in 72% yield as a colorless liquid, b.p. 94–96° (2 mm.); $n^{20}D$ 1.5425; $d^{20}_4$ 1.052; $MR_D$ (calcd.)[10] 45.12, $MR_D$ (obs.) 44.39; $\lambda_{max}$ 263 mμ, log ε 3.62; $\lambda_{min}$ 235 mμ, log ε 3.27.

*Anal.* Calcd. for $C_{10}H_{12}O$: C, 81.01; H, 8.16. Found: C, 80.74; H, 8.03.

The **N-(*p*-bromophenyl)-maleimide adduct** crystallized from methanol in colorless needles, m.p. 198–201°.

*Anal.* Calcd. for $C_{20}H_{18}O_3NBr$: C, 60.00; H, 4.53; N, 3.50. Found: C, 60.14; H, 4.54; N, 3.57.

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## Hydroxyalkylcyclosiloxanes

BY MAURICE PROBER

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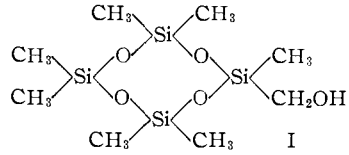
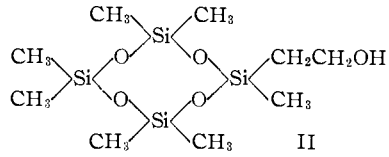
As part of a program on polar silicones, it was of interest to prepare cyclic tetramers containing hydroxyalkyl groups, notably hydroxymethylheptamethylcyclotetrasiloxane (I), and β-hydroxyethylheptamethylcyclotetrasiloxane (II).



I



II

Silicon compounds containing hydroxymethyl groups have been prepared by treating the chloromethyl compound with potassium acetate in acetic acid followed by methanolysis of the acetate.[1] With the chloromethyl cyclic tetramer, the reaction with potassium acetate–acetic acid led to ring opening and formation of polymeric siloxanes.[2] The preparation of I by neutral hydrolysis of the chloromethyl cyclic tetramer was unsuccessful.

Since the Grignard reagent of the chloromethyl cyclic tetramer has been described recently,[3] I was prepared by treating this reagent with oxygen; II was obtained by reaction with formaldehyde. The yields were low in both cases. Although improved yields are often obtained by coöxidizing a Grignard reagent in the presence of isopropylmagnesium bromide,[4] this technique did not increase the yield. Compound I is a crystalline solid, II a somewhat viscous liquid.

I and II were polymerized with ferric chloride and sulfuric acid and cross-linked gels were formed.[5]

### Experimental

**Hydroxymethylheptamethylcyclotetrasiloxane (I).**—Grignard reagents were prepared from 92.1 g. (0.750 mole) of isopropyl bromide in 700 ml. of diethyl ether and 165.3 g. (0.500 mole) of chloromethylheptamethylcyclotetrasiloxane in 700 ml. of diethyl ether. The solutions were mixed and dry oxygen bubbled through the stirred solution for two hours. The solution was allowed to stand overnight and then poured into a 10% ammonium chloride solution containing a trace of sulfuric acid. The ether solution was washed with water, dried and distilled, yielding 78.4 g., b.p. 49–100° at 1.5 mm., with a 59.2-g. residue. Upon rectification of the distillate, there was obtained 24.7 g. of hydroxymethylheptamethylcyclotetrasiloxane, b.p. 55–58° at 0.5 mm., m.p. 52–56°, yield 16%. Recrystallized from Dry Ice-chilled pentane, m.p. 56–57°. Compound I (as a pure solid) exhibited a strong infrared absorption maximum for OH at 2.92 μ.

*Anal.* Calcd. for $C_8H_{24}O_5Si_4$: C, 30.73; H, 7.74. Found: C, 30.6; H, 7.7.

A 13% yield was obtained in the absence of isopropylmagnesium bromide.

**β-Hydroxyethylheptamethylcyclotetrasiloxane** (II).—The Grignard reagent was prepared from 264.6 (0.800 mole) of chloromethylheptamethylcyclotetrasiloxane in diethyl ether, the solution chilled to −30 to −40° and 21.0 g. (0.700 mole) of formaldehyde[6] was distilled in. The reaction mixture was stirred overnight, the temperature slowly rising to room temperature, followed by hydrolysis with dilute sulfuric acid. The ether solution was dried and distilled, yielding 190.7 g., b.p. 70–130° at 2.5 mm., and a 56.4-g. residue. Rectification of the distillate gave 28.5 g. of β-hydroxyethylheptamethylcyclotetrasiloxane, b.p. 112–114° at 9 mm., $n^{20}D$ 1.4163, $d^{20}_4$ 1.020; $MR_D$ (calcd.) 80.96,[7] $MR_D$ (obsd.) 80.42; yield 13%. II (as a pure liquid) exhibited a strong infrared absorption maximum for OH at 2.98 μ.

