

minations have yielded four rather divergent results for the anhydrous particle weight.

| | |
|--------------------------------------|--------------------|
| Sedimentation equilibrium | 7.6×10^6 |
| Sedimentation velocity and diffusion | 10.6×10^6 |
| X-Ray analysis | 13.0×10^6 |
| Light scattering | 9.0×10^6 |

It is seen that the method of direct particle counts provides a mean value (9.4×10^6) not outside the limits of previous determinations.

Summary

1. A method of determining the particle weights of macromolecules suspended in a volatile medium is described. The method is called that of "direct particle counts," and consists essentially of counting, on electron micrographs, the numbers of macromolecules per unit volume of solution, and of obtaining the dry weight of an aliquot of the

unit volume. Representative specimen fields are obtained by a spray method.

2. Dow latex particles of polystyrene are used as reference particles to determine the volumes of the spray droplets, which also contain the macromolecules.

3. The dry weight of centrifugally purified particles of the bushy stunt virus has been determined, and is found to be $9.4 \pm 0.7 \times 10^6$ in molecular weight units.

4. Discussions of sources of error are given, and it is concluded that the primary source of error is in the determination of the weight concentration of the latex particles in the spray droplets.

5. Evidence bearing on the purity of the bushy stunt virus preparations is discussed, and it is concluded that the particle weights of the material in the two most highly purified pellets are the most reliable.

ANN ARBOR, MICH.

RECEIVED JUNE 9, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Configurational Correlation of (*levo*)-Glyceraldehyde with (*dextro*)-Lactic Acid by a New Chemical Method¹

BY M. L. WOLFROM, R. U. LEMIEUX,² S. M. OLIN² AND D. I. WEISBLAT

Freudenberg³ converted (*dextro*)-glyceric acid to (*dextro*)-lactic acid by methods which did not sever any of the bonds directly attached to the rotational center. (*levo*)-Glyceric acid was obtained by the oxidation of *D*-(*dextro*)-glyceraldehyde by Wohl and Schellenberg.⁴ These data effect a configurational correlation between *D*-(*dextro*)-glyceraldehyde and (*levo*)-lactic acid and establish the designation *D*-(*levo*)-lactic acid. We report herein a confirmation of this correlation by a chemical method similar in principle to that employed in correlating *L*-(*levo*)-glyceraldehyde with *L*-(*dextro*)-alanine.⁵

Tetraacetyl-2-methyl-*D*-glucose diethyl thioacetal (I) was reductively desulfurized to yield tetraacetyl-2-methyl-1-desoxy-*D*-glucitol (II). Deacetylation of II produced 2-methyl-1-desoxy-*D*-glucitol (III) which on periodate cleavage with subsequent oxidation yielded an *O*-methylactic acid (IV) that was isolated as its chromatographically purified *p*-phenylphenacyl ester⁶ of m. p. 74–75° and $[\alpha]_D^{23} - 16.5^\circ$ (*c* 2, benzene). This deriva-

tive was found to be identical with that obtained on the methylation of the *p*-phenylphenacyl ester of *L*-(*dextro*)-lactic acid (V), thus effecting a configurational correlation between *L*-glyceraldehyde and *L*-(*dextro*)-lactic acid. Carbon two in I is originally *D*_g, wherein the subscript⁷ denotes the reference standard glyceraldehyde, but this carbon becomes *L*_g in the product of periodate cleavage in which the aldehyde group of *D*-glucose has been reduced to the hydrocarbon stage. These operations effectively produce an interchange of groups on this rotatory center with a consequent reversal of configuration.

