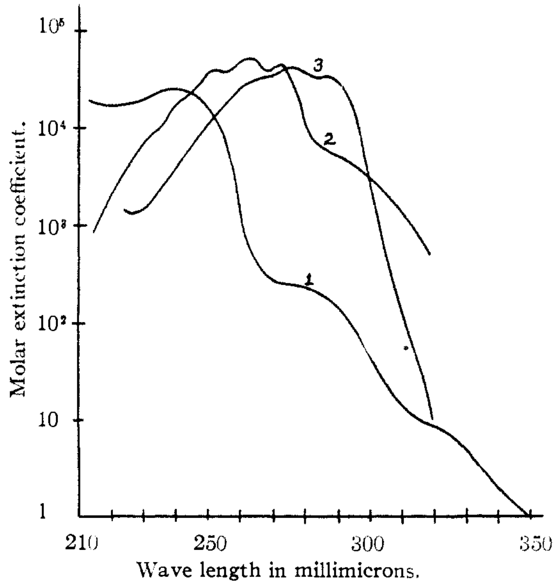

Fig. 2.—Ultraviolet absorption spectra in isoöctane at about 30°: 1, alloöcimene dimer; 2, 2,4,6-octatriene; 3, alloöcimene.

cule but it is equally possible that they are due to the presence of small amounts of ocimene and alloocimene dimer, with which alloöcimene is in equilibrium.[10]

In the course of the literature search preceding this investigation it was noted that Blout and Fields[11] had measured the ultraviolet absorption of 2,4,6-octatriene. Through the courtesy of Dr. Blout these data, previously unpublished in complete form, were made available to the authors and are shown in Fig. 2. This compound has the same resonance system as alloöcimene and it is not surprising to find a marked similarity between the absorption curves. 2,4,6-Octatriene has absorption peaks at 253, 263 and 274 mμ, the peak at 263 mμ being slightly higher than the other two. $\epsilon_{\max}$ at 263 mμ is 53,000. Each of the three main absorption bands in alloöcimene is thus shifted about 14 mμ toward longer wave lengths than in 2,4,6-octatriene.

Ultraviolet absorption data for alloöcimene dimer are shown in Fig. 2. The marked shift of the absorption peak from 277 to 240 mμ is indicative of a change from a triene to a diene chromophore as a result of dimerization. The spectral absorption curve clearly indicates the presence of a small amount of alloöcimene, most probably arising from depolymerization of the dimer,[10] and traces of the substance absorbing at 320 mμ.

Parker and Goldblatt[12] studied the thermal isomerization of alloöcimene in the vapor phase and reported that the products consisted almost entirely of cyclohexadienes having absorption maxima between 260–265 mμ. An examination of Fig. 2 indicates that cyclohexadienes are not major products of the liquid phase thermal isomerization, although their absence cannot be positively demonstrated on the basis of the ultraviolet absorption spectra available.

Values of $\lambda_{\max}$ for myrcene, ocimene and alloöcimene dimer calculated by Woodward's[5] method were compared with those observed experimentally. In the cases of ocimene and the alloöcimene dimer the values differ by more than the 5 mμ found by Woodward (see Table I).

TABLE I

Compound	$\lambda_{\max}$ calcd., mμ	$\lambda_{\max}$ obsd., mμ
Myrcene	222	224.5
Ocimene	227	237
Alloöcimene dimer	232	240

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[12] E. D. Parker and L. A. Goldblatt, *ibid.*, **72**, 2151 (1950).

Synthesis of α- and β-Lactose-1-phosphate[1,2]

BY F. J. REITHEL AND R. G. YOUNG

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Lactose-1-phosphate has been postulated as an intermediate in lactose synthesis in mammary gland[3] and has been suggested as an intermediate in lactose fermentation by yeasts.[4] A preliminary examination of the behavior of this compound in mammary gland homogenates has been reported.[5]

The method of Cori,[6] employing trisilver phosphate as a phosphorylating agent, was adapted to the preparation of α-lactose-1-phosphate, but yields were poor. The product obtained possessed constants approximating those reported in the experimental description following but an analytically pure product could not be prepared.

Similarly, attempts were made to use the procedures described by Posternak[7] in which silver diphenyl phosphate was used as a phosphorylating agent. Again, yields were low and the product was difficult to purify. However, it was possible to introduce modifications which allowed the successful application of the principles of this latter procedure.

The use of monosilver phosphate for the preparation of β-isomers of the sugar phosphates[8] was successfully applied to the preparation of β-lactose-1-phosphate.

