TABLE I

NUCLEAR HALOGENATED α,α-DIHYDROXYMALONANILIDE AND INTERMEDIATES

Derivative	Formula	M. p., °C.	Yield, %	Analyses, % Carbon Found	Carbon Calcd.	Hydrogen Found	Hydrogen Calcd.	Nitrogen Found	Nitrogen Calcd.
Nuclear Halogenated Isonitrosomalonanilide (II)									
4,4'-Dichloro	$C_{15}H_{11}N_3O_3Cl_2$	215	80	51.5	51.2	3.0	3.2	11.8	11.9
2,2',4,4'-Tetrachloro	$C_{15}H_9N_3O_3Cl_4$	215	67	42.7	42.8	2.0	2.1	10.1	10.0
4,4'-Dibromo	$C_{15}H_{11}N_3O_3Br_2$	226	38	41.0	40.8	2.6	2.5	9.4	9.5
N-Methyl Ether of Nuclear Halogenated Isonitrosomalonanilide (III)									
4,4'-Dichloro	$C_{16}H_{13}N_3O_3Cl_2$	185	69	52.7	52.5	3.6	3.6	11.8	11.5
2,2',4,4'-Tetrachloro	$C_{16}H_{11}N_3O_3Cl_4$	198	83	44.7	44.2	2.6	2.6	9.6	9.7
4,4'-Dibromo	$C_{16}H_{13}N_3O_3Br_2$	186	44	42.5	42.2	2.8	2.9	9.0	9.2
Nuclear Halogenated α,α-Dihydroxymalonanilide (IV)									
4,4'-Dichloro	$C_{15}H_{12}N_2O_4Cl_2$	208	21	51.0	50.7	3.2	3.4	8.2	7.9
2,2',4,4'-Tetrachloro	$C_{16}H_{10}N_2O_4Cl_4$	190	19	42.7	42.5	2.2	2.4	6.7	6.6
4,4'-Dibromo	$C_{16}H_{12}N_2O_4Br_2$	166	50	40.6	40.5	2.6	2.7	6.4	6.3
O-Methyl Ether of 4,4'-Dibromo-isonitrosomalonanilide (V)									
	$C_{16}H_{13}N_3O_3Br_2$	219	10	42.3	42.2	3.1	2.9	8.8	9.2

hydrogen chloride was passed through for one hour. The yellow solid gave way to a white precipitate. The solid (IV) was filtered off and crystallized from glacial acetic acid. The chloroform filtrate contained an additional quantity of IV. This was recovered by removing the solvent under reduced pressure on the water-bath and crystallizing the residue from glacial acetic acid.

In the preparation of 4,4'-dibromo-α,α-dihydroxymalonanilide a second compound was isolated in 16% yield from the chloroform filtrate. After removing the solvent under reduced pressure on the water-bath, the residue was crystallized from alcohol. There was obtained as colorless crystals a compound melting at 219°. Its chemical composition indicated the conversion of some N-methyl ether to the O-methyl form (V). This conversion was similar to that reported by Plowman and Whitely[1] in the preparation of mesox *p*-toluidide.

CHEMICAL CORPS TECHNICAL COMMAND
ARMY CHEMICAL CENTER, MD. RECEIVED APRIL 14, 1948

Branched Structure of Guaran

BY ROY L. WHISTLER, TSIANG KWANG LI AND WILLIAM DVONCH

The principal polysaccharide of guar endosperm has been shown to be homogeneous[1] as to composition and to consist of anhydrogalactose and anhydromannose units in the ratio of one to two.[1,2] For convenience in designation, this particular polysaccharide has been given the name guaran.[1,3] Its triacetate can be formed into strong films which can be elongated 550% and thereby become birefringent, but they do not develop crystallinity which can be detected by X-ray examination. This has been taken to indicate the presence either of linear molecules in which a random distribution of anhydrogalactose and anhydromannose units occur or of a chain predominantly linear but with branches of very short length.[1] Recently Swanson[4] has shown that the galactose units occur principally as chain ends since from the methylated polysaccharide approximately 90% of the galactose can be recovered as 2,3,4,6-tetramethylgalactose. Consequently, one galactopyranoside end unit occurs for approximately two anhydromannose units. On oxidation of guaran with periodate Moe, Miller and Iwen[2] found that one mole of oxidant is consumed for each anhydrosugar unit present. This led them to conclude that the polysaccharide contained 1,4-glycosidic units although the possibility of branching was not eliminated.

The structure suggested by Swanson[4] is supported by work reported here on the periodate oxidation of guaran. In agreement with Moe, Miller and Iwen it is found that one mole of oxidant is consumed for each anhydrosugar unit present; but, in addition, it is found that by use of the oxidation method of Hirst, *et al.*,[5] one mole of formic acid is produced for approximately 2.7 anhydroglycosidic units. This indicates a large number of pyranosidic non-reducing end units or about one for two units of the main chain. Hence, one way to account for both the yield of formic acid and the fact that the polysaccharide consumes but one mole of oxidant for each anhydroglycosidic unit is to assume that guaran consists predominantly of a chain of anhydroglycosidic units one-half of which bear a glycopyranoside unit in conformity with the structure proposed by Swanson. Such a structure would require that on periodate oxidation the single unit side chains are split twice with the formation of one molecule of formic acid, that one-half of

(1) E. Heyne and R. L. Whistler, THIS JOURNAL, **70**, 2249 (1948).