Incidental to the above transformations, we describe herein the crystalline tetraacetyl-2-methyl *aldehydo*-*D*-glucose obtained by demercaptalation of I according to general technics previously described.^{8,9}

Experimental¹⁰

Tetraacetyl-2-methyl-*D*-glucose Diethyl Thioacetal (I).¹¹—2-Methyl-*D*-glucose diethyl thioacetal¹² (20 g.) was acetylated by overnight treatment at room temperature with acetic anhydride (150 ml.) and pyridine (75 ml.). Crystalline material of fair purity was obtained on pouring the reaction mixture into 3 liters of ice and water; yield 27 g. Pure material was obtained on further crystalliza-

(1) This correlation was reported in *Abstracts Papers Am. Chem. Soc.*, **113**, 13Q (1948).

(2) Bristol Laboratories Research Associate (R. U. L.) and Research Fellow (S. M. O.) of The Ohio State University Research Foundation (Project 224).

(3) K. Freudenberg, *Ber.*, **47**, 2027 (1914).

(4) A. Wohl and R. Schellenberg, *Ber.*, **55**, 1404 (1922).

(5) M. L. Wolfrom, R. U. Lemieux and S. M. Olin, *THIS JOURNAL*, **71**, 2870 (1949).

(6) Following the general procedure of J. G. Kirchner, A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **18**, 31 (1946) recommended for the separation of fatty acids.

(7) E. J. Crane, *Chem. Eng. News*, **25**, 1364 (1947).

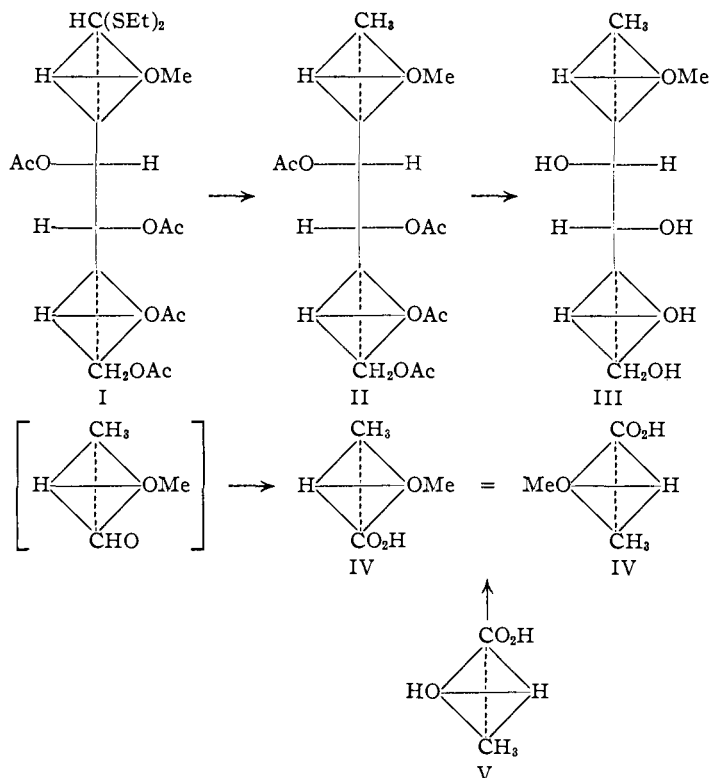
(8) M. L. Wolfrom, *THIS JOURNAL*, **51**, 2188 (1929).

(9) M. L. Wolfrom, M. Konigsberg and D. I. Weisblat, *ibid.*, **61**, 574 (1939).

(10) Unless otherwise noted, all experimental work was performed by Mr. S. M. Olin.

(11) Experimental work by D. I. Weisblat.

(12) T. Lieser and E. Leckzyck, *Ann.*, **511**, 137 (1934).



tion from methanol-water; yield 25 g., m. p. 45–46°, $[\alpha]^{25}_D +40^\circ$ (*c* 3, abs. CHCl_3), diamond-shaped plates. The substance was readily soluble in alcohol, ether and chloroform and was insoluble in water and petroleum ether.

Anal. Calcd. for $\text{C}_{11}\text{H}_{20}\text{O}_8(\text{CH}_3\text{CO})_4$: C, 48.70; H, 6.88; CH_3CO , 8.54 ml. 0.1 *N* NaOH per 100 mg. Found: C, 48.58; H, 6.70; CH_3CO , 8.93 ml.