Experimental

1-Bromoheptaacetyl-α-lactose.—To ensure purity of the final product it was found necessary to prepare a very pure starting material.

To 20 g. of pure octaacetyllactose in a stoppered flask was added 50 ml. of 30% hydrogen bromide in glacial acetic acid. The mixture was shaken occasionally during a reaction period of 1.5–2 hours and then poured slowly into a liter of ice-water with efficient stirring. The precipitate was collected on a buchner funnel and pressed dry. The cake was dissolved in absolute methanol[6] at 20°. This solution was filtered, cooled to 0°, and an equal volume of ice-water added with good stirring and efficient cooling.

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[1] This work was supported by the Office of Naval Research.

[2] Reported in part at the Northwest Regional Meeting of the ACS, June, 1951, Seattle, Washington.

[3] F. H. Malpress and A. B. Morrison, *Biochem. J.*, **46**, 307 (1950).

[4] M. Rogosa, *J. Biol. Chem.*, **175**, 413 (1948).

[5] F. J. Reithel, M. G. Horowitz, H. M. Davidson and G. W. Kittinger, *ibid.*, **194**, 839 (1952).

[6] M. E. Krahl and C. F. Cori, "Biochemical Preparations," Vol. 1, John Wiley and Sons, New York, N. Y., 1949, p. 33.

[7] T. Posternak, THIS JOURNAL, **72**, 4824 (1950).

[8] F. J. Reithel, *ibid.*, **67**, 1056 (1945).

Two additional repetitions of this methanol purification procedure yielded 8.2 g. of a product melting at 149° (uncor.) and having a rotation $[\alpha]^{20}\text{D}$ +107° (c 1.6, $CHCl_3$).

α-Lactose-1-phosphate.—To 15 ml. of dry benzene in a flask containing a magnetic stirring bar was added 1.0 g. of bromoacetyllactose. To the clear solution was added 0.52 g. of pure silver diphenyl phosphate[9] and the mixture refluxed with stirring for 30 minutes. After the addition of 0.1 g. of silver diphenyl phosphate the reaction mixture was refluxed again for 30 minutes. After filtration the benzene solution was evaporated. The resultant sticky mass was taken up in 10 ml. of absolute ethyl acetate and the excess silver diphenyl phosphate which crystallized was removed by filtration. The product, diphenylphosphonoheptaacetyllactose, could not be crystallized but was obtained only as a brittle glass.

Anal. Calcd. for $C_{38}H_{45}O_{21}P$ (868): P, 3.57. Found: P, 3.36.

The phenyl groups were removed by catalytic hydrogenation (Adams catalyst) in absolute ethyl acetate at room temperature and 2–6 cm. pressure. The volumes of hydrogen used were those calculated. The product, heptaacetyllactose-1-phosphoric acid, could not be crystallized and was impure.

Anal. Calcd. for $C_{26}H_{37}O_{21}P$ (716): P, 4.33. Found: P, 3.6.

In order to deacetylate, the product was dissolved in the smallest quantity of ethyl acetate permissible, 3 ml. of ice-water added, and titrated to the phenolphthalein end-point with 0.5 N sodium hydroxide at 0°. The emulsion was broken by centrifugation and the ethyl acetate removed. A small amount of barium phosphate was removed after the addition of 4 ml. of 10% barium acetate. The aqueous solution was evaporated *in vacuo* to dryness and the residue extracted with 95% ethanol. Addition of a catalytic amount of potassium methylate caused deacetylation and precipitation of barium lactose-1-phosphate. The reaction was allowed to proceed to completion at 0° overnight. The precipitate was centrifuged, drained, and extracted with small portions of 0.01 N acetic acid. The aqueous extract was adjusted to pH 8.2 with sodium hydroxide and two volumes of 95% ethanol, adjusted to pH 8.2, were added. This extraction–precipitation procedure was repeated until a product was obtained which dissolved completely in water. The pure barium salt was washed, by centrifugation, with ethanol and ether and dried *in vacuo* over P_2O_5 for five days. The yield was 0.26 g.

Anal. Calcd. for $C_{12}H_{21}O_{14}PBa\cdot 5H_2O$: C, 22.2; H, 4.78; P, 4.8. Found: C, 22.6; H, 4.5; total P, 4.85.

