ucts from which only β-estradiol could be isolated in pure form.

### Experimental

The estrone esters were prepared in excellent yield by the method of Miescher and Scholz.[8]

**β-Estradiol.**—A mixture of 400 mg. of estrone, 0.2 cc. of 20% sodium hydroxide solution and 20 cc. of methanol was added to a solution of 150 mg. of sodium borohydride in 20 cc. of methanol. After the evolution of hydrogen had ceased, the reaction mixture was poured into 30 cc. of water and neutralized with dilute hydrochloric acid. The precipitate was filtered, washed with water and immediately recrystallized from aqueous methanol; yield 360 mg. (90%), m.p. 172–174°. A mixed m.p. with an authentic sample of β-estradiol showed no depression.

**The Reduction of Estrone Trimethylacetate. A. By Lithium Aluminum Hydride.**—To 900 mg. (0.0025 mole) of estrone trimethyl acetate dissolved in 100 cc. of anhydrous ether was added with stirring 200 mg. (0.0052 mole) of lithium aluminum hydride in 15 cc. of anhydrous ether. The mixture was stirred for another hour, cooled and 10 cc. of water added. A precipitate formed, which was dissolved by the addition of 10 cc. of ice-cold 10% sulfuric acid.[9] The aqueous layer was separated and the ether layer concentrated to dryness *in vacuo*. The white, crystalline residue was suspended in 50% aqueous methanol and filtered; yield 700 mg. (100%), m.p. 175–177°. A mixed m.p. with an authentic sample of β-estradiol showed no depression.

When stoichiometric amounts of lithium aluminum hydride were used 85% of the estrone trimethylacetate was recovered unchanged. The remainder could not be purified further, but gave a very strong positive test for free phenolic groups with diazotized sulfanilic acid.

**B. By Calcium Hydride.**—A mixture of 900 mg. (0.0025 mole) of estrone trimethylacetate, 210 mg. (0.0050 mole) of calcium hydride and 75 cc. of 97% aqueous methanol was allowed to stand until the evolution of hydrogen had ceased. The filtrate was added to an equal volume of water, the precipitate filtered and 250 mg. of material collected, m.p. 156–160°. After recrystallization from methanol 200 mg. of the product was recovered; m.p. 165.5–166.5°. A mixed m.p. with an authentic sample of estrone trimethylacetate showed no depression. The aqueous methanol filtrate was chilled and 250 mg. of a solid collected, m.p. 247–255°. After recrystallization from methanol the material melted at 254–256°. A mixed m.p. with an authentic sample of estrone showed no depression.

**β-Estradiol 3-Trimethylacetate.**—To 250 mg. (0.0062 mole) of sodium borohydride in 10 cc. of methanol was added 900 mg. of estrone trimethylacetate dissolved in 10 cc. of hot methanol. After hydrogen evolution had ceased (*ca.* 30 minutes), 20 cc. of water was added slowly with stirring, the precipitate collected by filtration, washed with 50% methanol and immediately recrystallized from aqueous methanol; yield 600 mg., m.p. 179–181°. To the boiling filtrate water was added to a point just short of turbidity. On cooling, 200 mg. of product was obtained after filtration, m.p. 178–180°. The two fractions were combined; yield 800 mg. (89%); a mixed m.p. with a sample of β-estradiol 3-trimethylacetate obtained from the catalytic hydrogenation of estrone trimethylacetate[9] gave no depression. A test with diazotized sulfanilic acid showed the absence of free phenolic groups.

**β-Estradiol 3-*t*-Butylacetate.**—The procedure was identical with the one described for the preparation of the trimethylacetate. From 500 mg. of estrone ester there was obtained 350 mg. (70%) of the β-estradiol 3-ester, m.p. 127–129°. A mixed m.p. with a sample obtained from the catalytic hydrogenation of estrone *t*-butylacetate[9] gave no depression.

