reaction proceeded smoothly for one hour with evolution of hydrogen chloride. The reaction mixture was hydrolyzed by the addition with stirring of dilute hydrochloric acid. On removal of excess benzene from the organic layer 2.5 g. of crystalline material was collected. On recrystallization from 1:1 benzene-ethanol, the 1,1,4,4,5,5,8,8-octamethyl-1,2,3,4,5,6,7,8-octahydroanthracene separated in colorless needles which melted at 220-221°. A mixture of this octamethyloctahydroanthracene with the hydrocarbon $C_{22}H_{34}$ (m.p. 218.5-219°) melted at 220°.

The nitro derivative of the substituted anthracene was prepared by dissolving 1.7 g. in a solution of 83 cc. of acetic acid and 50 cc. of acetic anhydride at 85-95° and adding 2.5 cc. of fuming nitric acid slowly with stirring. After three hours heating at 85-95° the reaction mixture was cooled and the yellow crystals which separated were collected, washed with water, then alcohol; yield 1.1 g. After recrystallization from 1:1 benzene-ethanol, the nitro derivative of octamethyloctahydroanthracene melted at 263-265°. A mixture with the nitro derivative (m.p. 267°) of the high melting hydrocarbon melted at 263-264°.

Friedel-Crafts Alkylation of 1,3,5-Tri-*t*-butylbenzene. Run 1. The Action of Aluminum Chloride.—Ten grams of 1,3,5-tri-*t*-butylbenzene was dissolved in 50 cc. of carbon disulfide and cooled to −7°. Eight grams of aluminum chloride was added over a 0.5-hour period and the reaction mixture stirred below 0° for four hours when a small aliquot was taken and decomposed in water. After evaporation of the carbon disulfide an ultraviolet spectrum of the white crystals, m.p. 70°, was determined. It did not differ from the spectrum of pure 1,3,5-tri-*t*-butylbenzene.

Run 2. The Action of Isobutylene with Hydrogen Chloride.—Dry hydrogen chloride was added to the reaction mixture of run 1 at below 0° for two hours. Isobutylene was generated by dropping 85% phosphoric acid on warm *t*-butyl alcohol and passing the gas produced through an ice-trap, 85% phosphoric acid and a calcium chloride drying tube. For five hours, the isobutylene was passed into the reaction mixture which absorbed it rapidly. An aliquot of the reaction mixture was taken and decomposed in water. A spectrum of the viscous liquid remaining after evaporation of the carbon disulfide had the same absorption as 1,3,5-tri-*t*-butylbenzene and none of the characteristic peaks of octamethyloctahydroanthracene were present.

Run 3. The Addition of *t*-Butyl Chloride to the Reaction Mixture of Run 2.—*t*-Butyl chloride was added to the reaction mixture of run 2 below 0°. A gas which was not absorbed by 20% NaOH or concentrated sulfuric acid was evolved from the reaction mixture. After about an hour when 25 cc. of *t*-butyl chloride had been added, a small aliquot of the reaction mixture was taken and decomposed in water. The white solid left after the evaporation of the CS_2 melted at 210°.

Run 4. Preparation of Hydrocarbon $C_{22}H_{34}$ from 1,3,5-Tri-*t*-butylbenzene and Isolation of Isobutane.—Ten grams (0.04 mole) of 1,3,5-butylbenzene and 50 cc. of carbon disulfide were put in a flask so arranged that gases evolved during the reaction would pass through wash towers containing 20% sodium hydroxide and concentrated sulfuric acid, into a trap cooled in Dry Ice. The carbon disulfide solution was cooled to −5° and 8 g. (0.06 mole) of aluminum chloride added. Eleven grams (0.12 mole) of *t*-butyl chloride was added over a period of one hour, after which a gas was evolved continually which was not absorbed in the wash towers, but which condensed in Dry Ice. After four hours, the reaction mixture was decomposed by pouring into water, the organic layer extracted with ether, most of the ether and CS_2 evaporated and the solid which crystallized out was recrystallized from benzene-ethanol. The melting point of the colorless needles was 212-216°. A mixed melting point with the hydrocarbon (octamethyloctahydroanthracene) showed no depression. An infrared spectrum on the gas condensed in Dry Ice showed isobutane to be the only organic gas present.

