

Four almost equal fractions were obtained: 80–100°, 100–120°, 120–200°, and a non-distillable residue. The sublimate distilling at 120–200° was in the range in which one would expect to find the pregnanediols if present. As these are quite insoluble in acetone, each of the four fractions was dissolved in an equal volume of this solvent, but no product crystallized. The fraction also resisted crystallization from other solvents. Therefore, the total sublimate was combined and refluxed for ten hours with an equal weight of sodium in 100 cc. of dry xylene. If *allo*-pregnanediol had been present it would have been converted to the configuration at the 3-hydroxyl which would precipitate with digitonin. But when the isomerized product was treated with alcoholic digitonin, less than 100 mg. of insoluble digitonide was formed, showing the absence of *allo*-pregnanediol. This fraction was then oxidized with chromic acid in acetic acid at room temperature for thirty minutes. Only 4 g. of ketonic materials was obtained from 24 g. of carbinol, the remainder being acids. Upon sublimation of the ketones no crystalline product could be obtained.

The non-distillable carbinol fraction (9 g.) was oxidized by dissolving in 150 cc. of acetic acid and adding 4 g. of chromic anhydride in 25 cc. of 80% acetic acid. After

standing for thirty minutes, water was added. The product, isolated in the usual manner, was treated with Girard's reagent and the ketonic fraction (1.3 g.) sublimed in a high vacuum. The fraction distilling at 100–120° crystallized from dilute methanol, giving androstanedione, m. p. 125–128°, which gave no depression in melting point when mixed with an authentic sample.

*Anal.* Calcd. for  $C_{19}H_{28}O_2$ : C, 79.1; H, 9.8. Found: C, 79.0; H, 9.9.

### Summary

The steroidal content of steers' urine was investigated. Unlike bulls' urine, steers' urine contains no pregnanediols. A relatively large amount of cholesterol but no equistanol was found. The ketonic fraction gave androsterone, dehydroisoandrosterone and estrone in approximately the same proportions as found in bulls' urine. The non-distillable carbinols gave a small amount of androstanedione on oxidation. The characteristic urinary hydrocarbon,  $C_{28}H_{58}$ , was found.

STATE COLLEGE, PENNA. RECEIVED FEBRUARY 27, 1939

## NOTES

### Esterification of Highly Hindered Acids

BY REYNOLD C. FUSON, JOSEPH CORSE AND E. C. HORNING

Esterification of highly hindered acids is generally very difficult to accomplish by direct methods. Satisfactory yields of methyl esters have been obtained, however, by thermal decomposition of the corresponding tetramethylammonium salts according to the procedure of Prelog and Piantanida.<sup>1</sup> The method is based on observations of Lawson and Collie<sup>2</sup> and involves a reaction which is closely related to that which occurs when betaines are decomposed thermally.<sup>3</sup>

The method has now been applied to 2,4,6-trimethyl- and 2,4,6-triethylbenzoic acids to determine the influence of excessive hindrance. Tetramethylammonium hydroxide made by the method of Walker and Johnson<sup>4</sup> was employed to make the tetramethylammonium salts. These

(1) Prelog and Piantanida, *Z. physiol. Chem.*, **244**, 56 (1936).

(2) Lawson and Collie, *J. Chem. Soc.*, **53**, 631 (1888).

(3) Willstätter, *Ber.*, **35**, 587 (1902); Willstätter and Kahn, *ibid.*, **37**, 401, 1853 (1904); Prelog, *Coll. trav. chim. Tchech.*, **2**, 712 (1930); Kuhn and Giral, *Ber.*, **68**, 387 (1935).

(4) Walker and Johnson, *J. Chem. Soc.*, **87**, 955 (1905).

were decomposed by heating to 200–250°. The yields of pure methyl esters varied from 63 to 90% of the theoretical amounts.

The methyl 2,4,6-triethylbenzoate is a new compound. It boils at 114–115° (5 mm.);  $n_D^{20}$  1.5012;  $d_4^{20}$  0.982.

*Anal.* Calcd. for  $C_{14}H_{20}O_2$ : C, 76.86; H, 9.47. Found: C, 76.59; H, 9.48.

