mole) and 2-methylpentane (XIIb) (−41.66 kcal./mole) is 7.6 kcal./mole.[17] The discrepancy of 2.3 kcal. between these two values may be taken as the difference in strain between the compounds XIa and XIb. Unfortunately estimation of a corresponding factor for the olefins II and V is not possible by this method. It will be observed, however, that if the olefin strain difference does not exceed about 1 kcal./mole, the difference of 1.2 kcal./mole in the heats of hydrogenation of these compounds can be accounted for on the basis of steric effects alone.

In connection with this investigation we have also had an opportunity to examine the heats of hydrogenation of the *cis*- and *trans*-di-*t*-butylethylenes (2,2,5,5-tetramethyl-3-hexenes), samples of which were kindly provided by Prof. M. S. Newman. The values obtained for these substances are, respectively, −36.2 and −26.9 kcal./mole. The enthalpy difference of 9.3 kcal./mole in favor of the *trans* isomer is in this case comparable to that found in the *cis*-cycloöctene–*trans*-cycloöctene system (9.2 kcal./mole in favor of the *cis*-modification),[18] but is considerably lower than the value of 25.5 kcal./mole estimated by Brown, Gintis and Domash[19] for the strain energy in *o*-di-*t*-butylbenzene.

The heat of hydrogenation obtained for *cis*-di-*t*-butylethylene (−36.2 kcal./mole) represents the highest value that we have yet encountered. The spread of 15.5 kcal./mole between this figure and the *cis*-cyclodecene result (−20.7 kcal./mole),[18] which amounts to about 75% of the total heat change accompanying reduction of the latter olefin, serves to emphasize in a striking manner the important influence that steric phenomena may exert on thermochemical properties.

Experimental

The heats of hydrogenation reported in this paper were obtained in acetic acid solution (25°) by the previously described procedure.[11b]

Materials.—Samples of 2,2,5,5-tetramethyl-*cis*-3-hexene (*cis*-di-*t*-butylethylene) and of 2,2,5,5-tetramethyl-*trans*-3-hexene (*trans*-di-*t*-butylethylene) were prepared by Dr. W. H. Puterbaugh at Ohio State University. The *cis* isomer was obtained by partial hydrogenation of the corresponding acetylene and was purified by successive fractional distillation; b.p. 143°, $n^{20}D$ 1.4269 (literature values[20] b.p. 144.2–144.4°, $n^{20}D$ 1.4271). Hydrogenation of this sample proceeded with the absorption of only 95% of the theoretical amount of hydrogen and gave a ΔH value of −35.53 ± 0.06 kcal./mole (based on hydrogen uptake). It was subsequently determined by vapor phase chromatography that the material contained about 5% of a compound with the same retention time as 2,2,5,5-tetramethylhexane as well as a second minor impurity. Through the courtesy of Drs. Newman and Puterbaugh of Ohio State and Drs. Doering and Saunders at Yale a second sample was purified by vapor chromatography. The latter material showed a single chromatographic peak and was flask distilled immediately before use; $n^{20}D$ 1.4264. The hydrogenation values reported in Table I were obtained with this specimen. The *trans* compound was prepared by pyrolysis of 2,2,5,5-tetramethyl-3-hexyl acetate, and after repeated fractionation furnished a sample boiling at 125.1°, $n^{20}D$ 1.4117, f.p. −4.8° (literature values[21] b.p. 125.01°, $n^{20}D$ 1.4115, f.p. −4.75°).

The remainder of the substances employed in this investigation were American Petroleum Institute Standard Samples. The compounds together with the A.P.I. sample numbers and impurity indexes are listed in Table II.

TABLE II

AMERICAN PETROLEUM INSTITUTE SAMPLES

Pentene	Sample	Impurity, mole %
2,4-Dimethyl-1-	1044-5S	0.13 ± 0.09
2,4-Dimethyl-2-	1027-5S	.14 ± .04
2,4,4-Trimethyl-1-	545-5S	.09 ± .03
2,4,4-Trimethyl-2-	546-5S	.08 ± .05
4-Methyl-*cis*-2-	537-5S	.08 ± .07
4-Methyl-*trans*-2-	536-5S	.25 ± .07
4,4-Dimethyl-*cis*-2-	582-5S	.21 ± .11
4,4-Dimethyl-*trans*-2-	574-5S	.09 ± .03

The homogeneity of these samples was further checked by vapor phase chromatography.