(2) O. A. Moe, S. E. Miller and M. H. Iwen, *ibid.*, **69**, 2621 (1947).

(3) R. L. Whistler, *Chem. Ind.*, **62**, 60 (1948).

(4) W. Swanson, paper presented before the Division of Sugar Chemistry and Technology at the 112th meeting of the American Chemical Society, New York, 1947.

(5) F. Brown, S. Dunstan, T. G. Halsall, E. L. Hirst and J. K. N. Jones, *Nature*, **156**, 785 (1945); T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1399 (1947); and private communication from Dr. E. L. Hirst.

the units of the main chain are split once, and that the other half of the units of the main chain, or those at the points of branching, are not split at all.

Experimental

Periodate Oxidation.—Following the method of Hirst, a 400-mg. sample of guaran was dissolved in 100 ml. of potassium chloride solution (5 g. of potassium chloride per 100 ml. of water) in a 500-ml. glass-stoppered bottle. Then 10 ml. of 0.3 *M* sodium periodate solution and 10 ml. of water were added and the mixture shaken at 25° for about one hundred hours. At this point the reaction was complete and ethylene glycol was added to consume the excess periodate. The formic acid present was titrated with 0.01 *N* barium hydroxide solution. One mole of formic acid was produced for 2.7 anhydrohexoside units. Samples of 200 mg. and 100 mg. gave similar values.

The presence of formic acid was confirmed by oxidation with mercuric chloride by the method of Auerbach and Zeglin.[6] Formic acid (*ca.* 40 mg.) formed from a 400-mg. sample of guaran was removed from the final reaction mixture by extraction with ether in a liquid–liquid extractor for ten days. A slight excess of sodium hydroxide was added to the ether extract and the mixture concentrated to about 5 ml. to remove ether and then was diluted to 60 ml. with water. After neutralization with 1 *N* hydrochloric acid, 1 ml. excess of acid and 3 g. of sodium acetate were added. The solution was filtered into an Erlenmeyer flask and 20 ml. of 5% mercuric chloride solution was added. The flask was covered with an inverted beaker and the mixture heated on a steam-bath for two hours. Precipitated mercurous chloride was filtered on a medium porosity sintered glass crucible, washed with hot water and ethanol, dried at 100°, and weighed. The weight of the precipitate corresponded to 103% of the formic acid determined by the above method of direct titration.

(6) F. Auerbach and H. Zeglin, *Z. physik. Chem.*, **103**, 161 (1922).

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RECEIVED FEBRUARY 28, 1948

COMMUNICATIONS TO THE EDITOR

REVERSIBLE ACTION OF *macerans* AMYLASE[1]

Sir:

The action of *Bacillus macerans* amylase[2] on starch has been interpreted by Cori[3] as the exchange of a glycosidic bond in starch for a corresponding bond in a cyclic Schardinger dextrin (cycloamylose) molecule. In view of the small ΔF which would be expected for such an exchange, it might be expected that the reaction should be readily reversible. The reverse type reaction

$$\text{Cyclohexaamylose} + \text{Maltose} \xrightarrow{\textit{macerans}\ \text{amylase}} \text{Higher saccharides}$$

has been tested with crystalline substrates and verified; *macerans* amylase thus has a synthetic as well as degradative action.

Pure cyclohexaamylose,[4] 2.0 g., and C. P. maltose, 0.7 g., were dissolved in water, heated to complete mutarotation of the maltose, and treated with four units[2] of *macerans* amylase. The solution was made up to 100 ml. and the increase in rotation[5] was followed in the polarimeter: initial rotation, 7.87°; after two hours, 8.10°. At this point the enzyme was inactivated by boiling and the reaction products separated by fractional precipitation. The least soluble fraction, 0.14 g., sirupy, had $[\alpha]_D$ +163°; average chain length by alkaline ferricyanide,[6] 8.9 glucose units. These values indicate that the sample is probably a mixture of saccharides containing some non-carbohydrate impurities. It gave a slight deepening of the color of I_2–KI solution and no unchanged cyclohexaamylose could be detected by the Tilden micro test.[2] On treatment with *macerans* amylase the fraction was rapidly reconverted in part into cyclohexaamylose as indicated by the formation of the characteristic I_2–KI complex.

Results indicating a similar synthetic action of *macerans* amylase have been obtained from cyclohexaamylose with glucose, α-methylglucoside, sucrose, cellobiose or maltobionic acid as co-substrates; also from cycloheptaamylose[4] with maltose or glucose as co-substrates. These studies are being continued and will be reported in full at a later date.

(1) Journal Paper No. J-1581 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 964.

(2) Tilden and Hudson, *J. Bact.*, **43**, 527 (1942).

(3) Cori, *Federation Proc.*, **4**, 226 (1945).

(4) French and Rundle, THIS JOURNAL, **64**, 1651 (1942).

(5) McClenahan, Tilden and Hudson, *ibid.*, **64**, 2139 (1942).

(6) Levine, Foster and Hixon, *ibid.*, **64**, 2331 (1942).

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DEXTER FRENCH
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RECEIVED AUGUST 10, 1948

FORMATION OF FLUORESCING SUBSTANCES FROM AMINO ACIDS

Sir:

Tauber[1] has reported recently on the formation of a fluorescing compound formed by the reaction of tryptophan with perchloric acid at room tem-

(1) Tauber, THIS JOURNAL, **70**, 2615 (1948).