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### The Isolation of a Crystalline Substance from Starches Oxidized by Periodate

BY D. H. GRANGAARD, J. H. MICHELL AND C. B. PURVES

By degrading various periodate oxy-starches with acid methyl alcohol,<sup>1</sup> we have isolated a white, crystalline, levorotatory compound with the formula  $C_{13}H_{16}O_8(OCH_3)_4$  and m. p. 150–150.5° (corr.). Although stable to further oxidation with periodate or Fehling's solution, the substance

(1) Jackson and Hudson, *THIS JOURNAL*, **60**, 989 (1938).

readily reduced the latter after mild acid hydrolysis and appeared to be the methyl acetal of an aldehyde or ketone. This in turn was possibly a fragment of a still larger chemical unit which was oxidized with the starch. No conclusive evidence was obtained to indicate whether the crystals were derived from an integral part of the starch macromolecule or from an associated impurity.

The crystalline material was obtained in 0.7 to 0.9% yield from high grade commercial specimens of corn, wheat, potato and arrowroot starches. It was also isolated from soluble potato starch<sup>2</sup> which after being oxidized was dialyzed against water for four days. Preliminary experiments with xylan gave none of the substance while with cellulose the trace of apparently crystalline material observed was too minute to be identified with certainty.

This note is published because only two of us (J. H. M. and C. B. P.) intend to continue the investigation.

### Experimental

Sodium periodate, 34.0 g. or 0.116 mole of  $\text{Na}_3\text{H}_2\text{IO}_4$  was shaken at room temperature with 500 cc. of water and 50 cc. of glacial acetic acid until as much as possible had been dissolved. Starch, 20.25 g. air dry or 0.114 mole dry, was then added and the mixture, pH about 4.2, gently agitated until the next day, when titration<sup>3</sup> showed that all of the periodate had reacted. The oxy-starch was filtered off, washed free of iodate, completely dried in a desiccator and suspended in dry methanol containing 10% of hydrogen chloride. Five hours of heating under a reflux condenser completed the degradation and after the removal of acid as the lead salt the solvent was evaporated. The viscid, levorotatory residue was dissolved in 70 cc. of 0.2 *N* aqueous caustic soda, the solution was almost saturated with sodium chloride and extracted with a total volume of 1500 cc. of diethyl ether. After drying, the extract yielded a pale yellow oil which partly crystallized when alternately cooled in dry-ice and allowed to thaw. The same crystals also separated from the high-boiling fraction of the oil (b. p. 170–180° at 3–4 mm.). Two recrystallizations from ether left the m. p. constant at 150–150.5° (corr.) and not depressed by admixture with specimens derived from other starches. The yield of pure material was 0.15 to 0.17 g. for the different starches and either sodium or potassium periodate, buffered to pH 4.2 with acetate or phthalate, was used in the preparation.

*Anal.* Calcd. for  $\text{C}_{13}\text{H}_{16}\text{O}_8(\text{OCH}_3)_4$ : C, 48.11; H, 6.60;  $\text{OCH}_3$ , 29.24; mol. wt., 424. Found for two independent preparations: C, 48.21, 47.77, 47.75; H, 6.61, 6.59, 6.63;  $\text{OCH}_3$ , 28.87, 29.09, 29.20; mol. wt. (Rast), 419, 425, 428, 432 for 3–10% solutions.

The substance was sparingly soluble in water and petroleum ether but dissolved more readily in other solvents.

(2) Baird, Haworth and Hirst, *J. Chem. Soc.*, 1201 (1935).

(3) Treadwell and Hall, "Analytical Chemistry," 8th ed., Vol. II, John Wiley and Sons, Inc., New York, p. 616.

The specific rotation at 26° in dioxane was  $-7.1^\circ$  (sodium light, *C*, 0.911).

CONTRIBUTION No. 188

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## The Dissociation Constant of Hypobromous Acid

BY MANFRED KIESE AND A. BAIRD HASTINGS

In experiments on the catalytic effect of certain acids upon the hydration of carbon dioxide, it became necessary to know the dissociation constant of hypobromous acid. No data on this constant were found in the literature.

Hypobromous acid was prepared by allowing bromine in aqueous solution to react with mercuric oxide. The acid was purified by distillation in vacuum. Since hypobromous acid is not very stable, the experiments were carried out immediately after the preparation of the acid, in a constant temperature room, at a solution temperature of 5°.

The dissociation constant was estimated from data obtained by the titration of 0.07 *M* solutions of hypobromous acid with 0.7 *N* sodium hydroxide; pH was measured potentiometrically, using the glass electrode and a saturated calomel cell. The standard of reference used in the calculation of the pH values was "standard" acetate assuming its pH = 4.65.<sup>1</sup> Three titration curves were determined (obtaining from 15 to 20 points for each curve), and the dissociation constants calculated by application of the mass law equation. The average value of the apparent dissociation constant was found to be  $1.0 \times 10^{-9}$ , or expressed logarithmically  $pK' = 9.0 \pm 0.06$ .