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HOUSTON, TEXAS

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF NEBRASKA]

Reversible Transgalactosylation[1]

BY JOHN H. PAZUR, JEAN M. MARSH AND CARL L. TIPTON

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Allolactose (6-*O*-β-D-galactopyranosyl-D-glucose) is disproportionated to glucose, galactose and a new trisaccharide by an enzyme preparation from *S. fragilis*. In the disproportionation, galactosyl units of allolactose are transferred by the enzyme to accepting carbohydrate or water molecules. Evidence from isotope experiments indicates that the reactions are reversible.

The enzymatic conversion of disaccharides into new oligosaccharides *via* transglycosylation type reactions has been observed with enzymes from animal and microbial sources.[2,3] In a few instances, reversibility of enzyme action has been demonstrated.[4,5] Reversibility has now been observed for the transgalactosylation reactions catalyzed by a transferring enzyme from *Saccharomyces fragilis*. Because the transgalactosylation

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mechanism is important in the production of lactose in the mammary gland[6] in the synthesis of galactosyl oligosaccharides[7] and in the synthesis of plant galactosides[8] attention is drawn to the reversible nature of transgalactosylations.

Allolactose (6-*O*-β-D-galactopyranosyl-D-glucose) prepared as previously described[9] was subjected to the action of the transferring enzyme of *S. fragilis*. Periodic examination of the enzymatic digest revealed that the allolactose had been disproportionated to glucose, galactose and a reducing compound with chromatographic mobility of a trisaccharide. Structural information indicates that this trisaccharide is *O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-glucose. This compound has also been isolated from digests of lactose with the *S. fragilis* transferring enzyme.[10] A mechanism suggested for the action of the enzyme on allolactose is shown diagrammatically in the accompanying equations

$$\text{Gal–Gl} + \text{HE} \longrightarrow \text{Gal.E} + \text{Gl}' \quad (1)$$

$$\text{Gal.E} + \text{Gal–Gl} \longrightarrow \text{Gal–Gal–Gl} + \text{H E} \quad (2)$$

$$\text{Gal.E} + \text{HOH} \longrightarrow \text{Gal} + \text{H E} \quad (3)$$

Gal– represents galactosyl unit; –Gal–, a galactosyl unit substituted at position 6; Gl, glucosyl unit substituted at position 6; Gl′, glucose; Gal, galactose; HOH, water, and H E, the enzyme molecule.

Radioactive glucose and galactose were employed to test for the reversibility of the above reactions. Incubation of non-radioactive allolactose and glucose-1-C^{14} with the enzyme resulted in the rapid appearance of radioactivity in the allolactose (see Table I). The radioactive allolactose could be synthesized in this system either by a transfer of the galactosyl moiety of the disaccharide to glucose-1-C^{14} (a reversal of reaction 1) or by a resynthesis in which galactose liberated by hydrolysis of allolactose was recombined with glucose-1-C^{14}. Results of an experiment designed to test for the occurrence of the resynthesis reaction are shown in Table II. These data indicate that some resynthesis had occurred but that this type of reaction could account for only a small fraction of the allolactose-C^{14} synthesized from glucose-1-C^{14} and non-radioactive allolactose. It would appear that the major route for the synthesis of allolactose-C^{14} from glucose-1-C^{14} and allolactose is *via* transgalactosylation. Reaction 1 of the proposed mechanism is therefore reversible.

Incubation of the non-radioactive trisaccharide and glucose-1-C^{14} with the enzyme also resulted in the synthesis of allolactose-C^{14}. Results of a typical experiment are shown in Table III. Since only a small fraction of the allolactose-C^{14} could be produced from the galactose and glucose-1-C^{14} and since the concentration of galactose in the reaction mixture is low, the synthesis of allolactose-C^{14} most probably occurs by a transfer of the galactosyl moiety of the trisaccharide to glucose-1-C^{14}. Reaction 2 of the proposed mechanism is evidently also reversible.

TABLE I

RADIOACTIVITIES[a] OF PRODUCTS IN 5 μL. OF REACTION MIXTURE OF ALLOLACTOSE AND GLUCOSE-1-C^{14} IN PRESENCE OF *S. fragilis* ENZYME

Time, hr.	Glucose, c.p.m.	Allolactose, c.p.m.	Trisaccharide, c.p.m.
0	24,500	74	24
1	23,600	830	56
2	23,100	1050	62
4	22,700	1360	94
8	22,300	1590	105

[a] Radioactivities of the compounds were measured with a thin-walled G.M. tube directly on the paper chromatograms.