*Anal.* Calcd. for $C_9H_{26}O_5Si_4$: C, 33.09; H, 8.02. Found: C, 33.2; H, 8.3.

**Polymerization.**—Anhydrous ferric chloride and concentrated sulfuric acid (0.5% by weight) were used as catalysts.[8] The polymerizations were carried out at 80° except for the room temperature reaction of II and sulfuric acid. The polymers were gels which swelled but did not dissolve in toluene.

(1) J. L. Speier, B. F. Daubert and R. R. McGregor, THIS JOURNAL, **70**, 1400 (1948); **71**, 1474 (1949).

(2) J. L. Speier, U. S. Patent 2,550, 205 (August 24, 1951).

(3) M. Prober, THIS JOURNAL, **77**, 3224 (1955).

(4) M. S. Kharasch and W. B. Reynolds, *ibid.*, **65**, 50 (1943).

(5) The cross linking is due to etherification or the formation of trifunctional silicon. The latter could result from the interaction of acid and alcohol to form carbonium ions which undergo silicon–carbon bond cleavage: F. C. Whitmore, L. H. Sommer, J. R. Gould and R. E. Van Strien, *ibid.*, **69**, 1551 (1947); L. H. Sommer and F. J. Evans, *ibid.*, **76**, 1186 (1954).

(6) Monomeric formaldehyde was prepared according to J. F. Walker, "Formaldehyde," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1953, p. 14. Although an excess of formaldehyde had been prepared, less than the stoichiometric amount was distilled into the reaction flask because of polymerization in storage at −78°.

(7) A. I. Vogel, W. T. Cresswell, G. H. Jeffery and J. Leicester, *J. Chem. Soc.*, 531 (1952); A. I. Vogel, W. T. Cresswell and J. Leicester, *J. Phys. Chem.*, **58**, 177 (1954).

(8) J. Marsden and G. F. Roedel, U. S. Patent 2,469,883 (May 10, 1949).

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## Studies on the Decarboxylation of Radioactive Glucuronolactone by *in vitro* Systems[1–3]

BY JOSEPH L. RABINOWITZ

RECEIVED MAY 14, 1955

A soluble enzyme system from various tissues of the rat has been obtained which is capable of decarboxylating $C^{14}$-glucuronolactone as well as $C^{14}$-glucuronic acid. The concentration of this enzyme appeared to be highest in liver and kidney (Table I). Kidney appeared to be more constant in activity than did comparable liver preparations. Active extracts of the enzyme were obtained by treatment of kidney tissues with phosphate buffer at *p*H 7

(1) The radioactive materials were obtained on allocation from the United States Atomic Energy Commission.

(2) The $C^{14}$-glucuronolactone uniformly labeled was obtained through the generosity of the Corn Products Refining Company.

(3) Aided by a grant from the Muscular Dystrophy Association of America, Inc.

TABLE I

ENZYMATIC ACTIVITY OF VARIOUS TISSUES[a]

| Tissues | Expt. no. | | Radioactivity recovered as $CO_2$ Range, c.p.m./mg. in $BaCO_3$ |
|---|---|---|---|
| Kidney | 1 | Tissue slice | 10,000–15,000 |
| | | Homogenate | 16,000–18,000 |
| | 2 | Tissue slice | 14,000–15,000 |
| | | Homogenate | 27,000–29,000 |
| Liver | 1 | Tissue slice | 10,000–11,000 |
| | | Homogenate | 34,000–36,000 |
| | 2 | Tissue slice | 43,000–49,500 |
| | | Homogenate | 203,000–208,000 |
| Brain | 1 | Tissue slice | 850–910 |
| | | Homogenate | 920–950 |
| Heart (muscle) | 1 | Tissue slice | 560–590 |
| | | Homogenate | 480–600 |
| Leg muscle | 1 | Tissue slice | 560–630 |
| | | Homogenate | 375–625 |