It may be noted that the value of  $pK$  found for hypobromous acid lies between that reported for hypochlorous acid,  $pK = 7.6$ ,<sup>2</sup> and that for hypiodous acid,  $pK = 10.6$ .<sup>3</sup>

(1) D. I. Hitchcock and A. C. Taylor, *THIS JOURNAL*, **59**, 1812 (1937).

(2) References in Gmelin, *Handbuch der anorg. Chemie*, Vol. VI, 1927, p. 235.

(3) F. Fürth, *Z. Elektrochem.*, **28**, 57 (1922).

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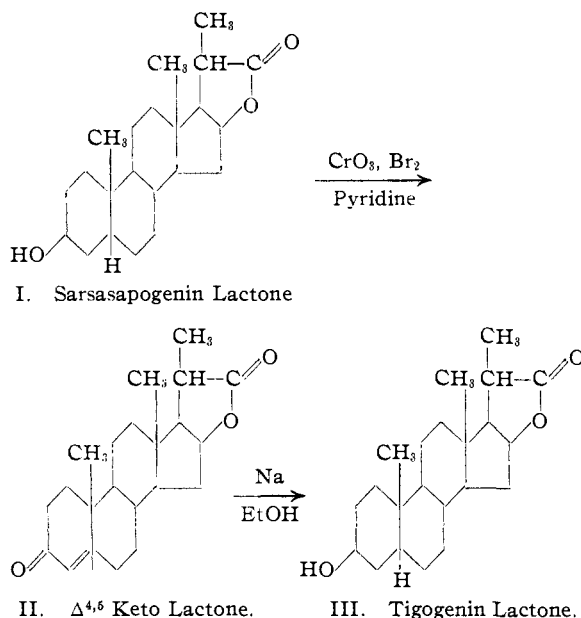
BOSTON, MASS. RECEIVED FEBRUARY 16, 1939

## Sterols. LXII. Position of the Hydroxyl Group in Tigogenin and Sarsasapogenin

BY RUSSELL E. MARKER AND EWALD ROHRMANN

Until the present time no substantial chemical evidence has been given which would distinguish

between positions at C-2 and C-3 for the hydroxyl group of tigogenin and sarsasapogenin. Likewise, it has not been shown that the hydroxyl group occupies the same position in both compounds. We have converted the hydroxy sarsasapogenin lactone into its *allo* form and find that this is identical with the hydroxy tigogenin lactone. This transformation indicates that the sarsasapogenin lactone differs from the tigogenin lactone only in regard to the configuration at C<sub>5</sub> and that the hydroxyl groups are located at C-3.



The keto lactone of sarsasapogenin first described by Farmer and Kon<sup>1</sup> was caused to react with one mole of bromine to give a bromo keto lactone, m. p. 194–195°. *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{31}\text{O}_3\text{Br}$ : C, 62.4; H, 7.4. Found: C, 62.3; H, 7.5. Treatment of this compound with boiling pyridine gave an unsaturated keto lactone, m. p. 213–214°. *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{30}\text{O}_3$ : C, 77.15; H, 8.8. Found: C, 77.1; H, 8.9. This was reduced with sodium and ethanol and the epimeric forms separated with digitonin. Decomposition of the insoluble digitonide yielded a hydroxy lactone, m. p. 234–235°. *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{34}\text{O}_3$ : C, 76.25; H, 9.9. Found: C, 76.3; H, 10.0. This formed an acetate, m. p. 217–218°, which gave no depression with an authentic sample of tigogenin lactone acetate, m. p. 217–218°, first described by Tschesche and Hagedorn.<sup>2</sup> *Anal.* Calcd. for  $\text{C}_{24}\text{H}_{36}\text{O}_4$ : C, 74.15; H, 9.4. Found: C, 73.9; H, 9.3. Mild oxidation of the lactone with chromic anhydride gave a keto lactone, m. p. 252–254°. *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{32}\text{O}_3$ : C, 76.7; H, 9.4. Found: C, 76.7; H, 9.6.

We wish to thank Parke, Davis and Company for their

(1) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).

(2) Tschesche and Hagedorn, *Ber.*, 68, 1412 (1935).

generous help and assistance in the various phases of this work.

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## On the Combination of Electrophoretic and Chromatographic Adsorption Methods

BY HAROLD H. STRAIN

Resolution of mixtures of water-soluble compounds on Tswett adsorption columns<sup>1</sup> is facilitated by application of electrical potential to the ends of the columns. This combination of chromatographic and electrophoretic methods extends and enhances the utility of both methods for the isolation of pure substances from mixtures.