TABLE II

RADIOACTIVITIES OF PRODUCTS IN 5 μL. OF REACTION MIXTURE OF GLUCOSE-1-C^{14} AND GALACTOSE IN PRESENCE OF *S. fragilis* ENZYME

Time, hr.	Glucose, c.p.m.	Allolactose, c.p.m.
0	24,200	23
0.5	24,200	66
1	24,100	135
2	23,900	194
4	23,900	256

TABLE III

RADIOACTIVITIES IN PRODUCTS IN 5 μL. OF REACTION MIXTURE OF GLUCOSE-1-C^{14} AND TRISACCHARIDE (GAL-GAL-GL) IN PRESENCE OF *S. fragilis* ENZYME

Time, hr.	Glucose, c.p.m.	Allolactose, c.p.m.	Trisaccharide, c.p.m.
0	20,400	83	40
0.5	19,800	350	52
1	19,500	564	108
2	19,200	728	165
4	19,400	620	122

In Table IV, radioactivities of the products in a digest of galactose-1-C^{14} and non-radioactive glucose are recorded. Some radioactive allolactose was produced in this system indicating reversibility of reaction 3. A comparison of the radioactivity values in Tables I, III and IV shows that the rate constant for reversal of reaction 3 is small in comparison to the constants for the reversal of reactions 1 and 2.

TABLE IV

RADIOACTIVITIES OF PRODUCTS IN 5 μL. REACTION MIXTURE OF GLUCOSE AND GALACTOSE-1-C^{14} IN PRESENCE OF *S. fragilis* ENZYME

Time hr.	Galactose, c.p.m.	Allolactose, c.p.m.
0	30,100	56
0.5	29,900	186
1	29,800	214
2	29,700	280
4	29,600	316

The action of the transferring enzyme of *S. fragilis* on allolactose appears to proceed by a two-step mechanism in which step 1 involves the formation of a galactosyl–enzyme complex and step 2 the transfer of the galactosyl unit to carbohydrate

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or to water acceptors. The former reaction results in the synthesis of new oligosaccharides while the latter in hydrolysis of allolactose. Evidence for reversibility of these reactions has been presented.

Experimental

Enzyme Material.—An enzyme preparation from *Saccharomyces fragilis* was kindly provided by Dr. W. M. Connors, National Dairy Research Laboratories, Long Island, New York. Solutions of the enzyme material exhibited hydrolytic and transferring activities. A measure of both types of activities was obtained by assaying the enzyme solution on lactose-1-C^{14}. In the assay procedure, 0.05 ml. of 0.6 *M* solution of lactose-1-C^{14} (0.36 μc./mg.) were treated with 0.05 ml. of the enzyme solution in 0.1 *M* phosphate buffer of *p*H 6.7. Samples of 5 microliters were analyzed at 0 hr. and after incubation at 30° for 1 hr. by the multiple ascent paper chromatographic method.[10] The radioactivities of the lactose and the glucose in the samples were measured directly on the paper chromatograms as previously described.[11] The rate of lactose disappearance is a measure of both hydrolytic and transferring activity while the rate of glucose production is a measure of the hydrolytic activity only. Under the conditions of the assay procedure, 0.065 mmole of lactose were converted to glucose and galactose and 0.026 mmole of lactose were converted to new oligosaccharides in the 1 hr. period by 1 mg. of enzyme preparation. This amount of activity has been defined as one unit of enzyme activity.

Carbohydrates.—Allolactose was prepared as previously described. The specific rotation of the compound was +25° and on paper chromatography in several solvent systems only one reducing spot was detectable in the preparation. Chromatographically pure *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-D-glucose has been previously prepared in this Laboratory[10] and was used in this study. Glucose-1-C^{14}, galactose-1-C^{14} and lactose-1-C^{14} were purchased from the National Bureau of Standards.