**The Reduction of Estrone Acetate with Sodium Borohydride.**—A solution of 500 mg. of estrone acetate in 10 cc. of methanol was added to 150 mg. of sodium borohydride in 10 cc. of methanol and the reaction mixture allowed to stand for 1 hour at room temperature, at which time no more hydrogen was evolved. Thirty cc. of water was then added slowly to the cooled solution and the resulting precipitate filtered and washed with cold 50% methanol; yield 400 mg., m.p. 170–175°. After recrystallization from aqueous methanol 350 mg. of a crystalline precipitate was obtained, m.p. 174–175°. A mixed m.p. with an authentic sample of β-estradiol showed no depression. The product coupled readily with diazotized sulfanilic acid showing the presence of a free phenolic group.

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[8] K. Miescher and C. Scholz, *Helv. Chim. Acta*, **20**, 263 (1937).

[9] An aqueous alcoholic acid solution of β-estradiol trimethylacetate showed no signs of hydrolysis after standing at room temperature for several hours.

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## The Oxidation of Glucose and Related Compounds with Oxides of Nitrogen[1]

BY ED. F. DEGERING[2] AND ROBERT A. BERNTSEN[3]

Saccharic acid has been prepared in moderate (40–46%)[4] yields by the oxidation of glucose with nitric acid.[4,5,6] The importance of saccharic acid[4] and the fact that acids have been obtained by the nitrogen tetroxide oxidation of certain compounds containing a primary hydroxyl group, such as cellulose,[7,8] starch[9] and galactose,[10] prompted this experimental work.

The accompanying experimental work shows that glucose when oxidized by nitrogen tetroxide at 35° in the presence of potassium carbonate gives potassium acid saccharate in 50% yield. Gluconic acid can be oxidized in a nitrogen tetroxide medium to give the same substance in 42% yield. Oxalic acid was not attacked. Potassium acid saccharate could be recovered in 85% yield after treatment with nitrogen tetroxide, but since the salt is relatively soluble in water the loss appears to be mechanical in nature.

### Experimental

**Materials.**—Anhydrous dextrose (C.P., Mallinckrodt) dried in a vacuum desiccator over phosphorus pentoxide or glucose (American Maize Products Co.) dried in a vacuum desiccator at 70° and 10 mm. pressure was used. Nitrogen tetroxide (Solvay Process Company) was dried over phosphorus pentoxide before use.

**Oxidation of Glucose in an Inert Medium.**—Glucose (0.1 mole) was added to 100 ml. of the inert medium (carbon tetrachloride, petroleum ether or toluene) in a 500-ml., round-bottom three-neck flask which contained a Dry Ice finger-type condenser and an efficient stirrer. The nitrogen tetroxide (0.09 or 0.17 mole) was added through a graduated ice water-cooled West condenser with attached stopcock and ground-glass joint. The experiments were carried out in a constant temperature water-bath at 25 ± 1° for a period of time from 1 to 96 hours. After the period of oxidation, the excess nitrogen tetroxide was blown out and the inert medium decanted off. The oxidation product was washed once with ether and then dissolved in water and the *p*H of the solution was adjusted to 9 using potassium carbonate, potassium hydroxide or both. After hydrolysis of the lactone ring, enough acetic acid was added to adjust the *p*H to 4. The resulting potassium acid saccharate was filtered off, air-dried, and weighed. The yield varied from 10 to 44%.

In a similar manner except for the presence of 0.1 g. of aluminum chloride, cobalt oxide, copper acetate, copper

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[1] From the Ph.D. Thesis of Robert A. Berntsen, Purdue University.

[2] Miner Laboratories, 9 South Clinton, Chicago 6, Ill.

[3] Augustana College, Rock Island, Ill.

[4] C. L. Mehltretter, U. S. Patent 2,436,659 (Feb. 24, 1948).

[5] A. A. Dietz, Ph.D. Thesis, Purdue University, 1941.

[6] J. W. Mench, Ph.D. Thesis, Purdue University, 1944.

[7] C. Unruh and W. O. Kenyon, THIS JOURNAL, **64**, 127 (1942).

[8] E. C. Yackel and W. O. Kenyon, *ibid.*, **64**, 121 (1942).