Tetraacetyl-2-methyl-aldehyde-D-glucose.¹¹—To 12.6 g. of tetraacetyl-2-methyl-D-glucose diethyl thioacetal dissolved in 45 ml. of acetone was added 25 g. of finely powdered cadmium carbonate and 5 ml. of water. To this was added with vigorous mechanical stirring, 25 g. of mercuric chloride dissolved in 40 ml. of acetone. Stirring was maintained for twenty hours at room temperature and then the reaction mixture was heated rapidly to boiling and gently refluxed for thirty minutes. The mixture was filtered into a flask containing cadmium carbonate (10 g.) and the residue was washed with acetone. The filtrate and washings were concentrated to dryness at 35° under reduced pressure in the presence of cadmium carbonate. The residue was extracted with three 100-ml. portions of warm chloroform (U. S. P.) and the extract was washed successively with water, 10% aqueous potassium iodide, and water until halide-free. The sirup obtained on solvent removal from the dried (decolorizing carbon) extract was crystallized from 25 ml. of ether; yield 6.3 g. (70%), m. p. 87–89°. Pure material was obtained on further crystallization from acetone-ether-petroleum ether (2:1:3); m. p. 89–90°, $[\alpha]^{25}_D +24^\circ$ (*c* 3, abs. CHCl_3), $[\alpha]^{25}_D +7.5^\circ$ (extrapolated) $\rightarrow +31^\circ$ (1.5 hr., *c* 3, methanol). The substance crystallized as elongated prisms that reduced Fehling solution. It was readily soluble in ether, alcohol, chloroform, acetone and hot water; it was insoluble in petroleum ether and cold water.

Anal. Calcd. for $\text{C}_7\text{H}_{10}\text{O}_6(\text{CH}_3\text{CO})_4$: C, 49.72; H, 6.12; CH_3CO , 11.04 ml. 0.1 *N* NaOH per 100 mg. Found: C, 49.78; H, 6.03; CH_3CO , 11.08 ml.

Tetraacetyl-2-methyl-1-desoxy-D-glucitol (II).—Raney nickel (25 g.) and tetraacetyl-2-methyl-D-glucose diethyl

thioacetal (5 g.) was refluxed in 70% aqueous ethanol (110 ml.). After five hours no ethanethiol odor could be detected on acidification of the warm alcoholic solution and the reaction was assumed to be complete. The catalyst was removed by centrifugation and washed with 95% ethanol. The centrifugate and washings were combined, treated with activated carbon and filtered through a bed of Celite.¹³ The sirup obtained on solvent removal under reduced pressure was crystallized from ethanol-water at 15°; yield 3.7 g. (92%), m. p. 62–65°, $[\alpha]^{25}_D +39^\circ$ (*c* 2, CHCl_3). Further crystallization enhanced the melting point to 67–68°.

Anal. Calcd. for $\text{C}_{15}\text{H}_{24}\text{O}_9$: C, 51.72; H, 6.93. Found: C, 51.92; H, 6.99.

2-Methyl-1-desoxy-D-glucitol (III).—Tetraacetyl-2-methyl-1-desoxy-D-glucitol (2.85 g.) was dissolved in anhydrous methanol (125 ml.) and a rapid stream of anhydrous ammonia was passed into the solution for fifteen minutes at 0°. The mixture was maintained at 25° for two hours and evaporated to dryness at reduced pressure. The crystalline residue was dissolved in warm methanol and crystallization was effected at 15°; yield 1.33 g. (2 crops, 92%), m. p. 133–134°, $[\alpha]^{25}_D +21.5^\circ$ (*c* 3, water). Further crystallization from methanol did not alter these constants.

Anal. Calcd. for $\text{C}_7\text{H}_{10}\text{O}_6$: C, 46.65; H, 8.95. Found: C, 46.70; H, 9.23.