[a] Each flask contained 1 g. of tissue or its equivalent amount of protein. Additions were 1 mg. each of ATP, DPN and $C^{14}$-glucurone uniformly labeled; 3-hour incubations at 36°; gas phase $O_2$.

fragments have not as yet been characterized; further studies along this line are in preparation. These results utilizing enzyme systems appear to be parallel to the findings observed by Douglas and King.[4]

TABLE IV

EFFECT OF AGING ON SOLUTIONS OF POTASSIUM GLUCURONATE[a]

| Age of substrate soln. | Substrate form of added solid uniformly labeled | Concn. of substrate, mg. | Radioactivity recovered in $BaCO_3$, c.p.m./mg. C Time of incubation 30 min. | 100 min. |
|---|---|---|---|---|
| 1 min. | $C^{14}$-Glucuronolactone | 1 | 18,000 | 172,000 |
| | | 3 | 26,000 | 185,000 |
| | | 5 | 17,500 | 95,000 |
| 2 hr. | $C^{14}$-Glucuronolactone | 1 | 21,000 | 196,000 |
| | | 3 | 18,000 | 185,000 |
| | | 5 | 12,000 | 85,000 |
| 1 min. | $C^{14}$-Potassium glucuronate | 1 | 44,000 | 222,000 |
| | | 3 | 52,000 | 208,000 |
| | | 5 | 42,000 | 185,000 |
| 2 hr. | $C^{14}$-Potassium glucuronate | 1 | 11,000 | 19,000 |
| | | 3 | 12,200 | 20,000 |
| | | 5 | 15,500 | 230,000 |

[a] Final volume of 1.7 ml. per flask. Additions were 1 mg. each of ATP and DPN; gas phase $O_2$.

TABLE II

ENZYMATIC ACTIVITY OF KIDNEY PREPARATION[a]

| System utilized | | Radioactivity recovered as $CO_2$ in $BaCO_3$, c.p.m./mg. C Expt. no. 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Tissue slices | (T) | ..... | ..... | 9,500 | 15,000 | 32,000 |
| Cell-free whole homogenate[b] | (WH) | 27,000 | 138,000 | 96,000 | 99,000 | 176,000 |
| Washed mitochondrial fraction[c] | (M) | 300 | ..... | ..... | ..... | ..... |
| Lysed mitochondrial fraction | ($M_1$) | 10,000 | ..... | ..... | ..... | ..... |
| Supernatant fraction[d] | ($S_5$) | 159,000 | 465,000 | ..... | ..... | ..... |
| Supernatant fraction[e] | ($S_{10}$) | ..... | ..... | 335,000 | 200,000 | 150,000 |
| Microsomal fraction[f] | (MS) | 300 | 9,600 | ..... | ..... | ..... |
| $S_5 + M_1$ | | 117,000 | 510,000 | ..... | ..... | ..... |
| $S_{10} + M_1$ | | 129,000 | 480,000 | ..... | ..... | ..... |
| $S_{10} + M_1 + MS$ | | ..... | 525,000 | ..... | ..... | ..... |

[a] Each flask contained an equivalent amount of the original tissue used. The additions were 1 mg. each of ATP, DPN and $C^{14}$-glucurone. Incubation was for 3 hours at 36°. Gas phase $O_2$. [b] Supernatant fluid after 7 minutes at 600 × g. [c] Residue of the 40,000 × g. [d] From 40,000 × g. [e] From 100,000 × g. [f] Residue of 100,000 × g.

(Table II). For maximum efficiency, nicotinamide, uridine triphosphate, adenosine monophosphate and diphosphopyridine nucleotide, as well as magnesium ions, were required. 6-$C^{14}$-Glucuronolactone yielded $CO_2$ having six times the radioactivity of that obtained from uniformly labeled $C^{14}$-glucuronolactone (Table III). These results suggest that carbon six is probably the only carbon involved in this decarboxylation. The remaining

TABLE III

DECARBOXYLATION OF UNIFORMLY LABELED $C^{14}$ AND 6-$C^{14}$-LABELED SUBSTRATES[a]

| Radioactive precursor, S.A. 1 mc./mmole | Recovered radioactivity as $CO_2$ in $BaCO_3$, range, c.p.m./mg. C |
|---|---|
| Glucurone-6-$C^{14}$ | 158,000–163,000 |
| Glucurone uniformly labeled-$C^{14}$ | 25,500–37,000 |
| Potassium glucuronate 6-$C^{14}$ | 185,000–194,000 |
| Potassium glucuronate uniformly labeled-$C^{14}$ | 34,000–39,000 |

[a] To 3 ml. of enzyme system, additions were 1 mg. each of ATP, DPN, UTP and 3 mg. of the radioactive substrate; incubation was for 3 hr. at 36°; gas phase $CO_2$.