[9] J. W. Mench with Ed. F. Degering, *Proc. Ind. Acad. Sci.*, **55**, 69 (1946).

[10] K. Maurer and G. Drefahl, *Ber.*, **75**, 1489 (1942).

powder, fused vanadium pentoxide, iron filings, manganese dioxide, potassium nitrite or sodium fluoride in the carbon tetrachloride medium, the yields of potassium acid saccharate varied from 12 to 44%.

**Oxidation of Glucose in Nitrogen Tetroxide Medium.**—In a similar manner except for a 100-ml. round-bottom flask and 60 ml. of nitrogen tetroxide as solvent, a 25 to 44% yield of potassium acid saccharate was obtained by the oxidation of glucose (0.1 mole). A solution of urea was used to remove the last traces of the oxides of nitrogen. When 5 g. of potassium carbonate was added to the reaction mixture the yield of potassium acid saccharate, neut. equiv. 247.6, increased to 45%. When 5 g. of potassium carbonate was added to the reaction mixture at 35 ± 0.2°, the yield of potassium acid saccharate, neut. equiv. 247.4, was 50%.

**Treatment of Gluconic Acid and Its Lactone with Nitrogen Tetroxide.**—D-Gluconolactone (0.05 mole) was dried over phosphorus pentoxide under a vacuum and then treated with nitrogen tetroxide (0.17 mole) in 150 ml. of carbon tetrachloride for 24 hours at 25 ± 1°. The product was treated as was the glucose oxidation run to obtain 4.6 g. (37%) of potassium acid saccharate, neut. equiv. 246.5. Gluconic acid (0.1 mole) was dried in a vacuum over phosphorus pentoxide and treated with nitrogen tetroxide (0.35 mole) in 150 ml. of carbon tetrachloride for 9 hours at 25 ± 1°. The product was treated as was the glucose oxidation run to obtain 8.39 g. (34%) of potassium acid saccharate, neut. equiv. 252.5. Gluconic acid (0.1 mole) was also treated with nitrogen tetroxide in a nitrogen tetroxide medium for 3 hours at 25 ± 1°. The product was treated as was the glucose oxidation run to obtain 10.4 g. (42%) of potassium acid saccharate, neut. equiv. 248.2.

**Treatment of Oxalic Acid with Nitrogen Tetroxide.**—Anhydrous oxalic acid (0.05 mole) was treated with nitrogen tetroxide (0.17 mole) in carbon tetrachloride at a temperature of 25 ± 1° for 24 hours. After the period of oxidation, the excess nitrogen tetroxide was blown out and the water soluble component was extracted and transferred to a 250-ml. volumetric flask. To aliquots was added ammonium hydroxide to a *p*H of approximately 4. The calcium salt was precipitated out using calcium acetate. Following isolation and the dissolution of the calcium oxalate in sulfuric acid, the solution was titrated with standard potassium permanganate. The amount of oxalic acid found agreed within experimental error with that initially treated with nitrogen tetroxide.

**Treatment of Potassium Acid Saccharate with Nitrogen Tetroxide.**—Potassium acid saccharate (0.1 mole) and 6 ml. of glacial acetic acid were treated with nitrogen tetroxide (0.35 mole) in 150 ml. of carbon tetrachloride at 25 ± 1° for 8 hours. The product was treated to obtain 22.3 g. (90%) unreacted potassium acid saccharate, neut. equiv. 251.4. Potassium acid saccharate (0.1 mole) was also treated with nitrogen tetroxide in a nitrogen tetroxide medium for 3 hours at 25 ± 1°. The product was treated to obtain 20.9 g. (84%) of potassium acid saccharate, neut. equiv. 249.3.

CHICAGO, ILLINOIS RECEIVED AUGUST 14, 1950

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## L-Rhamno-2,4-dinitrophenylhydrazone

BY JORGE ALEJANDRO DOMÍNGUEZ

In 1927 Glaser and Zuckermann[1] reported the preparation of glucose and galactose 2,4-dinitrophenylhydrazones. Subsequently, Allen[2] and Brady,[3] unaware of this work, claimed that 2,4-dinitrophenylhydrazine did not give precipitates with the sugars.