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Synthesis of Disaccharides with Pea Preparations

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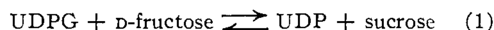
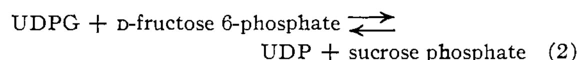
Leloir and Cardini[1,2] showed that enzyme preparations from wheat, corn, peas and bean germs catalyze the reversible formation of sucrose from UDPG and free fructose



UDPG + D-fructose ⇄ UDP + sucrose (1)

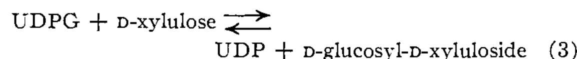
They also demonstrated[3] that some pea preparations contain an enzyme which will form sucrose phosphate when fructose 6-phosphate is substituted for D-fructose



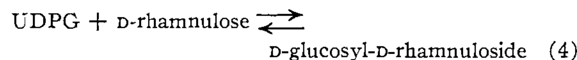
UDPG + D-fructose 6-phosphate ⇄ UDP + sucrose phosphate (2)

the enzymes causing reactions (1) and (2) could not be completely separated, but sufficient evidence was presented to conclude that two enzymes are involved.

In the present communication, using preparations from green peas, we confirmed the synthesis of sucrose from UDPG and D-fructose, and of sucrose phosphate from UDPG and D-fructose 6-phosphate. We have also shown that these preparations produce three other disaccharides, D-glucosyl-D-xyluloside, D-glucosyl-D-rhamnuloside[4] and D-glucosyl-L-sorboside, according to the reactions



UDPG + D-xylulose ⇄ UDP + D-glucosyl-D-xyluloside (3)



UDPG + D-rhamnulose ⇄ D-glucosyl-D-rhamnuloside (4)

UDPG + L-sorbose ⇄ D-glucosyl-L-sorboside (5)

The disaccharide in reaction (3) is probably identical with the one formed by the action of an enzyme present in *Pseudomonas saccharophila* from D-glucose 1-phosphate and D-xylulose.[5]

Experimental

Synthesis of Sucrose and Sucrose Phosphates.—Five hundred grams of fresh peas was placed in 150 ml. of water, homogenized in a Waring blendor, the homogenate was centrifuged and the supernatant solution was fractionated with ammonium sulfate. The precipitate occurring between 20 and 50% ammonium sulfate saturation was dissolved in a minimum of water, dialyzed for 2 hr. against distilled water and then overnight against 0.05 *M* phosphate buffer, *p*H 7. The solution was adjusted to *p*H 5, the resulting precipitate dissolved in water, dialyzed again against 0.05 *M* sodium Versenate (ethylenediamine tetraacetate), *p*H 7, overnight, and then against phosphate buffer at the same *p*H to remove the Versene. The final solution, which had a volume of 7 ml., contained 5 mg. N per/ml.

The complete reaction mixture contained 0.01 ml. of

(1) L. F. Leloir and C. E. Cardini, THIS JOURNAL, **75**, 6084 (1953).
(2) L. F. Leloir and C. E. Cardini, *J. Biol. Chem.*, **214**, 149 (1955).
(3) L. F. Leloir and C. E. Cardini, *ibid.*, **214**, 157 (1955).
(4) The D-rhamnulose was obtained by Drs. N. Palleroni and M. Doudoroff through isomerization of D-rhamnose with a bacterial enzyme (mannose isomerase) preparation from *P. saccharophila*.
(5) W. Z. Hassid, M. Doudoroff, H. A. Barker and W. H. Dore, THIS JOURNAL, **68**, 1465 (1946).

