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).

TABLE I

2-HYDROXY-3-R-QUINOXALINES

R	M.p., °C.	Yield, %	Solvent	Appearance	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
$-CH_2CO_2C_2H_5$	204–205	85	Alcohol	Yellow	...	...	..	..	12.06	12.18
$-CH(CH_3)CO_2C_2H_5$	160	95	Alcohol	Colorless	...	...	..	..	11.38	11.45
$-CH(CH_2)_2CH_2$ (CO)	256–258 dec.	90	Alcohol	Dark red	68.42	68.31	5.30	5.32	12.28	12.30
$-CH(CH_2)_2CHCOCO_2Et$ (CO)	>360	70	Pyridine	Dark purple	62.18	62.08	4.91	5.12	8.54	8.70

low *p*H, and the prevailing high dilution. In general, however, quinoxalinols are formed rapidly and in almost quantitative yields and thus provide the best available derivatives of α-keto esters.

Experimental

General Procedure.—Equimolar proportions of α-keto ester and *o*-phenylenediamine were refluxed in alcoholic solution (with or without added glacial acetic acid) for several hours. Ten minutes heating sufficed in the cases of less soluble quinoxalinols. The products were isolated by filtration or evaporation, followed by recrystallization. The data are collected in Table I.

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The Displacement of Positive Halogen from Tri-(*p*-nitrophenyl)-methyl Halides[1]

BY M. FREDERICK HAWTHORNE

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During the course of an investigation of the chemistry of tri-(*p*-nitrophenyl)-methyl nitrate[2] it was observed that the treatment of this nitrate ester with iodide ion in acetone solution produced the green tri-(*p*-nitrophenyl)-methyl radical and iodine. This reaction was similar to that reported by Leffler[3] for the corresponding bromide and both reactions were examined in greater detail.

One of the possible paths for the production of the triarylmethyl radical from triarylmethyl bromide and iodide ion is the nucleophilic displacement of "positive" bromine by iodide ion to produce the tri-(*p*-nitrophenyl)-methide ion which, in turn, may be oxidized by the liberated halogen to the free radical. Alternatively, the reaction could take the course of a nucleophilic attack on carbon by iodide ion followed by the spontaneous homolytic dissociation of the resulting triarylmethyl iodide.

$$R_3CBr + I^\ominus \longrightarrow R_3C^\ominus + IBr \longrightarrow R_3C\cdot + I\cdot + Br^\ominus$$

R = *p*-nitrophenyl

The production of triarylmethyl radical from the nitrate ester, under identical circumstances, could proceed through the agency of the similar reaction sequence

$$R_3CONO_2 + I^\ominus \longrightarrow R_3CI + NO_3^\ominus$$
$$R_3CI + I^\ominus \longrightarrow R_3C^\ominus + I_2 \longrightarrow R_3C\cdot + I\cdot + I^\ominus$$

or

$$R_3CI \longrightarrow R_3C\cdot + I\cdot$$

R = *p*-nitrophenyl

In order to choose from among the numerous possible mechanisms for these reactions (nucleophilic attack on carbon *versus* nucleophilic attack on halogen) the reactions of triarylmethyl bromide and nitrate with iodide ion were examined in acetic acid–methylene chloride. In this medium the nucleophilic attack of iodide ion on halogen should still occur, with the liberation of iodine, as in acetone solution but the resulting triarylmethide ion intermediate should be effectively captured as hydrocarbon due to the presence of a proton donor and little radical should result. This reaction path was indicated to be correct by the isolation of high yields of tri-(*p*-nitrophenyl)-methane, iodine and some carbinol from the reaction of the bromide and nitrate esters with iodide ion under these conditions. A similar experiment conducted by Leffler[3] was unsuccessful due to the relatively low concentration of acetic acid employed. The nitrate ester produced a small amount of triarylmethyl radical and this is attributed to the homolytic dissociation of the intermediate iodide. The presence of carbinol among the reaction products is explained on the basis of acetolysis of the triarylmethyl nitrate or iodide followed by hydrolysis of the resulting acetate during chromatography on alumina. A control experiment showed that tri-(*p*-nitrophenyl)-methyl is not reduced by iodide ion to the methide ion.

Experimental

The Reaction of Tri-(*p*-nitrophenyl)-methyl Nitrate with Iodide Ion in Acetic Acid–Methylene Chloride.—Two grams (0.0044 mole) of tri-(*p*-nitrophenyl)-methyl nitrate, prepared as previously described,[2] was dissolved in 200 ml. of 50–50 (volume) acetic acid–methylene chloride and 3.50 g. of sodium iodide quickly added. Iodine was immediately released and the solution was allowed to stand at room temperature for one hour and then quickly filtered to remove a small amount of green triarylmethyl radical (0.38 g., weighed as peroxide, m.p. 212–214°). The filtrate was flooded with water, washed with excess sodium thiosulfate solution, washed again with water and the organic layer dried over magnesium sulfate. Evaporation of the solution to dryness produced an orange solid which was dissolved in a minimum of 50–50 benzene–methylene chloride and placed on a 15-cm. non-alkaline, activity grade I alumina column. The column was eluted with methylene chloride to produce 0.97 g. (0.0026 mole) of pure tri-(*p*-nitrophenyl)-methane (m.p. 210–212°, base soluble with blue coloration) followed by 0.25 g. (0.00065 mole) of tri-(*p*-nitrophenyl)-carbinol (m.p. 180–183° after one recrystallization from ethanol–water).

In order to prove that tri-(*p*-nitrophenyl)-methyl is not reduced by iodide ion under the above reaction conditions, 1.00 g. of freshly prepared radical was treated with 5.0 g. of sodium iodide in 50 ml., of reaction solvent. The green radical persisted for at least several hours.

The Reaction of Tri-(*p*-nitrophenyl)-methyl Bromide with Iodide Ion in Acetic Acid–Methylene Chloride.—This experiment was conducted in exactly the same manner as that of the nitrate ester except that 2.80 g. of bromide (0.0079 mole) was used with 4.0 g. of sodium iodide. No free radical was obtained, but 1.56 g. (0.0043 mole) of the tri-(*p*-

(1) This research was carried out under Army Ordnance Contract W-01-021-ORD-334.

(2) M. F. Hawthorne, THIS JOURNAL, **77**, 5523 (1955).

(3) J. E. Leffler, *ibid.*, **75**, 3598 (1953).