Enzymatic Digest of Allolactose.—To 0.08 ml. of 0.15 *M* allolactose solution were added 0.04 ml. of enzyme solution (total activity, 1.2 units) buffered to *p*H 6.7 with 0.1 *M* phosphate buffer. Samples of 5 microliters were placed on paper chromatograms at 0, 0.5, 1, 2, 4 and 8 hr. and immediately inactivated by heating at 100° for 5 minutes. The compounds in the samples were separated on the paper and located by spraying with copper sulfate and molybdic acid reagents. Glucose and galactose appeared in the digest in 0.5 hr. and increased in concentration up to 8 hr. A new compound with chromatographic mobility of a trisaccharide appeared in 0.5 hr., increased in concentration at 1 and 2 hr., decreased at 4 hr. and disappeared at 8 hr. Allolactose decreased progressively in concentration at 0.5, 1, 2, 4 and 8 hr.

For isolation of the trisaccharide, a digest of 0.8 ml. of allolactose (0.15 *M*) and 0.4 ml. of enzyme (12 units) was prepared and inactivated after reaction time of 1.5 hr. The reducing compounds in the digest were separated chromatographically and isolated as previously described.[10] Partial acid hydrolysis of the trisaccharide yielded allolactose and galactobiose while complete hydrolysis yielded glucose and galactose in molar ratio of 1:2. This information and the chromatographic behavior of the compound indicate that the trisaccharide is probably *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-D-glucose.

Enzymatic Digests of Allolactose and Glucose-1-C^{14}.—To 0.05 ml. of a solution of allolactose (0.3 *M*) and glucose-1-C^{14} (0.3 *M* and with specific activity 1.92 μc./mg.) were added 0.05 ml. of enzyme solution (1.5 units). Samples of 5 microliters were placed on a chromatogram after 0, 1, 2, 4 and 8 hr. reaction time and treated as above. The compounds in these aliquots were located on the chromatogram with copper sulfate and molybdic acid sprays and the radioactive products were detected by radioautography. Radioactivity values for glucose, allolactose and the trisaccharide were obtained with a conventional counting apparatus and are recorded in Table I.

Enzymatic Digest of Galactose and Glucose-1-C^{14}.—To 0.05 ml. of solution of galactose (0.3 *M*) and glucose-1-C^{14} (0.3 *M*) was added 0.05 ml. of enzyme solution (1.5 units). Analyses for reducing and radioactive products were performed on aliquots of the digest after 0, 0.5, 1, 2 and 4 hr. as described above. The results are contained in Table II.

Enzymatic Digest of Trisaccharide and Glucose-1-C^{14}.—To 0.05 ml. of solution of trisaccharide (0.07 *M*) and glucose-1-C^{14} (0.3 *M*) were added to 0.05 ml. of enzyme (1.5 units). Samples of 5 microliters were obtained at 0, 0.5, 1, 2 and 4 hr. and were analyzed for reducing and radioactive products by the methods described above. The radioactivities of the products are recorded in Table III.

Enzymatic Digest of Glucose and Galactose-1-C^{14}.—A digest of 0.1 ml. was prepared containing glucose (0.15 *M*), galactose-1-C^{14} (0.15 *M* and specific activity, 2.24 μc./mg.) and 1.5 units of enzyme. Samples of 5 microliters were analyzed at 0, 0.5, 1, 2 and 4 hr. Results of radioactivity measurements on the products are recorded in Table IV.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Isomerization of D-Glucose-1-C^{14} to D- and L-Sorbose-C^{14} by a Strong Base Resin[1]

BY JOHN C. SOWDEN AND ROBERT R. THOMPSON

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The D- and L-sorbose-C^{14} formed by the action of a strong base resin on D-glucose-1-C^{14} contain over 90% of the label in carbons 1 and 6. This fact, as well as the relative distributions of the label between C-1 and C-6 of the enantiomorphs, indicates that the racemic ketose is formed by the enolization–ketonization route rather than by fragment recombination.

(DL + D)-Sorbose recently has been established as a product of the action of potassium hydroxide on D-fructose[2] and of a strong base resin on D-glucose.[3] Since the cleavage of hexose to triose[4] and the condensation of DL-glycerose to racemic sorbose and fructose[5] are known to occur in alkaline solution, it seemed likely[2,3] that the D-hexoses formed racemic sorbose in the above two instances by the fragmentation–racemization–recombination route. However, the results reported herein with D-glucose-1-C^{14} indicate that the racemic ketose is formed almost exclusively by the enolization–ketonization mechanism, and that fragment recombination plays only a minor role in the isomerization.

D-Glucose-1-C^{14} was isomerized by the action of Amberlite IRA-400[6] at 50–60°, and the resulting (DL + D)-sorbose was isolated, converted

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