UDPG (0.45 μmole), 0.03 ml. of fructose (0.44 μmole) and 0.05 ml. of the enzyme solution. The reaction was allowed to proceed for 30 minutes and then inactivated by heating for one minute at 100°. The residual fructose was destroyed by heating for 10 minutes in 2 ml. of 0.2 *M* NaOH at 100°. The sucrose produced was estimated by determining the fructose of the disaccharide by a modified Roe reaction.[6] For comparison, standards containing varying amounts of sucrose, fructose and enzyme solution were used. Conversion of UDPG to UDP was determined by chromatographing aliquots of the reaction mixtures, using an ammonium acetate–alcohol solvent, *p*H 7.5, elution of the ultraviolet absorbing spots and reading the absorption at 260 mμ.

Using the above mixture of UDPG and fructose, 0.18 μmole of sucrose and 0.2 μmole of UDP were produced. When this reaction was carried out in the presence of 10^{-3} *M* $MgCl_2$, 0.24 μmole of sucrose and 0.2 μmole of UDP formed. These results indicate that Mg^{++} has a stimulating effect on sucrose synthesis.

The production of sucrose was confirmed by chromatographing the products of the reaction two-dimensionally in butanol–acetic acid–water and then in water-saturated phenol. Upon elution of the disaccharide spot and hydrolysis with invertase, glucose and fructose were obtained, which were also identified by paper chromatography.

When glucose 6-phosphate was substituted for fructose in the reaction with UDPG, using the same enzyme preparation from peas in the presence of 10^{-3} *M* Mg^{++}, 0.08 μmole of sucrose phosphate was formed instead of free sucrose. No reaction took place in the absence of this ion. The methods used for the analyses of the reaction products were the same as those used to demonstrate the synthesis of free sucrose.

The formation of sucrose phosphate was demonstrated by paper chromatography. An aliquot of the incubating mixture was inactivated and spotted on paper. After development in butanol–acetic acid–water mixture, the area normally corresponding to glucose 6-phosphate was cut out from the unsprayed area, treated with alkaline intestinal phosphatase and chromatographed two-dimensionally in the usual solvents. On spraying with *p*-anisidine hydrochloride, two spots were identified, one corresponding to sucrose and the other to glucose.

The apparent stimulation of sucrose synthesis from free fructose by Mg^{++} may be due to the additive effect of the sucrose phosphate reaction. Under conditions of the reaction a significant amount of fructose 6-phosphate may be formed which in the presence of UDPG would lead to the formation of sucrose phosphate.

Synthesis of D-Glucosyl-D-xyluloside and D-Glucosyl-D-rhamnuloside.—For the preparation of the D-glucosyl-D-xyluloside a mixture consisting of 0.9 μmole of UDPG, 8 μmole of D-xylulose and 0.4 ml. of enzyme preparation from peas was incubated for 6 hr. The enzyme was inactivated by adding 5 volumes of ethanol and heating the digest. The mixture was centrifuged, the solution evaporated and the resulting sirup chromatographed two-dimensionally on paper, using butanol–acetic acid–water and phenol-saturated water as developing solvents. After spraying with *p*-anisidine hydrochloride, a red spot appeared on the chromatogram which coincided with that of D-glucosyl-D-xyluloside prepared by the action of sucrose phosphorylase from *P. saccharophila* on D-glucose 1-phosphate and D-xylulose. A control without UDPG produced only a D-xylulose spot. Elution of the disaccharide spot and hydrolysis with 0.1 *M* HCl at 100° for 10 minutes and chromatography of the products yielded spots which were identified by their R_f values as D-glucose and D-xylulose.

The rate of D-glucosyl-D-xyluloside formation relative to that of sucrose synthesis was estimated by determining its synthesis at several time intervals under similar conditions. Samples consisting of 2.25 μmoles of UDPG, 40 μmoles of D-xylulose and 0.25 ml. of enzyme solution in a total volume of 0.45 ml. were incubated at 37°, and the D-glucosyl-D-xyluloside produced was determined colorimetrically at various intervals by a modification of the Dische and Borenfreund[7] reaction. Analysis of the samples showed the formation of 0.60, 0.95 and 1.2 μmoles disaccharide after 30, 60 and 120 minutes, respectively. Under similar conditions D-glucosyl-D-xyluloside is synthesized at a considerably slower rate than sucrose. Using the same UDPG concentration and a D-xylulose concentration 16 times greater than that of D-fructose in the sucrose reaction, the rate of formation of D-glucosyl-D-xyluloside was still slower than that of sucrose.