***p*-Phenylphenacyl Ester of O-Methyl-L-lactic Acid (IV) from 2-Methyl-1-desoxy-D-glucitol (III).**—A solution of 1.18 g. of 2-methyl-1-desoxy-D-glucitol in 20 ml. of water was treated at 25° for sixty minutes with 25 ml. of *N* periodic acid. The solution was then made just alkaline to phenolphthalein with aqueous barium hydroxide. The filtered solution was kept overnight with strontium carbonate (10 g.) and bromine (5 g., shaken until dissolved). The filtered solution was then acidified with 2.5 *N* hydrochloric acid (2.5 ml.) and extracted with ether for seventy-two hours in a continuous extractor containing barium carbonate and 25 ml. of water in its distillation flask. The ether was then distilled from the suspension, which was then heated on a boiling water-bath for two hours. Barium carbonate was removed by filtration and the aqueous filtrate was concentrated to dryness under reduced pressure. The residual barium salt was dissolved in 4 ml. of water, acidified with 0.5 ml. of 6 *N* hydrochloric acid and refluxed for sixty minutes with 12 ml. of a solution of 0.6 g. of *p*-phenylphenacyl bromide in 12 ml. of 95% ethanol. Crystalline material separated on pouring the cooled solution into 20 ml. of water; yield 0.8 g.

An amount of 0.5 g. of the above material was dissolved in 25 ml. of benzene and added at the top of a 200 × 55 mm. (diam.) column of precipitated silicic acid¹⁴ (160 g.) and developed with 1 liter of 2:2 benzene:petroleum ether (b. p. 85–100°, volume ratio). Two zones, at 16–17 mm. and 20–21 mm. from the top, were detected on the extruded column by ultraviolet light. Crystalline material was obtained from the acetone eluate of the sectioned top zone; yield 0.4 g., m. p. 72–74°, $[\alpha]^{25}_D -16.0^\circ$ (*c* 2, benzene). Pure material was obtained on recrystallization from ethanol-water; m. p. 74–75° unchanged on admixture with the product obtained from O-methyl-L-lactic acid as described below; $[\alpha]^{25}_D -16.5^\circ$ (*c* 2, benzene).

(13) No. 535, Johns-Manville, New York, N. Y., a siliceous filter aid.

(14) Mallinckrodt Chemical Works, St. Louis, Missouri.

Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.40; H, 6.03.

***p*-Phenylphenacyl O-Methyl-L-lactate from L-(*dextro*)-Lactic Acid (V).**—An authentic sample of L-(*dextro*)-lactic acid¹⁵ was converted to the *p*-phenylphenacyl ester (m. p. 139–140°¹⁶) and 0.76 g. of this ester, 7.5 g. of silver oxide and 20 ml. of methyl iodide were shaken mechanically at room temperature for four hours. The silver salts were removed by filtration and washed with warm methanol. The filtrate and washings were concentrated to a crystalline residue under reduced pressure; yield 0.70 g., m. p. 70–73°, $[\alpha]^{25}_D -15.5^\circ$ (*c* 3, benzene). Recrystallization from ethanol-water yielded pure material with constants unaltered by further crystallization or by chromatographic purification effected as described above; yield 0.54 g., m. p. 74–75°, $[\alpha]^{25}_D -16.2^\circ$ (*c* 3, benzene).

(15) A product of high purity made by the fermentation of carbohydrate material and for which we are indebted to the Clinton Industries, Inc., Clinton, Iowa.

(16) N. L. Drake and J. Bronitsky, *THIS JOURNAL*, **52**, 3715 (1930), record 145° as the melting point of the *p*-phenylphenacyl ester of lactic acid (presumably D,L).

Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.49; H, 6.06.

Summary

1. Reductive desulfurization of tetraacetyl-2-methyl-D-glucose diethyl thioacetal (I) yielded tetraacetyl-2-methyl-1-desoxy-D-glucitol (II) which on deacetylation (III) and periodate cleavage with subsequent oxidation produced an O-methyl-lactic acid (IV) whose *p*-phenylphenacyl ester was identical with that obtained by esterification and methylation of L-(*dextro*)-lactic acid (V). This effects a direct chemical correlation of L-(*levo*)-glyceraldehyde with L-(*dextro*)-lactic acid.