The compound did not reduce alkaline copper reagents. The phosphate linkage was completely hydrolyzed by normal sulfuric acid at 100° in seven minutes. The rotation of the anhydrous lactose-1-phosphoric acid was calculated to be $[\alpha]^{23}\text{D}$ +99.5° (c 0.4, H_2O) from measurements on the pentahydrate barium salt. Failure attended all attempts to prepare brucine, strychnine or cyclohexylamine derivatives.

β-Lactose-1-phosphate.—Monosilver phosphate was prepared by adding 0.18 ml. of 90% phosphoric acid to 0.6 g. of freshly prepared, pure, dry trisilver phosphate in a round bottom centrifuge tube containing a stirring magnet. Three milliliters of dry ether was added and the tube and contents were cooled to 0°. Dropwise 3.0 g. of bromoacetyllactose in 8 ml. of dry chloroform was added with efficient stirring and the mixture allowed to react for 30 minutes at 0°. The precipitate was removed. To the supernatant liquid was added 10 ml. of water and normal sodium hydroxide until pH 8.0 resulted. After removing the chloroform layer, 2 g. of barium acetate in 10 ml. of water was added and the pH adjusted to 9. The precipitate was removed and the aqueous solution evaporated to dryness *in vacuo*. The residue was dissolved in 95% ethanol and deacetylated with catalytic amounts of potassium methylate. The resultant barium salt of the β-isomer of lactose-1-phosphate was then purified and dried as described above for the α isomer. This procedure yielded 0.57 g. of a compound whose specific rotation, calculated for the anhydrous acid form, was $[\alpha]^{23}\text{D}$ +31.5° (c 0.5, H_2O).

Anal. Calcd. for $C_{12}H_{21}O_{14}PBa\cdot 5H_2O$: C, 22.2; H, 4.78; P, 4.8. Found: C, 22.54; H, 4.5; P, 4.75.

The compound did not reduce alkaline copper reagents. The phosphate linkage was completely hydrolyzed by normal sulfuric acid at 100° in seven minutes.

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[9] M. E. Foss and C. S. Gibson, *J. Chem. Soc.*, 3079 (1949).

Concerning Hydrogen Transfer in a Reaction Sensitized by Chlorophyll[1]

BY JOHN W. WEIGL AND ROBERT LIVINGSTON

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While it is known that chlorophyll is capable of sensitizing a variety of oxidation–reduction reactions in solution,[2] the primary act has not been established unambiguously for any of these reactions. It has been postulated that either the energy of excitation is transferred, in all or in part, to one or both of the reactants, or else that the excited chlorophyll is oxidized or reduced to an unstable intermediate by one of the reactants. If the chlorophyll were first reduced, the intermediate could react rapidly with the oxidizing agent to regenerate the chlorophyll. Conversely, if the primary reaction were one in which the excited chlorophyll gave up a hydrogen atom to the oxidizing agent, the intermediate could be rapidly hydrogenated by the reductant. Only in the latter case, would the sensitized reaction result in a transfer of hydrogen from the reductant to the sensitizer.

The experiments described in this note were performed to determine whether a proton–deuteron exchange between chlorophyll and the reductant accompanied a sensitized oxidation–reduction reaction. The reaction studied was the chlorophyll-sensitized reduction of an azo dye, butter yellow, by deuterated ascorbic acid. When this reaction occurs in methanol, its limiting quantum yield is about one-half and its products are aniline, dimethylphenylenediamine and (presumably) dehydroascorbic acid.[3] To avoid the possibility of exchange between chlorophyll and the solvent, dry dioxane was substituted for methanol in the present experiments. Unfortunately, the yield in dioxane is 20- to 100-fold less than it is in methanol; accordingly, there can be no certainty that the reaction proceeds by the same mechanism in the two solvents.

The reaction mixtures contained approximately $2.0 \times 10^{-4}m$ chlorophyll-a, $2.0 \times 10^{-3}m$ butter yellow and $2.7 \times 10^{-2}m$ deuterated ascorbic acid. The solution, at a temperature of 45°, was illuminated with red light for 5 to 7 hours, until about 75% of the butter yellow was reduced. This corresponded to an average of 4–7 butter yellow molecules reacted per molecule of sensitizer present. The chlorophyll was then separated from the reactants and products by chromatography on a paper pulp column. The deuterium content of the chlorophyll was determined with a mass spectrograph. In no case was the amount of deuterium found more than 4% of that which would be ex-

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[1] This work was made possible by the support of the Office of Naval Research (NR 059,028, Contract N6ori-212, T. O. I) to which the authors are indebted.

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