**L-Rhamno-2,4-dinitrophenylhydrazone.**—Five ml. of glacial acetic acid and 0.8 g. of 2,4-dinitrophenylhydrazine were added to a solution of 1 g. of rhamnose in 5 ml. of water. This mixture was heated at 50° during 20 minutes, and allowed to stand at room temperature for 12 hours. The precipitated orange mass was collected, washed with water and a little cold alcohol, and recrystallized from hot alcohol. The yield of the dry yellow crystals was 1.0 g. (71%) m. p. 164–165° (uncor.). The melting point did not change after three recrystallizations.

*Anal.* Calcd. for $C_{12}H_{16}O_8N_4$: N, 16.27. Found: N, 16.18.

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[1] E. Glaser and N. Zuckermann, *Z. physiol. Chem.*, **167**, 37 (1927).

[2] C. F. H. Allen, THIS JOURNAL, **52**, 2955 (1930).

[3] O. L. Brady, *J. Chem. Soc.*, **133**, 756 (1931).

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## The "Exponential" Adsorption Isotherms of Fatty Acids on Activated Charcoal

BY E. GLUECKAUF

F. H. M. Nestler and H. G. Cassidy[1] have investigated the simultaneous adsorption of acetic, propionic and butyric acids on active charcoal from aqueous solution. They have tried to interpret these data by utilizing the binary exponential isotherm by Glueckauf[2] which should apply when the exponential form is caused by a statistical distribution of adsorbing centers of different affinity.[2,3] However, they were able to obtain agreement with the observations only, by introducing an extra constant not provided by the theory. This note is intended to show that the exponential single-solute isotherms in this case need not be due to non-uniformity of the adsorbent, but can be caused by the strong affinity of activated charcoal for hydrogen ions, and that Nestler and Cassidy's binary adsorption data can be explained as an anion exchange process.

It has been shown by Steenberg[4] that charcoal in a mixture of strong acids displays all the characteristics of an anion-exchanger, the primarily adsorbed group being the $H_3O^+$ ion. The chromatographically obtained data of Nestler and Cassidy fully support this view also for the weaker fatty acids, as the values obtained for the exchange factor $k_3$ are independent of the concentrations both for the pairs of acetic + propionic and propionic + butyric acids (see Table I). This feature is characteristic of exchange and of Langmuir isotherms, but is quite improbable for exponential ones.

TABLE I

ACETIC (1) AND PROPIONIC (2) ACID

$$k_3 = \frac{q_1c_2}{c_1q_2} = 1 - \frac{c_{1,1}(w_2 - w_1^0)}{c_1^0w_2} \qquad (1)$$

$c_{1,1}$ and $w_1^0$ = concentration and breakthrough volume of solute 1 (concentrations in wt. %). $c_1^0$ = concentration of solute 1 at breakthrough of solute 2. $w_2$ = breakthrough volume of solute 2 (see ref. 1)

| $c_1^0$ | $c_{1,1}$ | $w_2$ | $w_1^0$ | $k_3$ |
|---|---|---|---|---|
| 0.982 | 1.521 | 18.35 | 9.47 | 0.25 |
| .759 | 1.145 | 22.35 | 11.05 | (.24) |
| .752 | 1.153 | 22.75 | 11.79 | .26 |
| .503 | 0.751 | 27.70 | 14.24 | .28 |
| .379 | .566 | 32.96 | 16.83 | .27 |
| .251 | .376 | 41.35 | 20.75 | .26 |
| .152 | .210 | 55.60 | 27.0 | .29 |

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[1] F. H. M. Nestler and H. G. Cassidy, THIS JOURNAL, **72**, 680 (1950).

[2] E. Glueckauf, *J. Chem. Soc.*, 1322 (1947).

[3] G. D. Halsey and H. S. Taylor, *J. Phys. Chem.*, **15**, 624 (1947).

[4] B. Steenberg, Thesis, Uppsala University, Sweden, 1944.