alone hydrochloride, 77 mg., and noradrenaline, 53 mg., a 28% yield. The activity was 8.5×10^4 c.p.m. per mg. Identity and purity of the noradrenaline were established by microanalysis, pharmacological activity and isotope dilution assay.

Full experimental details for this synthesis are available on microfilm.[3]

(3) For full experimental details of this synthesis order Document 3847 from American Documentation Institute, c/o Library of Congress, Washington 25, D. C., remitting $1.25 for microfilm (images 1 inch on standard 35-mm. motion picture film) or $1.25 for photostats readable without optical aid.

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Temperature Coefficients of Rotation of Some *o*- and *p*-Nitrophenyl Glycosides and their Polyacetates[1]

BY JACK A. SNYDER AND KARL PAUL LINK

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Pigman[2] has suggested that the anomalous positive rotations of the *ortho*-substituted phenyl β-D-glycoside tetraacetates are due to "interactions between the acetyl and aglycon groups. Such interaction might take the form of weak bonds between these groups or it might operate through steric hindrance to free rotation of the aglycon group about the glycosidic linkage." This was postulated on the basis of the large temperature coefficients of rotation of these glycosides in contrast to those of their *m*- and *p*-isomers. We have determined the rotations of several *o*- and *p*-nitrophenyl glycosides and their polyacetates over the range 15–45°, and find that the *ortho* compounds have large temperature coefficients while the *para* compounds have normal coefficients. This indicates that the acetate groups are not directly concerned with the production of large temperature coefficients and favors their explanation on the basis of steric hindrance.

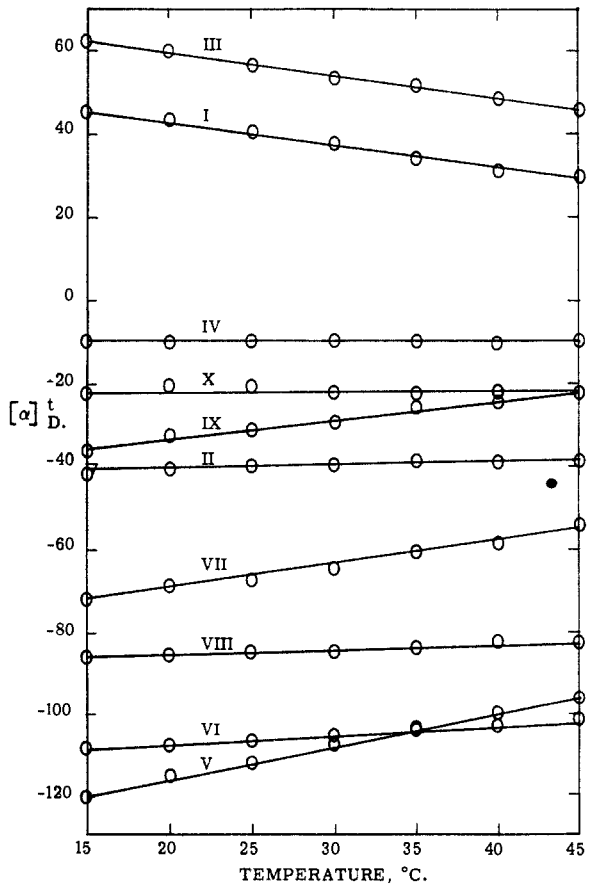

Fig. 1.—Influence of temperature on specific rotation of some *o*- and *p*-nitrophenyl glycosides and their polyacetates.

Experimental

Change of Specific Rotation with Temperature.—The method of preparation of the compounds studied has been reported previously.[3] Rotations were determined with a Schmidt and Haensch polarimeter No. 52-b with monochromator. A 2-dm. jacketed tube was used, with water, maintained at $t \pm 0.2°$ by means of a thermostatically controlled water-bath, as the circulating fluid. No correction was made for liquid density change with temperature.

TABLE I

SOLVENTS AND CONCENTRATIONS IN DETERMINATION OF CHANGE OF SPECIFIC ROTATION WITH TEMPERATURE

Compound	Solvent	Concn., %
o-Nitrophenyl β-D-glucoside tetraacetate (I)	Chloroform	1.966
p-Nitrophenyl β-D-glucoside tetraacetate (II)	Chloroform	1.884
o-Nitrophenyl β-D-galactoside tetraacetate (III)	Chloroform	1.865
p-Nitrophenyl β-D-galactoside tetraacetate (IV)	Chloroform	1.983
o-Nitrophenyl β-D-glucoside (V)	Water	0.828
p-Nitrophenyl β-D-glucoside (VI)	Water	0.987
o-Nitrophenyl β-D-galactoside (VII)	Water	1.065
p-Nitrophenyl β-D-galactoside (VIII)	Water	0.980
o-Nitrophenyl α-L-arabinoside (IX)	Water	.290
p-Nitrophenyl α-L-arabinoside (X)	Water	.265