The addition of citrate, α-ketoglutarate, etc., did not affect the reaction.

Although the same quantitative results were obtained when freshly prepared glucuronolactone was compared with aged preparations of glucuronolactone, the utilization of potassium glucuronate solutions appeared dependent upon the storage time. Freshly prepared solutions were more readily utilized than were older solutions (Table IV).

Because of the rapid rates of isomerization of this substance in aqueous solution it is impossible to decide which form is the active substrate. The enzyme appeared to be most efficient at a *p*H range of 6.8 and 7; efficiency falls off sharply on either side of the optimal *p*H. The enzyme system was saturated with substrate at the concentrations employed as indicated in Table IV.

### Experimental

Male Wistar rats were sacrificed by cervical fracture and the organs quickly removed. Work was carried on in a

(4) J. F. Douglas and C. G. King, *J. Biol. Chem.*, **203**, 889 (1953).

walk-in refrigerator at 0°. The buffer utilized was: $K_2HPO_4$, 0.067 *M*; $KH_2PO_4$, 0.042 *M*; $MgCl_2$, 0.06 *M*; nicotinamide 0.03 *M*, *p*H 7.[5] Surviving tissue slices were prepared in the usual way with a Stadie slicer. Cell-free homogenates were prepared from tissue minces following rapid homogenization for 20 seconds in a loose fitting Potter–Elvehjem glass homogenizer. Two and a half volumes of buffer were employed. Cell debris, cells and nuclei were removed by centrifugation at 2000 r.p.m. for 7 minutes. The remaining particulate fraction was subjected to centrifugation at 20,000 r.p.m. for 30 minutes (40,000 *g*) and the resulting mitochondria were washed with cold buffer solution and resedimented (fraction M). The mitocnondria were extracted by the method of deDuve.[6] The microsomal fraction was obtained after centrifugation at 36,000 r.p.m. for 30 minutes (100,000 g.). A Spinco preparative ultracentrifuge was utilized for all the separations and the speeds indicated refer to dial readings. Flask additions were 1 milligram each of uridine triphosphate, diphosphopyridine nucleotide, adenosine monophosphate and $C^{14}$-glucurone (1 mc./mmole); except when specified. The time interval between preparation of the glucurone solution and the incubation of the solution is given for each experiment. The evolved $CO_2$ was absorbed by a saturated $Ba(OH)_2$ solution layered with toluene. Measurements of radioactivity were performed in a flow counter; the results were corrected to infinite thinness.

**Acknowledgments.**—The author is deeply indebted to Dr. R. M. Dowben and Dr. S. Gurin for their kind encouragement and advice. The author is happy to acknowledge the technical assistance of Mrs. J. Mullin and Miss O. Oleksyshyn.

(5) J. L. Rabinowitz and S. Gurin, *J. Biol. Chem.*, **208**, 307 (1954).

(6) C. deDuve, J. Berthet and F. Applemans, *Nature*, **167**, 389 (1951).

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## Biosynthesis[1] of $C^{14}$-Labeled Cotton Cellulose from D-Glucose-1-$C^{14}$ and D-Glucose-6-$C^{14}$

BY F. SHAFIZADEH AND M. L. WOLFROM

RECEIVED MAY 12, 1955

To study the mechanism of controlled ignition of cellulose nitrate which results in the formation of a variety of decomposition products,[2] we were interested in the production of specifically labeled cellulose-$C^{14}$. The biosynthesis of labeled cellulose has been investigated in the cultures of *Acetobacter xylinum*[3] and *Acetobacter acetigenum*,[4] in a maturing cotton boll,[5] and in growing wheat seedlings.[6,7] Greathouse and associates have reported that the biosynthesis in a maturing cotton boll required the introduction of D-glucose-1-$C^{14}$ at the time of maximum sugar translocation (21 days after fertilization of the flower) and the product cotton cellulose-$C^{14}$ gave on hydrolysis a 44% radiochemical yield of D-glucose-1-$C^{14}$ with 99.97% of the activity at position one[5]; the culture of *Acetobacter xylinum*, in a medium containing D-glucose-1-$C^{14}$ and ethanol, produced bacterial cellulose-$C^{14}$ in a 3.7% radiochemical yield with 70% of the label at carbon 1 and 28.7% at positions 3 and 4.[3] The *Acetobacter acetigenum*, when grown in a medium containing DL-lactate-1-$C^{14}$, gave a product cellulose which was labeled mainly in the middle positions of the D-glucose molecules.[4] Conversely, the radioactive D-glucose unit of the cellulose that was biosynthesized from D-glucose-1-$C^{14}$ and D-glucose-6-$C^{14}$ by the growing wheat seedlings, had about 90% of its activity at the terminal positions (approximately 70% at the original terminal position and 20% at the other).[7] Of these products, labeled cotton cellulose was best suited for our requirements.

