be used as the criterion of basicity there is no doubt but that substitution of phenyl by xenyl intensifies this property.

The second link in the chain of arguments is that substituents in the 4'-position of the biphenyl nucleus have their influence propagated through two phenyl nuclei, thus further enhancing the basicity of the central carbon atom. For example, trimethyltrixenyl- and trimethoxytrixenyl-carbinols previously[5] have been observed to show color in acid which is approximately one-tenth and one-hundredth, respectively, the strength needed for trixenylcarbinol. There is also a shift in wave length from 542 for the unsubstituted compound to 575 and 638 mμ for the two substituted compounds, respectively. The evidence is conclusive that there is a definite transfer of influence of substituents over two phenyl bridges and that marked changes in basicity of the central carbon atom can be observed. Indeed, as shown in competitive titrations, the basicity of trimethoxytrixenylcarbinol is not far below that of a dimethylamino group present in a compound of the triarylmethane type.

It then appears reasonable that a dimethylamino substituent in the 4'-position of a carbinol

(1) Previous papers on triarylcarbinols are: Morton and Stevens, THIS JOURNAL, **53**, 2244, 4028 (1931); Morton and Peakes, Jr., *ibid.*, **55**, 2110 (1933); Clapp and Morton, *ibid.*, **59**, 2074 (1937); Morton and Emerson, *ibid.*, **59**, 1947 (1937); **60**, 284 (1938).

(2) Dilthey and Wizinger, *J. prakt. Chem.*, [2] **118**, 321 (1928).

(3) Wizinger, "Organische Farbstoffe," Ferd. Dümmlers Verlag, Bonn, 1933.

(4) Ziegler and Boye, *Ann.*, **458**, 229 (1927).

(5) Morton and Emerson, THIS JOURNAL, **60**, 284 (1938).