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The Sedimentation Constant of Insulin in Acid Solution: A Re-examination[1]

BY FRANK TIETZE AND HANS NEURATH

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On the basis of their observations on the sedimentation and diffusion constants of bovine insulin in acid solution, Fredericq and Neurath[2] concluded that the minimum molecular weight of this protein was about 6000. Although this conclusion has received support from the more recent work of Harfenist and Craig[3] on counter-current distribu-

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(2) W. W. Pigman, *J. Research Natl. Bur. Standards*, **33**, 129 (1944).

(3) J. A. Snyder and K. P. Link, THIS JOURNAL, **74**, 1883 (1952).

(1) This work has been supported by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, to whom we are also indebted for the supply of crystalline insulin.

(2) E. Fredericq and H. Neurath, THIS JOURNAL, **72**, 2684 (1950).

(3) E. J. Harfenist and L. Craig, *ibid.*, **74**, 3087 (1952).

tion studies of insulin partially substituted by dinitrofluorobenzene, other investigations, notably osmotic pressure[4] and light scattering[5,6] measurements, have failed to yield a minimum molecular weight of less than 12,000.[7] In view of these discrepancies it was deemed of importance to re-examine the sedimentation behavior of insulin under conditions similar to those of Fredericq and Neurath.[2] These results of these more detailed measurements are the subject of the present communication.

In the present measurements, certain improvements in technique over those of the preceding study[2] were made in an effort to increase the precision of sedimentation analyses of low molecular weight portions. These have included (1) use of a Gaertner microcomparator for measurement of peak displacements and (2) use of the "synthetic boundary cell" of Harrington, Schachman and Pickels[8] in place of the conventional cell. With regard to (1), it may be remarked that the previous practice of determining peak displacements from enlarged projections of the photographic plates was found to lack precision particularly when low protein concentrations and slow sedimentation rates were involved, which give rise to rapid boundary blurring. As regards (2), use of the synthetic boundary cell served to eliminate one of the chief difficulties previously encountered with insulin, particularly in dilute solutions: when sufficient time was allowed to elapse for the boundary to separate from the meniscus so as to reveal the region of the maximum gradient, boundary spreading had occurred to such an extent as to impair the precise location of the boundary peak. With the synthetic boundary cell, however, the boundary becomes visible almost immediately after the centrifuge has attained full speed and photographs can be taken while the gradient curve is still sharp and its position well defined.

Preliminary experimentation with the synthetic boundary cell revealed that the rotor speed at which boundary formation occurred was a critical factor. Irregular results were obtained when boundary formation took place outside the limits of 5000 to 10,000 r.p.m. and hence all such data were discarded. While this procedure is admittedly arbitrary and influenced by the characteristics of the particular cell which has been used, it did serve to minimize scatter of the data.

All runs were performed in a Spinco Model E ultracentrifuge at 59,780 r.p.m. at an average rotor temperature of 24–27°. Each individual run was extended over a time not exceeding 1 hour during which the average temperature rise was not more than 1°. Sedimentation constants were calculated in the usual manner from the slope of a plot of log x *vs.* time, where x is the distance in cm. of the maximum ordinate of the gradient curve from the center of rotation. Correction of the observed sedimentation constants to standard conditions (20°, water as solvent) was carried out in the customary manner.[9]

Phosphate buffers, *p*H 2.6, of ionic strength 0.1, 0.2 and 0.4, respectively, were prepared by the addition of phosphoric acid to calculated quantities of potassium dihydrogen phosphate. Crystalline zinc insulin, Lot T-2842, was used since the supply of Lot T-2344, employed by Fredericq and Neurath,[2] was exhausted. These two lots, however, appear to be of the same degree of purity.

The results of sedimentation measurements in phosphate buffer of varying ionic strength are shown in Fig. 1. For comparison, the experimental data previously reported[2] are also included in Fig. 1. Although considerable scatter of the data is evident at 0.1 ionic strength, particularly at lower protein concentrations, this curve appears to lie convex relative to the axis of the abscissas. This somewhat anomalous behavior, previously observed,[4] is in accord with the suggestion that at a salt concentration of 0.1 ionic strength the sedimentation potential is not entirely suppressed[4] throughout the range of insulin concentrations employed here.[10] In higher insulin concentrations the resulting decrease in sedimentation rate is apparently overcome by the increased aggregation of the protein, for the slope of the curve relating sedimentation constant to protein concentration becomes positive beyond 0.8% insulin. In view of these observations, no linear extrapolation of these data to zero protein concentration was attempted.