Small quantities of D-glucose-1-$C^{14}$ were introduced into ten selected cotton bolls according to the method of Greathouse[5] and the treated bolls were allowed to continue in their normal course of development. The crops of radioactive cotton were separated from the seeds and the dried capsules, pooled together and purified; the seeds and dried capsules were found to be radioactive. A representative sample of the radioactive cotton was hydrolyzed by the procedure of Monier-Williams.[8] The resulting D-glucose was chromatographed over carbon[9] and oxidized to potassium D-gluconate.[10]

Sometime later, another like series of experiments was carried out with D-glucose-6-$C^{14}$ but under better growth conditions. Radioactive assay of the $C^{14}$-labeled potassium D-gluconates indicated a radiochemical yield of approximately 10.6% for the biosynthesis of cellulose-$C^{14}$ from D-glucose-1-$C^{14}$ and 23.5% from D-glucose-6-$C^{14}$, based on the assumption that purified cotton consists of 100% pure cellulose. The distribution of the label between the terminal and middle positions of the anhydro-D-glucose units, which are given in Table I, was established by periodate oxidation of the potassium D-gluconates according to the method of Eisenberg.[11] As noted above, similar results have been obtained by Hassid and associates for the incorporation of D-glucose-1-$C^{14}$ and -6-$C^{14}$ into the cellulose formed by growing wheat seedlings.

TABLE I

$C^{14}$-LABELED COTTON CELLULOSE; DISTRIBUTION OF RADIOACTIVITY IN THE COMPONENT ANHYDRO-D-GLUCOSE UNITS

| | Activity in μc./mole[a] | | |
|---|---|---|---|
| | Cellulose from D-glucose-1-$C^{14}$ | | Cellulose from D-glucose-6-$C^{14}$ |
| Experiment | A[b] | B[c] | C |
| D-Glucose | 29.5 | 128.5 | 129.1 |
| Carbon 1 | 19.1 | 60.5 | 28.6 |
| Carbons 2–5 | 4.4 | 34.3 | 13.5 |
| Carbon 6 | 6.6 | 38.7 | 92.0 |

[a] Microcuries per mole. [b] Preferred greenhouse growth conditions; 10 bolls combined. [c] Less favorable growth conditions; 1 boll.

(1) This work was carried out under contract (DA-33-019-ord-1476; technical supervising agency, Ballistic Research Laboratories, Aberdeen Proving Ground, Maryland) between the Office of Ordnance Research of the U. S. Army Ordnance Corps and The Ohio State University Research Foundation (Project 591).

(2) M. L. Wolfrom, *Abstracts Papers Am. Chem. Soc.*, **127**, 9E (1955).

(3) F. W. Minor, G. A. Greathouse, H. G. Shirk, A. M. Schwartz and M. Harris, THIS JOURNAL, **76**, 1658, 5052 (1954); G. A. Greathouse, H. G. Shirk and F. W. Minor, *ibid.*, **76**, 5157 (1954); F. W. Minor, G. A. Greathouse and H. G. Shirk, *ibid.*, **77**, 1244 (1955).

(4) E. J. Bourne and H. Weigel, *Chemistry & Industry*, 132 (1954).

(5) G. A. Greathouse, *Science*, **117**, 553 (1953).

(6) S. A. Brown and A. C. Neish, *Can. J. Biochem. Physiol.*, **32**, 170 (1954).

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Greathouse[5] has suggested that his data indicate a direct polymerization of intact D-glucose-1-$C^{14}$

(8) G. W. Monier-Williams, *J. Chem. Soc.*, **119**, 803 (1921).

(9) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(10) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 293 (1940).

(11) F. Eisenberg, Jr., THIS JOURNAL, **76**, 5152 (1954).