Colored substances are separated upon adsorption columns by electrophoresis in the following manner. An electrode of coiled wire covered loosely with cotton is placed in the constricted end of a glass tube and the tube is filled with the adsorbent, usually cotton, Hyflo Super Cel (heat-treated siliceous earth) or mixtures of this with talc. Before use, the adsorption column is attached to a suction flask and filled with water. A little of the mixture of charged or ionized colored substances to be separated is then drawn into the upper portion of the column where it forms a rather homogeneous colored band. The suction is released; a second electrode is placed at the top of the column and covered with water; and potential is applied to the two electrodes. This causes the pigments to migrate toward the electrodes of opposite charge and gives rise to a series of colored bands or zones each of which contains a single pigment.

Compounds separated upon adsorption columns by electrophoresis may be isolated in two ways. The bands of colored substances may be removed separately from the column and the pigments themselves eluted from the adsorbent with additional water, ethanol, alkalis or acids, or the pigments may be washed through the columns and collected separately in successive portions of the percolate.

In striking contrast to the effect obtained in the usual electrophoretic experiments where the bands of colored substances migrate with little or no widening, the materials on Tswett columns spread out rapidly until rather homogeneous colored zones are obtained. The pigments in

(1) M. Tswett, *Ber. deut. botan. Ges.*, 24, 384 (1906).

these regions appear to migrate along the surface of the adsorbent. This latter phenomenon was not observed in columns filled with weakly adsorptive, impervious materials such as sand and small glass beads.

The width of the bands of adsorbed substances formed in columns by electrophoresis depends upon the quantity of pigment present and upon the adsorptive capacity of the adsorbent. In experiments at room temperature with large columns, the bands of migrating pigments always exhibit well-defined boundaries throughout the mass of the adsorbent. The adsorbent thus serves to prevent mixing of the colored substances as well as to aid in their separation.

A pronounced electro-osmotic effect has been observed in all the experiments carried out thus far. Since the water usually moves toward the cathode, this electrode is placed at the top of the column so that the head of water may be adjusted to balance the electro-osmotic pressure. Passage of the water through the adsorbent also may be regulated, as customary in the Tswett method, by application of suction to the base or of pressure to the top of the column.

A great number of colored, water-soluble, organic substances such as nitro, hydroxy, amino, azo, carboxy and sulfonic compounds can be isolated from mixtures by electrophoresis upon Tswett columns. Combination of colorless organic compounds with colored, water-soluble radicals before adsorption would doubtless facilitate the separation of mixtures of colorless substances by the electrophoretic and chromatographic methods.<sup>2</sup>

Thus far, compounds separable by electrophoresis but inseparable by chromatographic adsorption have not been found. However, with columns of the same size and with equal quantities of pigments, electrophoresis often produces greater separation of the pigments than percolation alone.

The mixtures separated on Tswett columns by electrophoresis (the first compound named in each group remaining nearest the cathode) were: aminoazobenzene and indigo carmine, 3-nitro-4-aminoanisole and indigo carmine, methyl orange and methyl red, methyl orange and 2,6-dichlorophenol indophenol, methyl orange and methyl red and indigo carmine, picric acid and methyl orange. For the separation of these compounds, potentials ranging from 175 to 200 volts and

(2) H. H. Strain, *THIS JOURNAL*, **57**, 758 (1935).

currents from 0.5 to 2 m. a. were used with columns ranging in size from 2 by 13 cm. to 3 by 23 cm. The quantity of each pigment adsorbed varied from 2 to 15 mg.

From weakly acid solutions, two forms of the indicators were separated on Tswett columns both by chromatographic adsorption and by electrophoresis. The forms present in acid solutions were much more strongly adsorbed than those present in weakly alkaline solutions. Indicators adsorbed from acid solutions were gradually converted into the isomers observed in alkaline solutions upon prolonged washing of the columns with water or upon extended passage of the electric current. These methods provide another means for investigation of the tautomerism of indicators.

Resolution of mixtures by adsorption upon Tswett columns depends upon the properties of the adsorbent, of the solvent and of the compounds in the mixture. Since the properties of the adsorbent vary with the method employed in its preparation and since the properties of the solvent are altered by small quantities of impurities, considerable experience is often required to adapt this effective adsorption technique to the isolation of new compounds from mixtures.

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### The Temperature of Maximum Density of D<sub>2</sub>O and of its Mixtures with H<sub>2</sub>O

BY ELIJAH SWIFT, JR.