When a mixture of 2.25 μmoles of UDPG, 30 μmoles of D-rhamnulose and 0.25 ml. of enzyme was incubated in a total volume of 0.45 ml. under the same conditions as the mixture containing the D-xylulose, 0.70 μmole of D-glucosyl-D-rhamnuloside was obtained after 30 minutes and 0.91 μmole after 60 minutes. The rate of formation of this disaccharide was similar to that of the D-glucosyl-D-xyluloside.

D-Glucosyl-L-sorboside was synthesized from a mixture of 2.25 μmoles UDPG, 27 μmoles of L-sorbose and 0.20 ml. of enzyme solution in a total volume of 0.35 ml. After 60 minutes of incubation, 0.45 μmole of the disaccharide was produced under these conditions. The synthesis of the glucosyl sorboside was demonstrated by the previously described methods, and later confirmed as follows: approximately 1 μmole of randomly C^{14}-labeled sorbose[8] (11 μc.) was incubated for 2 hr. with 2 μmoles of UDPG and 0.2 ml. of enzyme solution.

The reaction mixture was chromatographed two-dimensionally in the usual solvents. One spot, containing 1.4% of the total C^{14}-activity, was found to be situated closely to the position of the sucrose spot on the chromatogram. This spot was eluted, the product hydrolyzed with dilute acid, inactive L-sorbose added to the hydrolysis products, and the mixture was chromatographed two-dimensionally. A radioautogram prepared from this chromatogram produced a spot which coincided with the colored spot produced by the sorbose when the chromatogram was sprayed with *p*-anisidine hydrochloride reagent. Glucose was identified as the other component of the disaccharide.

Thus, it appears that peas contain an enzyme (probably the same as the one responsible for the reaction of UDPG with D-fructose to form sucrose) which is capable of producing D-glucosyl-D-xyluloside from UDPG and xylulose; D-glucosyl-D-rhamnuloside and D-glucosyl-L-sorboside from the same nucleotide and D-rhamnulose.

(6) J. S. D. Bacon and D. J. Bell, *Biochem. J.*, **42**, 397 (1948).

(7) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(8) The C^{14}-labeled L-sorbose was prepared by Dr. E. W. Putman by reduction of D-fructose with borohydride and subsequent oxidation with *A. suboxidans*.

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2-Quinoxalinols as Derivatives of α-Ketocarboxylic Esters

BY P. H. GORE AND G. K. HUGHES[1]

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In view of recent notes[2a,b] dealing with the formation of 3-substituted 2-quinoxalinols, we wish to record parallel experiments carried out some years ago. These compounds have been obtained in good yields[2b,3] by the condensation of α-keto acids with *o*-phenylenediamines.

It is now found that 2-quinoxalinols may similarly be obtained analogously from α-keto esters, both simple esters (*e.g.*, ethyl oxalacetate) or those derived from cyclic ketones (*e.g.*, ethyl cyclopentanone 2-glyoxylate[4]). By treating diethyl cyclopentanone 2,5-bisglyoxylate[4] with two molar proportions of *o*-phenylenediamine, the expected bisquinoxaline system was not formed, due to insolubility of the initial product Ic. An attempt to introduce a second quinoxaline ring into Ic in boiling pyridine failed, due perhaps to the insufficiently

(1) Deceased.

(2) (a) M. Goldweber and H. P. Schultz, THIS JOURNAL, **76**, 287 (1954); (b) D. C. Morrison, *ibid.*, **76**, 4483 (1954).

(3) O. Hinsberg, *Ann.*, **237**, 327 (1887).

(4) S. Ruhemann, *J. Chem. Soc.*, **101**, 1729 (1912).