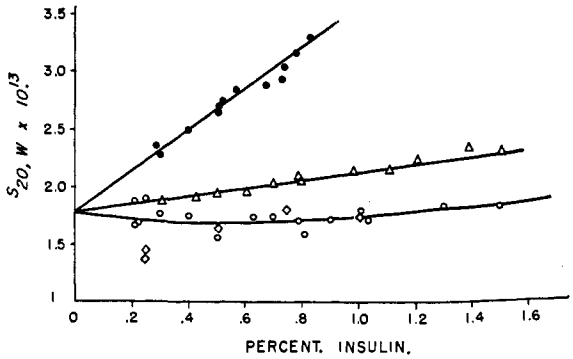

Fig. 1.—Concentration dependence of the sedimentation rate of insulin in phosphate buffers, *p*H 2.6; the ionic strength was: ●, 0.4; △, 0.2; ○, 0.1. The previous data of Fredericq and Neurath[3] are indicated by ◇.

At ionic strengths of 0.2 and 0.4 the sedimentation constants increase with increasing protein concentration throughout the entire range. The concentration dependence, moreover, appears to be linear in each case and permits unambiguous extrapolation to zero concentration. The extrapolated sedimentation constant is 1.78 Svedberg units (S). This value is considerably higher than that previously reported[3] (1.2 S) but is in fair agreement with that reported by Gutfreund[4] for a 0.25% solution of Boots insulin (1.65 S). While it is conceivable

(4) H. Gutfreund, *Biochem. J.*, **42**, 544 (1948); **50**, 564 (1952).

(5) F. Tietze and H. Neurath, *J. Biol. Chem.*, **194**, 1 (1952).

(6) P. Doty, M. Gellert and B. Rabinovitch, THIS JOURNAL, **74**, 2065 (1952); P. Doty and G. E. Myers, *Trans. Faraday Soc.*, in press.

(7) The sedimentation analyses of Ellenbogen and Oncley (J. L. Oncley, E. Ellenbogen, D. Gitlin and F. R. N. Gurd, *J. Phys. Chem.*, **56**, 85 (1952)) are likewise in agreement with a molecular weight of 12,000; however, these measurements were not performed under conditions of maximum dissociation of the protein and were not extended to as low a range of protein concentration as the other investigations previously cited.

(8) W. F. Harrington, H. K. Schachman and E. G. Pickels, Abstracts of the 122nd Meeting, American Chemical Society, 51C (1952); E. G. Pickels, W. F. Harrington and H. K. Schachman, *Proc. Nat. Acad. Sci., U. S.*, **38**, 943 (1952). We are indebted to Dr. E. G. Pickels for placing an experimental model of this cell at our disposal.

(9) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940.

(10) K. O. Pedersen, *Cold Spring Harbor Symposium Quant. Biol.*, **14**, 140 (1950).

that at ionic strengths 0.2 and 0.4 the measurements have not been extended to sufficiently low protein concentrations to exclude a further downward curvature to lower sedimentation constants, such a possibility is minimized by the behavior at 0.1 ionic strength. Although the latter data cannot be precisely extrapolated, they do show a trend toward a common intercept with the data obtained at the higher ionic strengths. It must be concluded, therefore, that in the presence of phosphate buffer *p*H 2.6, the extrapolated sedimentation constant of bovine insulin is in agreement with a molecular weight of 12,000.

Acknowledgment.—We are indebted to Mr. Roger M. Wade for the performance of sedimentation measurements.

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The Relative Catalytic Activity of Nickel Produced by the Reduction of Nickel(II) Bromide with Liquid Ammonia Solutions of Different Alkali Metals[1]

BY GEORGE W. WATT AND PEGGY I. MAYFIELD

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Burgess and co-workers have reported marked differences in both the chemical and catalytic activity of silver and nickel precipitated by the reduction of salts with solutions of metals in liquid ammonia at its normal boiling temperature. Thus, the reduction of certain silver salts with solutions of potassium yielded silver far more active than that which resulted when sodium was employed.[2] Similar differences were observed in studies involving the reduction of silver salts with solutions of calcium[3] and in the reduction of nickel salts with sodium, potassium and calcium.[4] No explanation of these differences was proposed by Burgess, *et al.*, and since similar observations have been made in our laboratories it seemed worthwhile to carry out somewhat more definitive experiments.