The temperature of maximum density of deuterium oxide has been reported as 11.4<sup>1</sup> and 11.6<sup>2</sup>, the latter being the generally accepted value. This measurement was made on a sample of only 0.12 ml. volume in a small dilatometer, and the temperature taken as the temperature of maximum density was apparently that at which the meniscus was at its minimum position in the capillary. A temperature obtained in this manner is not the true temperature of maximum density, but what might be termed *the apparent temperature of maximum density in glass*, the difference between the two amounting to about 0.5° in the case of Pyrex.<sup>3</sup> It was hoped that a rede-

(1) T. Takeuchi and T. Inai, *Japan J. Phys.*, **11**, 67 (1936).

(2) G. N. Lewis and R. T. Macdonald, *THIS JOURNAL*, **55**, 3057 (1933).

(3) "Pyrex" brand chemical glass.

termination of the true temperature of maximum density might allow a definite and significant value to be assigned to it.

The measurements necessary to determine this value were made in a 20-ml. pycnometer with a capillary of about 1 mm. diameter, with a single very fine scratch on the exterior.<sup>4</sup> The technique of the measurements consisted in filling the pycnometer to a point just above the mark and measuring the height of the meniscus above the mark at a series of temperatures close to the temperature of maximum density. The measurements were made with a travelling microscope reading to 0.005 mm., and the temperatures were measured with thermometers calibrated at the ice-point and at 5 and 11° against Bureau of Standards calibrated thermometers kindly loaned by Professor Grinnell Jones.

The observed heights of the meniscus were plotted against the temperature and a smooth curve drawn. This was then corrected for the thermal expansion of the glass,<sup>5</sup> the resulting curve then showing a minimum at the true temperature of maximum density. In order to check the accuracy of this method the temperature of maximum density of distilled water was measured. This was found to be  $3.85 \pm 0.1^\circ$ , somewhat lower than the accepted value of 3.98, the difference probably being due in part to the presence of dissolved air, carbon dioxide, etc., since

no attempt was made to remove all traces of dissolved impurities.

The temperature of maximum density of two different samples of nearly pure deuterium oxide was determined. The first was purified by chemical means and by distillation<sup>4</sup> and the second, loaned by Dr. Fred Stitt, was prepared from deuterium gas and atmospheric oxygen. The temperature of maximum density of the two samples was  $11.1 \pm 0.1^\circ$ , there being no detectable difference between the two. It is probable that the temperature of maximum density of pure D<sub>2</sub>O is not more than a few thousandths of a degree above this value. The temperature at which the meniscus was at its minimum position was  $11.65^\circ$ , in close agreement with the value of  $11.6^\circ$  reported by Lewis and Macdonald<sup>2</sup> as the temperature of maximum density.

The temperatures of maximum density of five mixtures of light and heavy water were also found by the same method. These mixtures behaved in the same manner as the pure components as far as could be told from these measurements and all showed temperatures of maximum density, the apparent temperature of maximum density being about half a degree above the true value in every case investigated. While the temperatures of maximum density of the mixtures were intermediate between those of the pure components, there was not quite a linear relationship between temperature and mole fraction, the temperatures being from 0.2 to 0.5° above the line.

The maximum densities of these mixtures were also measured and are included in the table.

Stokland, Ronaess and Tronstad have just published [*Trans. Faraday Soc.*, **35**, 312 (1939)] a value of  $11.23 \pm 0.02^\circ$  for the temperature of maximum density of D<sub>2</sub>O as the result of a much more precise investigation than this one. While the value which they found is somewhat higher than the value obtained here, it is barely outside the limits of experimental error, and both results are in agreement in indicating that the results of earlier investigations were much too high.

TABLE I  
TEMPERATURES OF MAXIMUM DENSITY OF D<sub>2</sub>O-H<sub>2</sub>O MIXTURES

$N_{D_2O}$	$T$	$T_{\text{apparent}}$	$d_4^t$
0.9985	$11.1 \pm 0.1$	$11.65 \pm 0.1$	1.10577
.9959	11.1	...	1.10522
.9353	10.8	...	1.09909
.7780	9.7	...	1.08264
.6051	8.7	9.2	1.06411
.3784	7.0	7.5	1.04019
.1866	5.6	...	1.01978
.0002	3.85	4.3	1.00000

(4) E. Swift, Jr., *THIS JOURNAL*, **61**, 198 (1939).

(5) This correction was obtained by finding the volume of the pycnometer at a series of temperatures and calculating the effect of the thermal expansion of the glass on the height of the meniscus from the volume of the capillary per unit length.

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