2. Tetraacetyl-2-methyl-aldehydo-D-glucose is described.

COLUMBUS, OHIO

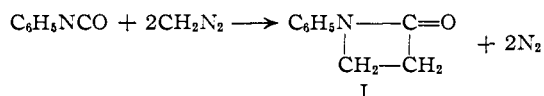
RECEIVED MAY 31, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Reaction of Diazomethane with Isocyanates and Isothiocyanates

BY JOHN C. SHEEHAN AND PATRICK T. IZZO¹

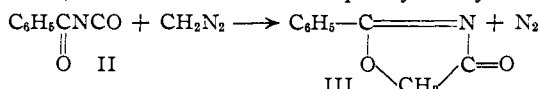
In a recent communication from this Laboratory the action of diazomethane on phenyl isocyanate was reported.² In a reaction analogous to the formation of cyclobutanone from ketene and diazomethane, the β -lactam of N-phenyl- β -alanine is formed.



The preparation of this same β -lactam, 1-phenyl-2-azetidinone (I), by cyclization of an ester of N-phenyl- β -alanine with a Grignard reagent has since been described.³ Phenyl isocyanate and diazomethane were reported by v. Pechmann⁴ to yield an oily product which was not further characterized.

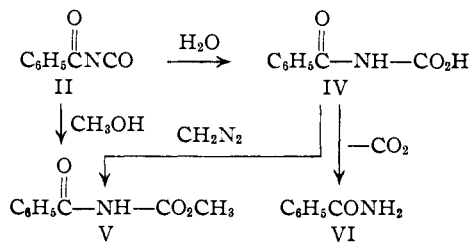
With *p*-bromophenyl isocyanate a β -lactam also is obtained. Under similar conditions no β -lactam was isolated from the treatment with diazomethane of α -naphthyl, *p*-nitrophenyl, benzyl and benzoyl isocyanates, or methyl and phenyl isothiocyanates.

Benzoyl isocyanate (II) reacted very rapidly at ice-bath temperature to give, in 68–70% yield, 2-phenyl-4-oxazolone (III). Unlike the formation of the β -lactam in the case of phenyl isocyanate,



benzoyl isocyanate added only one methylene group, bridging its two reactive carbonyl groups.

The structure of 2-phenyl-4-oxazolone was proved by cleavage with boiling water. Hydrolysis took place at the carbon–nitrogen double bond, forming benzoylglycolic acid amide, which was identified by comparison with an authentic sample.⁵ About 10–15% of the total reaction products was found to be N-benzoyl methyl carbamate (V), which was also obtained by interaction of methanol and benzoyl isocyanate. Absorption of a small amount of moisture during the addition of diazomethane to benzoyl isocyanate would explain the formation of this compound. A portion of the unstable N-benzoylcarbamic acid (IV) formed by the addition of water to the isocyanate presumably was methylated by diazomethane, and thus its decarboxylation to benzamide (VI) was prevented. However, some decomposition to VI apparently took place as benzamide was found in small amounts among the reaction products.



Phenyl isothiocyanate and diazomethane have been reported by v. Pechmann⁴ to form an equimolar adduct, m. p. 172.5°, in unspecified yield. We obtained the addition product in 60% yield,

(1) Swift Postdoctoral Fellow, 1947–1948. Present address: American Cyanamid Company, Linden, New Jersey.

(2) J. Sheehan and P. Izzo, *THIS JOURNAL*, **70**, 1985 (1948).

(3) Clarke, Johnson and Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 977.

(4) H. v. Pechmann, *Ber.*, **28**, 861 (1895).

(5) Aloy and Ch. Rabaut, *Bull. soc. chim.*, **13**, 458 (1913).