In view of the presently accepted interpretation of the physical nature of solutions of metals in liquid ammonia,[5] it seems unlikely that differences in the properties of these reduction products are attributable to any inherent differences in the nature of the metal solutions. Rather it is more likely that both the chemical and catalytic activities of the reduction products are determined by rate factors and solubility relationships.

Although both the rates of solution of the alkali and alkaline earth metals in ammonia and the rates of the ensuing reactions with nickel(II) bromide are too rapid for accurate measurements, our experiments show qualitatively that both of these rates increase from lithium to cesium. Furthermore, the solubilities of the by-products (alkali bromides and amides) increase in the same direction. Thus, one obtains from the corresponding reactions, elemental nickel that is different in only one important respect, namely, surface area. This is shown by the fact that for the products obtained using lithium, sodium, potassium, rubidium and cesium as the reducing metals, catalytic activity per unit surface area is substantially constant. The relative insolubility of the by-products obtained using calcium obviated a rigorous comparison including this metal.

Burgess and Eastes[4] have attributed the pyrophoric character of the elemental nickel so-produced to the presence of adsorbed hydrogen. While all of the products prepared in our studies were pyrophoric in a degree that increased from lithium to cesium, the corresponding quantities of adsorbed hydrogen per unit weight of metal showed no consistent trend.

Experimental

Materials.—Hexamminenickel(II) bromide was prepared as described by Watt.[6] All other materials were commercial reagent grade chemicals.

Reduction Reactions.—The equipment and procedures employed were in all respects the same as those described previously[7] except that lithium was maintained in an atmosphere of nitrogen prior to addition to the solution and suspension of nickel(II) bromide, and that rubidium and cesium were added in fragile glass ampoules that were subsequently crushed.

When samples of hexamminenickel(II) bromide of the order of 2.5 g. in 15–20 ml. of liquid ammonia at −33.5° were treated with alkali metals (*ca.* 10% in excess of that required for complete removal of bromide ion), both the rates of solution of the alkali metals and the rates of the ensuing reactions with the bromide were quite evidently dependent upon the alkali metal employed. *Approximate* total times that elapsed between the addition of the alkali metal and the disappearance of the blue color characteristic of solutions of these metals in ammonia were as follows: Li, 5 min.; Na, 20 sec.; K, 10 sec.; Rb, < 10 sec.; Cs, << 10 sec. Following completion of the reactions, the ammonia-insoluble products were washed with liquid ammonia, with ethanol, and thereafter handled out of contact with the atmosphere and under strictly anhydrous conditions.

Properties of the Reduction Products.—By methods previously described,[7] the highly pyrophoric ammonia-insoluble products were analyzed for nickel, nitrogen, bromine and

TABLE I

PROPERTIES OF PRODUCTS FROM THE REDUCTION OF NICKEL (II) BROMIDE WITH ALKALI METALS IN LIQUID AMMONIA

	Ammonia-insoluble product				
Alkali metal	Ni, %	H_2, cc./g.	Surface area, $m.^2/g.$	Reaction rate	Rate/unit area
Li	82.3	17.6	30[a]	1.6	0.05
Na	93.6	7.5	27	3.1	.11
K	92.0	18.7	54	3.8	.07
Rb	90.4	10.4	105	8.8	.08
Cs	83.9	2.1	127	9.1	.07

[a] This value was determined using a sample washed with liquid ammonia but not with ethanol and involves a correction for an initial rapid uptake of ammonia during the surface area determinations. This was attributed to the ammonation of impurities present and the validity of this procedure was confirmed by a surface area estimate obtained from electron photomicrographs of an ethanol-washed product which showed an average particle radius of 88 Å. and led to a computed surface area of 38 $m.^2/g.$

(1) This work was supported, in part, by the Office of Naval Research, Contract N6onr-26610.

(2) W. M. Burgess and F. R. Holden, THIS JOURNAL, **59**, 459 (1937).

(3) W. M. Burgess and F. R. Holden, *ibid.*, **59**, 462 (1937).

(4) W. M. Burgess and J. W. Eastes, *ibid.*, **63**, 2674 (1941).

(5) W. C. Johnson and A. W. Meyer, *Chem. Revs.*, **8**, 273 (1931); *cf.* W. L. Jolly, *ibid.*, **50**, 351 (1952).

(6) G. W. Watt, "Inorganic Syntheses," Vol. II, McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 194.

(7) G. W. Watt, W. F. Roper and S. G. Parker, THIS JOURNAL, **73**, 5791 (1951).